Effect of Hypercholesterolemia on Vascular Reactivity in the Rabbit. II. Influence of Treatment With Dipyridamole on Endothelium-Dependent and Endothelium-Independent Responses in Isolated Aortas of Control and Hypercholesterolemic Rabbits

Tony J. Verbeuren, Marie-Claire Coene, François H. Jordaens, Cor E. Van Hove, Ludo L. Zonnekeyn, and Arnold G. Herman

The effects of cholesterol-feeding in the presence of dipyridamole (0.60 g daily) on contractile responses and on endothelium-dependent and endothelium-independent relaxations in isolated rabbit aortas are described. The investigations were performed simultaneously with those described in Part I (Circ Res 1986;58:552-564), where the effects of cholesterol feeding on vascular reactivity in rabbit arteries (n = 8 in each group) selected at random from the same group of animals was studied. In the hypercholesterolemic rabbits treated with dipyridamole for 8 or 16 weeks, both the increases in plasma cholesterol and the formation of fatty streaks were significantly less pronounced than in the hypercholesterolemic rabbits not receiving the drug. Segments of the isolated arteries were mounted in organ chambers for isometric tension recording. The contractions caused by acetylcholine, prostaglandin F2α, norepinephrine, clonidine, and serotonin and the endothelium-independent relaxations to nitroglycerin were not significantly altered by the hypercholesterolemia in rabbits treated with dipyridamole, even after 16 weeks of treatment. Thus, the decreased responses to norepinephrine, clonidine, and nitroglycerin and the augmented responses to serotonin noted in aortas of hypercholesterolemic rabbits in Part I were absent in the dipyridamole-treated hypercholesterolemic animals. The endothelium-dependent relaxations to ATP and acetylcholine were not affected after 8 weeks of hypercholesterolemia in presence of dipyridamole, while after 16 weeks the relaxations to ATP and acetylcholine were attenuated only in the more severely affected arteries. The effects of hypercholesterolemia + dipyridamole on endothelium-dependent relaxations were significantly less pronounced than those induced by hypercholesterolemia alone. We thus conclude from our studies that in the rabbit 1) dipyridamole can partially inhibit the augmentation in plasma cholesterol levels caused by a cholesterol-rich diet; 2) dipyridamole can slow down the process of formation of intimal aortic lesions caused by the cholesterol feeding; 3) dipyridamole inhibits the cholesterol-induced changes in vascular reactivity, and 4) the degree of formation of intimal aortic lesions determines the degree to which the vascular responses, especially the endothelium-dependent relaxations, are affected by the cholesterol-rich diet. (Circulation Research 1986;59:496-504)

In Part I of this study1 we evaluated the effects of hypercholesterolemia on contractions and relaxations in isolated rabbit arteries. Those experiments illustrated that a cholesterol-rich diet caused an augmented contractile responsiveness to serotonin in severely affected arteries and a decreased responsiveness to norepinephrine and clonidine. The hypercholesterolemia also inhibited the endothelium-mediated relaxations to acetylcholine and ATP in a manner dependent on the degree of fatty streak formation while the endothelium independent relaxations to nitroglycerin were only affected in the most severely damaged arteries. The release of the endothelium-derived relaxant factor (EDRF; see Furchgott and Zawadzki,2 Furchgott3) was not altered in hypercholesterolemic abdominal aortas (see Verbeuren et al1). Blood platelets are believed to play an important role in the genesis of atherosclerosis; at sites of endothelial injury, mitogenic factors are released from aggregating platelets causing proliferation of arterial smooth muscle cells, thus initiating or maintaining the atherosclerotic process.4-7 Important support for the involvement of platelets comes from the experiments performed on pigs with homozygous von Willebrand disease; these animals are resistant to spontaneous and cholesterol-induced atherosclerosis that might be related to the impairment of the platelet mechanisms.8 It has been proposed that drugs which inhibit platelet function can slow down the progress of the atherosclerotic disease.9 Dipryridamole, an inhibitor of phosphodiesterase is known for its ability to stabilize platelets9,10; the drug was able to slow down the formation of atherosclerotic plaques in arteries of rabbits fed a
cholesterol rich-diet, and it was recently shown that dipyridamole can delay the progression of peripheral occlusive arterial disease in man. We have therefore investigated the effect of dipyridamole on the development of atherosclerosis evoked by hypercholesterolemia in rabbits and have, more particularly, studied the effects of a cholesterol-rich diet to which was added dipyridamole on the contractions and relaxations in isolated rabbit aortas. The results described in this part of our study have then been compared with those reported in Part I.

Materials and Methods

In a first series of introductory experiments, two groups of 8 male rabbits were fed either the atherogenic or the control diet to which dipyridamole was added for 8 weeks. For the major part of the investigation, the diets were administered to two groups of 8 male New Zealand rabbits for 16 weeks. During the selected periods, all animals received 150 g of diet daily. All the diets were prepared by Altromin (West Germany). The atherogenic diet was prepared by adding 0.45 g of cholesterol to 150 g of the commercially available diet; determination of the cholesterol content of the atherogenic diets revealed that 0.27% of cholesterol was present in both the diets used for the 8- and 16-week experiments. This value was identical to that obtained in the control study. To 150 g of diet, 0.60 g of dipyridamole was added.

The present study was conducted simultaneously with the control study on rabbits that entered either the control groups (control diet or cholesterol diet) or the groups treated with dipyridamole. Two animals entered the trial daily; they were selected at random for one of the 4 diets (e.g., on Day 1, a control and a cholesterol diet; on Day 2, a control and a dipyridamole diet; on Day 3, a cholesterol and a cholesterol + dipyridamole diet; etc.). Each animal received its respective diet for exactly 8 or 16 weeks. After this period, the histological and organ chamber studies were performed on aortic tissues of 2 animals each day.

Tissue Preparation

After 8 or 16 weeks of receiving the respective diets, the rabbits were anesthetized with sodium pentobarbital (30 mg/kg IV). Before the rabbits were killed a blood sample was obtained for determination of plasma cholesterol and triglyceride levels; in the rabbits used for the 16-week experiments a second blood sample was taken for the determination of plasma levels of dipyridamole and its glucuronide metabolites. Segments of the aortic arch, the thoracic aorta, and the abdominal aorta were carefully removed from the rabbits and placed in physiological salt solution (mM composition: NaCl 118.3; KCl 4.7; CaCl, 2.5; KH,PO, 1.2; MgSO, 1.2; NaHCO, 25.0; calcium EDTA 0.026, and glucose 11.1). Segments of the thoracic aorta were used for macroscopic and microscopic examination of the intimal vessel wall.

Macroscopic Examination

A segment (5 mm in length) of each of the 3 aortas was cut open longitudinally and the intimal surface of the blood vessels was investigated for the occurrence of visible fatty streaks. These streaks (visible as white spots) were then evaluated on a scale from 0 to 5; an artery segment showing no visible streaks received a score of zero, while a segment of which the total intimal surface was covered with lesions received a score of five (see detailed description in legend of Table 2). For the introductory studies (8 weeks of cholesterol), the macroscopic evaluation was performed only on segments of the thoracic aorta. Except for the thoracic aorta, the segments selected for this evaluation were taken from the same area of the blood vessels as the segments used for the organ chamber experiments (see also Part I, Verbeuren et al).

Histological Examination

Segments of the thoracic aorta (either adjacent to the aortic arch or adjacent to the abdominal aorta) were investigated histologically for the occurrence of fatty streaks. Cross-sections of the blood vessel segments, stained with the haemotoxylin & eosin (HE) method or with the Periodic-acid Schiff method (PAS-reaction) were investigated. The part of the intimal outline covered with foam cell plaques was measured and expressed as a percent of the total intimal outline of the section; this value was taken to represent the surface area covered with lesions. The thickness of the fatty streaks was then measured and expressed as a percent of the unaffected wall thickness; since the thickness of the fatty streaks varied over the area affected, a mean value was estimated.

Organ Chamber Experiments

Segments (3 mm in length) of the arteries were mounted in organ chambers (50 ml) filled with Krebs-Ringer bicarbonate solution (37°C) as described previously. The isometric tension development was continuously monitored by means of force transducers (Statham UC2). After equilibration (30 minutes), the arterial segments were placed at the optimal point of their length-tension relation using a standard concentration of norepinephrine (3 × 10⁻⁷ M; see Vanhoutte and Leusen). The tissues were then allowed to equilibrate at their optimal length for 45 minutes prior to experimentation. After equilibration, two major experimental protocols were used in the present study. These protocols were described in detail in Part I.

Briefly, in segments of the arteries (aortic arch and abdominal aorta) obtained from rabbits fed the diets for 8 weeks, dose–response curves to serotonin were performed and relaxations to acetylcholine and ATP were studied during sustained contractions caused by norepinephrine. In segments of the arteries (aortic arch and abdominal aorta) obtained from rabbits fed the diets for 16 weeks, the contractile effects of prostaglandin F₂α, norepinephrine, clonidine, serotonin, and high concentrations of acetylcholine and the relaxations evoked by acetylcholine, ATP, and nitroglycer-
in during sustained contractions caused by prostaglandin F₂₀ were investigated.

The experiments on the arteries of the 16-week rabbits were performed in the presence of indomethacin (3 \( \times 10^{-5} \) M) since it has been shown recently that endogenous prostaglandins can modulate the vascular reactivity of rabbit blood vessels.¹⁷

A major difference between the 8-week and the 16-week studies was that in the former group, the relaxations to acetylcholine and ATP were tested during contractions caused by norepinephrine (3 \( \times 10^{-7} \) M), while in the latter group, the initial contraction was induced by prostaglandin F₂₀ (2 \( \times 10^{-6} \) M). This different approach did not affect the relaxations caused by the vasodilators in the control tissues.¹

**Drugs**

The following pharmacological agents were used: acetylcholine (Sigma), ATP (Sigma), clonidine (Boehringer), dipyridamole (Boehringer), indomethacin (Merck, Sharp & Dohme), nitroglycerin (1% commercial solution in ethanol), norepinephrine (Sigma), prostaglandin F₂₀ (Upjohn), serotonin (Janssen Chimica).

**Statistical Analysis**

The data are expressed as means ± SEM. For statistical analysis, the Student's t test for paired or unpaired observations was used: \( p \)-values < 0.05 were considered to be significant. For the contractile agents, the potency of the compounds was evaluated by calculating \( ED_{50} \) values. The \( ED_{50} \)-values were determined graphically after linear regression of the 20–80% region of the dose–response curves.¹⁸

**Results**

**Body Weight and Plasma Levels of Cholesterol, Triglyceride, and Dipyridamole**

The body weight of the rabbits at the start of the experiments was the same for the 4 groups of animals used; the weight of the 16-week cholesterol animals increased significantly more than that of the control rabbits (Table 1).

The plasma levels of dipyridamole and its glucuronide metabolites were determined in the rabbits used for the 16-week experiments; Table 1 shows that these levels were not different when control and cholesterol-fed animals were compared.

The plasma triglyceride concentrations were not significantly altered after 8 or 16 weeks of hypercholesterolemia (Table 1) whereas the plasma levels of cholesterol were significantly augmented in rabbits fed cholesterol for 8 or 16 weeks (Figure 1). These plasma cholesterol levels noted in the hypercholesterolemic rabbits treated with dipyridamole were significantly lower than those obtained in the hypercholesterolemic rabbits not treated with the drug (Figure 1).

**Macroscopic and Microscopic Evaluation of Fatty Streak Formation**

The macroscopic evaluation of the fatty streaks is illustrated in Table 2. Fatty streaks were not observed in the control arteries. After 8 weeks of the cholesterol-rich diet containing dipyridamole, the intimal side of the thoracic aorta was not significantly affected by the hypercholesterolemia (Table 2). This contrasts to the significant visible damage already noted after 8 weeks of cholesterol-feeding in the rabbits not treated with dipyridamole.¹ After 16 weeks of cholesterol-rich diet the aortic tissues were significantly affected by the hypercholesterolemia with a tendency for the aortic arch to be the most affected (Table 2). For the aortas obtained from dipyridamole treated hypercholesterolemic rabbits the scores were lower than for those obtained from untreated hypercholesterolemic rabbits,¹ but the values were not statistically different.

After 8 weeks of hypercholesterolemia + dipyridamole, the only changes observed at the intimal surface of the thoracic aorta was the infiltration of a few isolated foam cells; the thickness of these lesions was never more than one cell layer. In the thoracic aorta of the rabbits fed the cholesterol-rich diet + dipyridamole for 16 weeks, about 20% of the intimal surface was lesioned; the thickness of these lesions was about 20% of the initial wall thickness (Figure 2). The microscopic examination of the thoracic aorta revealed significantly less damage in the arteries obtained from the

<table>
<thead>
<tr>
<th>Table 1. Body Weight and Plasma Levels of Cholesterol, Triglycerides, and Dipyridamole in Control and Hypercholesterolemic Rabbits Treated With Dipyridamole*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma dipyridamole (µg/ml)</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Control group (8 weeks)</td>
</tr>
<tr>
<td>Cholesterol-rich (8 weeks)</td>
</tr>
<tr>
<td>Control group (16 weeks)</td>
</tr>
<tr>
<td>Cholesterol-rich (16 weeks)</td>
</tr>
</tbody>
</table>

*Values shown as means ± SEM.
†Value significantly different from respective control (\( p \) < 0.05; Student's t-test for unpaired observations.)
ND = not determined.
Verbeuren et al  Dipyridamole and Hypercholesterolemia in the Rabbit

Effect of dipyridamole (0.60 g daily) on plasma cholesterol levels in rabbits fed control or cholesterol-rich (0.3%) diet for 8 or 16 weeks. The data "without dipyridamole" are redrawn from Verbeuren et al.1 Value significantly different from that obtained in rabbits not treated with dipyridamole (p < 0.05; Student's t test for unpaired observations).

Vascular Responses

Effect of Dipyridamole Treatment Per Se on Vascular Reactivity. Dipyridamole treatment per se did not alter the contractile responses or the relaxations obtained with the different agents used (see Figures 3 and 4 and Verbeuren et al1).

RABBITS FED THE CHOLESTEROL-RICH DIET + DIPYRIDAMOLE FOR 8 WEEKS. The contractions caused by serotonin (10^-8 to 10^-5 M) in segments of the aortic arch and the abdominal aorta obtained from rabbits fed cholesterol + dipyridamole for 8 weeks were not different from those obtained in arteries of rabbits fed only dipyridamole as illustrated by the ED50-values and the maximal contractions (Tables 3 and 4). In the aortas obtained from the 8-week hypercholesterolemic rabbits treated with dipyridamole, acetylcholine (10^-9 to 3 \times 10^-6 M), and ATP (10^-4 to 10^-4 M) caused concentration dependent relaxations that were not significantly different from those observed in arteries obtained from control rabbits treated with dipyridamole, as seen in Figures 3 and 4. These figures also illustrate that in aortas from rabbits fed cholesterol without dipyridamole for 8 weeks, the relaxations to acetylcholine and

Table 2. Macroscopic Evaluation of Fatty Streak Formation in Arteries of Control and Hypercholesterolemic Rabbits Treated With Dipyridamole*

<table>
<thead>
<tr>
<th>Artery</th>
<th>Control</th>
<th>8 Weeks Cholesterol†</th>
<th>16 Weeks Cholesterol†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic arch</td>
<td>0</td>
<td>ND</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0</td>
<td>ND</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>0</td>
<td>0.4 ± 0.3</td>
<td>1.4 ± 0.6</td>
</tr>
</tbody>
</table>

*Scale: 0-5
0. no visible fatty streaks.
1. only a few, small, widely spread fatty streaks
2. a few fatty streaks spread over the intimal surface
3. a lot of small fatty streaks that do not overlap
4. fatty streaks separated by little spots of "intact" intimal surface
5. the total intimal surface of the artery is covered by fatty streaks
†The results are shown as means ± SEM; n = 8 in each group.
ND = not determined.

Figure 1. Effect of dipyridamole (0.60 g daily) on plasma cholesterol levels in rabbits fed control or cholesterol-rich (0.3%) diet for 8 or 16 weeks. The data "without dipyridamole" are redrawn from Verbeuren et al.1

Figure 2. Microscopic evaluation of fatty streak formation: effect of dipyridamole on the surface area covered with lesions and on the thickness of the lesions in rabbit aortic tissues obtained from control or hypercholesterolemic rabbits. In cross sections of the vessel wall segments the part of the intimal outline covered with foam cell plaques was expressed as percent of the total intimal outline of the section; this value was taken to represent the surface area covered with lesions. The thickness of the fatty streaks was expressed as a percent of the unaffected wall thickness. In some of the tissues obtained from the dipyridamole treated rabbits used for the 8-week experiments, a few foam cells were detected in the intima; the thickness was never more than one cell layer. The data "without dipyridamole" are redrawn from Verbeuren et al.1 The effect of dipyridamole was significant (p < 0.05; Student's t test for unpaired observations).
Figure 3. Relaxations to acetylcholine (upper), nitroglycerin (middle), and ATP (lower) in aortic arches of control (○—○), 8-week (□—□) and 16-week (△—△) hypercholesterolemic rabbits. Filled symbols (● ■) indicate that the difference between the control and the hypercholesterolemic artery is significant. In the right panels, the results obtained in aortic arches of rabbits treated for either 8 or 16 weeks with 0.60 g of dipyridamole daily, are shown. For details of the procedures used see "Materials and Methods." The results are shown as means; n = at least 6 in each group. In the aortic arches of the hypercholesterolemic rabbits treated with dipyridamole, the endothelium-dependent relaxations (to acetylcholine and ATP) and the endothelium-independent relaxations (to nitroglycerin) are either not altered or much less inhibited than those obtained in arches of hypercholesterolemic rabbits not treated with dipyridamole.

Figure 4. Relaxations to acetylcholine (upper), nitroglycerin (middle) and ATP (lower) in abdominal aortas of control (○—○), 8-week (□—□) and 16-week (△—△) hypercholesterolemic rabbits. Filled symbols (● ■) indicate that the difference between the control and the hypercholesterolemic artery is significant. In the right panels, the results obtained in abdominal aortas of rabbits treated for either 8 or 16 weeks with 0.60 g of dipyridamole daily, are shown. For details of the procedures used, see "Materials and Methods." The results are shown as means; n = at least 6 in each group. In the aortas of the hypercholesterolemic rabbits treated with dipyridamole, the endothelium-dependent relaxations (to acetylcholine and ATP) and the endothelium-independent relaxations (to nitroglycerin) are either not altered or much less inhibited than those obtained in aortas of hypercholesterolemic rabbits not treated with dipyridamole.

Rabbits fed the cholesterol-rich diet + dipyridamole for 16 weeks. Contraction: Increasing concentrations of prostaglandin F2α (10^-7 to 3 x 10^-5 M), acetylcholine (10^-9 to 10^-3 M), norepinephrine (10^-9 to 10^-5 M), clonidine (10^-4 to 10^-3 M), and serotonin (10^-4 to 10^-3 M) caused contractions in segments of the aortic arch (Table 3) and the abdominal aorta (Table 4) of control and hypercholesterolemic rabbits treated with dipyridamole. As illustrated by the maximal responses and the ED50 values, no significant differences in the responsiveness to these agonists were noted when the control and hypercholesterolemic tissues were compared (Tables 3 and 4). Thus, the decreased responses to norepinephrine and clonidine and the enhanced responses to serotonin, noted in aortas of hypercholesterolemic rabbits not receiving dipyridamole, were no longer detected when rabbits were given dipyridamole.

Relaxations: Acetylcholine (10^-9 to 3 x 10^-6 M), ATP (10^-4 to 10^-3 M), and nitroglycerin (10^-4 to 10^-3 M) evoked concentration-dependent relaxations in the aortic arch and in the abdominal aorta.
Table 3. Evaluation of Contractile Responses in Aortic Arches of Control and Hypercholesterolemic Rabbits Treated With Dipyridamole*

<table>
<thead>
<tr>
<th>Maximal response (g)</th>
<th>Aortic arch segments of rabbits treated with dipyridamole</th>
<th>8-Week</th>
<th>Control</th>
<th>16-Week</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hypercholesterolemia</td>
<td>hypercholesterolemia</td>
<td>hypercholesterolemia</td>
<td>hypercholesterolemia</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td>5.1 ± 1.1(5)</td>
<td>5.7 ± 1.2(5)</td>
<td>7.8 ± 1.1 (8)</td>
<td>5.7 ± 1.3 (8)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td>13.2 ± 2.2 (8)</td>
<td>11.2 ± 2.1 (8)</td>
<td>13.2 ± 2.4 (8)</td>
<td>11.2 ± 2.1 (8)</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>6.6 ± 1.0 (8)</td>
<td>3.7 ± 1.0 (8)</td>
<td>6.6 ± 1.0 (8)</td>
<td>3.7 ± 1.0 (8)</td>
</tr>
<tr>
<td>Prostaglandin F&lt;sub&gt;2α&lt;/sub&gt;</td>
<td></td>
<td>11.8 ± 1.6 (7)</td>
<td>8.3 ± 1.9 (6)</td>
<td>11.8 ± 1.6 (7)</td>
<td>8.3 ± 1.9 (6)</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td>0.49 ± 0.14(8)</td>
<td>0.88 ± 0.28(8)</td>
<td>0.49 ± 0.14(8)</td>
<td>0.88 ± 0.28(8)</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt; value (× 10&lt;sup&gt;−7&lt;/sup&gt;M)</td>
<td></td>
<td>5.88 ± 0.69</td>
<td>4.71 ± 0.92</td>
<td>3.44 ± 0.66</td>
<td>2.15 ± 0.28</td>
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<tr>
<td>Serotonin</td>
<td></td>
<td>0.85 ± 0.31</td>
<td>1.66 ± 0.42</td>
<td>0.85 ± 0.31</td>
<td>1.66 ± 0.42</td>
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<tr>
<td>Norepinephrine</td>
<td></td>
<td>6.22 ± 2.43</td>
<td>13.4 ± 3.10</td>
<td>6.22 ± 2.43</td>
<td>13.4 ± 3.10</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>24.8 ± 7.0</td>
<td>22.7 ± 1.9&lt;sup&gt;*&lt;/sup&gt;</td>
<td>24.8 ± 7.0</td>
<td>22.7 ± 1.9&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td>258 ± 58</td>
<td>352 ± 52</td>
<td>258 ± 58</td>
<td>352 ± 52</td>
</tr>
</tbody>
</table>

*Values shown as means ± SEM; the number of experiments in each group is shown between brackets.

The relaxations to acetylcholine were significantly attenuated only in the aortic arch (Figure 3) and in the abdominal aorta (Figure 4) of the 16 weeks hypercholesterolemic rabbits treated with dipyridamole; these relaxations to acetylcholine were significantly more pronounced than those noted in the tissues obtained from hypercholesterolemic rabbits not treated with dipyridamole (Figures 3 and 4). In aortic arches of control and hypercholesterolemic animals treated with or without dipyridamole, the maximal relaxation to acetylcholine was plotted as a function of the degree of intimal lesion formation. Figure 5 illustrates that a linear correlation between both parameters was obtained indicating that the relaxation is progressively inhibited as the degree of intimal damage increases.

The relaxations to nitroglycerin were not significantly altered in the aortas obtained from hypercholesterolemic rabbits treated with dipyridamole (Figures 3 and 4); for comparison, in the aortic arch of hypercholesterolemic rabbits not treated with dipyridamole, the relaxations to nitroglycerin were significantly attenuated as compared to those noted in control arteries (Figure 3).

The relaxations to ATP were significantly attenuated only in the aortic arch (Figure 3) obtained from the rabbits treated for 16 weeks with cholesterol + dipyridamole; these relaxations to ATP were more pronounced than those noted in arteries obtained from hypercholesterolemic rabbits not treated with dipyridamole (Figure 3). In the abdominal aortas hypercholesterolemic rabbits treated with dipyridamole and the abdominal aorta previously contracted with prostaglandin F<sub>2α</sub> (Figures 3 and 4).

Table 4. Evaluation of Contractile responses in Abdominal Aortas of Control and Hypercholesterolemic Rabbits Treated With Dipyridamole*

<table>
<thead>
<tr>
<th>Maximal response (g)</th>
<th>Abdominal aorta segments of rabbits treated with dipyridamole</th>
<th>8-Week</th>
<th>Control</th>
<th>16-Week</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hypercholesterolemia</td>
<td>hypercholesterolemia</td>
<td>hypercholesterolemia</td>
<td>hypercholesterolemia</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td>5.2 ± 1.0(5)</td>
<td>6.9 ± 1.5(5)</td>
<td>4.9 ± 0.4 (8)</td>
<td>4.1 ± 0.6 (8)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td>9.0 ± 1.5 (8)</td>
<td>7.4 ± 0.8 (8)</td>
<td>9.0 ± 1.5 (8)</td>
<td>7.4 ± 0.8 (8)</td>
</tr>
<tr>
<td>Clonidine</td>
<td></td>
<td>4.6 ± 1.0 (8)</td>
<td>3.3 ± 0.6 (8)</td>
<td>4.6 ± 1.0 (8)</td>
<td>3.3 ± 0.6 (8)</td>
</tr>
<tr>
<td>Prostaglandin F&lt;sub&gt;2α&lt;/sub&gt;</td>
<td></td>
<td>6.8 ± 1.2 (8)</td>
<td>5.3 ± 1.1 (8)</td>
<td>6.8 ± 1.2 (8)</td>
<td>5.3 ± 1.1 (8)</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td>0.41 ± 0.13(8)</td>
<td>0.70 ± 0.28(8)</td>
<td>0.41 ± 0.13(8)</td>
<td>0.70 ± 0.28(8)</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt; value (× 10&lt;sup&gt;−7&lt;/sup&gt;M)</td>
<td></td>
<td>6.64 ± 1.47</td>
<td>4.01 ± 1.08</td>
<td>4.53 ± 0.65</td>
<td>3.06 ± 0.59</td>
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<td>Serotonin</td>
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<td>0.84 ± 0.10</td>
<td>1.06 ± 0.20</td>
<td>0.84 ± 0.10</td>
<td>1.06 ± 0.20</td>
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<tr>
<td>Norepinephrine</td>
<td></td>
<td>6.71 ± 1.52</td>
<td>8.56 ± 1.89</td>
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<td>8.56 ± 1.89</td>
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<tr>
<td>Clonidine</td>
<td></td>
<td>33.1 ± 6.9</td>
<td>30.4 ± 5.8</td>
<td>33.1 ± 6.9</td>
<td>30.4 ± 5.8</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td>316 ± 77</td>
<td>267 ± 84</td>
<td>316 ± 77</td>
<td>267 ± 84</td>
</tr>
</tbody>
</table>

*Values shown as means ± SEM; the number of experiments in each group is shown between brackets.
terolome + dipyridamole did not affect the relaxations to ATP (Figure 4), even after 16 weeks of treatment.

**Discussion**

In Part I of our study about the influence of hypercholesterolemia on vascular reactivity, we were able to illustrate profound effects of cholesterol-feeding on contractions and relaxations (especially on endothelium-dependent relaxations) in isolated arteries of the rabbit. The second part of our investigation was designed to test whether dipyridamole, a phosphodiesterase inhibitor that delays the breakdown of cyclic AMP and stabilizes the platelets, can interfere with the consequences of developing atherosclerosis on vascular reactivity in the rabbit. Since blood platelets are believed to play an important, if not a decisive, role in the morphogenesis of atherosclerosis, drugs such as dipyridamole, which inhibit platelet aggregation, are believed to slow down the progress of the disease.

In the rabbits treated for 8 or 16 weeks with 0.60 g of dipyridamole daily, no significant changes in plasma cholesterol levels were noted as compared to the levels reported for control rabbits not receiving the drug. In rabbits fed the cholesterol-rich diet + dipyridamole for 8 or 16 weeks, a significant increase in plasma cholesterol levels was obtained; however, this increase was significantly less pronounced than that obtained in cholesterol-fed rabbits not receiving dipyridamole. This observation differs from that reported by Hallermayer; he illustrated that in rabbits fed a cholesterol-rich diet (2%) for 6 weeks, increasing doses of dipyridamole did not decrease the plasma cholesterol levels.

Although our present results do not provide an explanation for these different findings, it should be noted that in our study the rabbits received a higher dose of dipyridamole (0.60 g versus ± 0.20 g daily) but a lower dose of cholesterol (0.3% vs. 2%) and that we used male rabbits whereas those used by Hallermayer were female animals.

After 8 weeks of cholesterol-rich diet + dipyridamole, no significant lesion formation was noted in the thoracic aorta of the rabbits neither when investigated macroscopically nor when studied microscopically. When these results are compared with those obtained in the thoracic aorta of rabbits treated for 8 weeks with cholesterol alone, they indicate that the treatment with dipyridamole caused a profound inhibition of the lesion formation. Similarly, after 16 weeks of treatment with cholesterol + dipyridamole, the formation of lesions in the aortic tissues was less pronounced than after 16 weeks of cholesterol. Thus, dipyridamole is able to counteract the formation of fatty streaks. Our results are also in agreement with those of a recent clinical study in which it was shown that combined treatment of hypercholesterolemic patients with acetylsalicylic acid and dipyridamole was more effective than acetylsalicylic acid alone in slowing the progression of peripheral occlusive atherosclerosis.

A likely explanation for our results is that the reduced lesion formation noted in the cholesterol-fed rabbits treated with dipyridamole is caused by the lower plasma cholesterol levels obtained in these animals. However, since the plasma cholesterol levels were not altered in Hallermayer’s experiments, a reduced plasma cholesterol content does not seem to be a prerequisite to obtain a decreased fatty streak formation. Support for that conclusion also comes from recent studies with Ca$^{2+}$-antagonists; indeed certain Ca$^{2+}$-antagonists can reduce the diet-induced atherosclerosis in monkeys and rabbits without normalizing the serum cholesterol levels.

Since treatment of the rabbits with dipyridamole can slow down the process of lesion formation caused by cholesterol in the aortic tissues, the question arises whether this treatment also affects the changes in vascular reactivity caused by hypercholesterolemia. In aortas of control rabbits treated for 8 or 16 weeks with 0.60 g of dipyridamole daily, the contractile responses to norepinephrine, clonidine, serotonin, prostaglandin F$_{2a}$, and to high concentrations of acetylcholine and the relaxations to acetylcholine, ATP, and nitroglycerin were not significantly different from those noted in arteries obtained from rabbits not receiving the drug. These results indicate that the administration of dipyridamole by itself did not interfere with the contractions and relaxations of the vascular smooth muscle cells, and that the drug did not alter the endothelium-dependent relaxations in the aortas.

In the aortas of rabbits treated with cholesterol +
dipyridamole, the contractile responses to the different agonists studied were not significantly altered as compared to those obtained in aortas of rabbits treated with dipyridamole alone. In Part I of our study, we could demonstrate that in the most severely affected artery (the aortic arch) the contractile responses to the lowest concentrations of serotonin were augmented; we also showed that the contractions induced by noradrenaline and clonidine were decreased in arteries obtained from hypercholesterolemic rabbits. Our present results with the arteries of the dipyridamole treated rabbits show that the drug can prevent the changes in the contractile responses induced by 16-week cholesterol feeding.

The endothelium-independent relaxations to nitroglycerin were significantly attenuated in the more severely affected arteries of hypercholesterolemic rabbits (see Figure 3 and Verbeuren et al). However, in the aortic tissues of the hypercholesterolemic rabbits treated with dipyridamole, the relaxations to nitroglycerin were not significantly different from those obtained in control rabbits treated with dipyridamole only, indicating that the drug prevents the effect of 16 weeks of cholesterol feeding on the ability of the vascular smooth muscle cells to relax.

In aortas of the hypercholesterolemic rabbits treated with dipyridamole, the endothelium-dependent relaxations to acetylcholine and ATP were significantly attenuated only after 16 weeks of cholesterol feeding; those to ATP were even then still unaltered in the abdominal aortas (Figures 3 and 4). When these results are compared with those obtained in rabbits not treated with dipyridamole (see Verbeuren et al' and Figures 3 and 4), it is obvious that dipyridamole slows down the inhibition of these endothelium-mediated relaxations caused by the high cholesterol intake.

In summary: Adding dipyridamole to the cholesterol-rich diet of the rabbits, illustrates that the drug 1) inhibits the elevation of the plasma cholesterol levels, 2) inhibits the formation of atherosclerotic lesions in the aortic tissues, and 3) inhibits the effects of cholesterol-feeding on the contractions and relaxations of the aortas. How dipyridamole exerts these different effects remains to be determined.

It has been speculated that the inhibition of the phosphodiesterase and/or uptake of adenosine by the platelets, resulting in an inhibition of thrombotic processes and smooth muscle cell proliferation, might contribute to the anti-atherosclerotic effect of the drug. Both inhibition of phosphodiesterase and inhibition of adenosine uptake into platelets are achieved with the plasma concentrations of dipyridamole found in the present study (± 0.8 μg/ml; about 2 μM). By blocking the platelet phosphodiesterase enzyme dipyridamole has been shown to potentiate the increase in cyclic adenosine-5'-monophosphate and the platelet inhibition evoked by prostacyclin. Inhibition of adenosine uptake by platelets is an unlikely possibility since in the rabbit the platelet uptake of adenosine is negligible; indeed, in the rabbit adenosine is mainly taken up by and deaminated in red blood cells. Aortic endothelial cells have also been shown to accumulate adenosine by a high affinity transport system that can be blocked by dipyridamole; in the pig, the concentrations of dipyridamole required to block this adenosine uptake are in the micromolar range, and thus inhibition of uptake of adenosine into endothelial cells could contribute to the beneficial effects of dipyridamole. Other effects of dipyridamole such as inhibition of adenosine kinase and of adenosine deaminase are probably not involved since very high concentrations of dipyridamole are required to achieve these actions. Dipyridamole is often used in combination with aspirin; since in the rabbit the suppression of the lesion formation caused by dipyridamole is more pronounced when aspirin is also added to the diet, it has been speculated that prostaglandin metabolism may be involved.

Hypertension is considered to be a risk factor that could evoke endothelial lesions leading to atherosclerosis. Dipyridamole is known to decrease blood pressure as a result of the dilatation of the systemic resistance vessels. Such a decreased blood pressure could theoretically also explain the beneficial effects of dipyridamole on the development of atherosclerosis. It should be mentioned, however, that feeding rabbits a diet containing 2% cholesterol during 10 weeks did not change their mean arterial blood pressure.

It is clear from the above described possibilities that a variety of mechanisms may help to explain the anti-atherosclerotic effects of dipyridamole. It is also noteworthy that other drugs, in particular Ca2+-antagonists such as nifedipine, verapamil and diltiazem, certain anti-calciifying agents, lanthanum, and indomethacin can also interfere with atherogenesis in experimental animals; these agents did not influence serum cholesterol levels but did normalize serum Ca2+-levels and did decrease Ca2+-deposition in aortic tissues. Whether the effect of these antagonists and that of dipyridamole share a common mechanism, e.g., at the level of the platelets, remains to be investigated; indeed, platelet aggregation is a Ca2+-dependent phenomenon and certain Ca2+-antagonists can inhibit platelet aggregation both in vitro and in vivo. In 1979 an increased atherosclerotic plaque formation in the rabbit aorta caused by dipyridamole was reported. In that study dipyridamole was administered by intramuscular injections to rabbits that were already on the high cholesterol-diet for 4 months. The different methodology used in our study may be one reason for the difference in results obtained. It should also be pointed out that those authors based their conclusions on observations made in 3 animals in each group and our present investigation shows an important variability in the formation of lesions in the aortic tissues of the cholesterol-treated group (see, e.g., Figure 5).

It seems logical to conclude that the inhibition of fatty streak formation caused by dipyridamole is the primary reason why the contractile responses and the relaxations of the vascular preparations are more or less restored in the hypercholesterolemic rabbits treat-
ed with dipyridamol. As regards the endothelium-dependent relaxations to acetylcholine, this conclusion is supported by the results shown in Figure 5, where a significant correlation is illustrated between the degree of visual lesions and the maximal relaxation to acetylcholine in aortic arches obtained from control and hypercholesterolemic animals treated with or without dipyridamole; as the degree of damage to the intimal surface augments, the maximal relaxation to acetylcholine (expressed as a percent of the initial contraction) decreases. These observations thus support our conclusions also suggested in Part I of this study, i.e., the more severely the intimal surface is damaged by the hypercholesterolemia, the more the endothelium mediated relaxations are inhibited.

Note: During the revision process of this manuscript, we became aware of a study by Habib et al. (Circ Res 1986;58:305-309, in which the preservation of endothelium-mediated relaxations is described. The authors wish to acknowledge the technical assistance of Y. Onsea and are grateful to L. Van den Eynde for typing the manuscript. They also are indebted to Prof. D. L. Lepoutre (University Hospital of Antwerp) for determination of cholesterol and triglyceride levels, to Prof. D. A. Dresse (University of Liege) for the determination of dipyridamole levels and to Dr. H. Püschnerr (Dr. Karl Thomae GMBH) for performing the histological examination of the thoracic aorta segments.

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