Differential Alteration of Vascular Reactivity in Rabbit Aorta With Modest Elevation of Serum Cholesterol

Linda A. Merkel, Luz M. Rivera, Glenda E. Bilder, and Mark H. Perrone

The effect of diet-induced, moderate elevation of serum cholesterol on vascular reactivity in isolated rabbit abdominal aortic rings was examined by using a series of vasoconstrictor and vasodilator agonists. Serum cholesterol of rabbits that were fed a cholesterol-free, casein-rich diet for 10 weeks was elevated approximately 4.5-fold compared with values found in control rabbits that were fed standard lab chow (223±41 versus 51±5 mg/dl, respectively). Relaxation responses to carbachol chloride and (+)-isoproterenol hydrochloride in vessels from hypercholesterolemic rabbits were markedly inhibited in the presence of norepinephrine, prostaglandin F2α, 5-hydroxytryptamine, and angiotensin II but not in the presence of phorbol 12,13-dibutyrate. The depressed vasodilation in hypercholesterolemic vessels appeared to depend on the agonist initiating the contraction. Sodium nitroprusside–induced relaxations were unchanged in rings from hypercholesterolemic rabbits compared with rings from control rabbits for all contractile agonists except KCl. Isolated aortic rings from hypercholesterolemic rabbits exhibited a slight but significantly increased vasoconstrictor sensitivity to 5-hydroxytryptamine and KCl but not to norepinephrine, prostaglandin F2α, angiotensin II, or phorbol 12,13-dibutyrate compared with aortic rings from control rabbits. These results demonstrate that modest elevation of serum cholesterol is sufficient to depress vasodilator and enhance vasoconstrictor responses to certain agonists. Vasodilator effects are impaired to a greater extent by a small increase in serum cholesterol than are responses to vasoconstrictor agonists. It is postulated that the induction of differential alterations in vascular reactivity with moderate increase in serum cholesterol may represent important early events predisposing arteries to vasospasm. (Circulation Research 1990;67:550–555)

Vasospasm is an important clinical entity that is thought to contribute to precipitation of angina, myocardial infarction, and sudden death. Since the presence of atherosclerotic lesions results in a loss of endothelium-dependent relaxation, and an increased or decreased β-adrenergic receptor activity, atherosclerosis purportedly predisposes vessels to vasospasm. Previous reports of altered reactivity in atherosclerosis recorded vascular effects in vessels with overt atherosclerotic pathology and/or in vessels from animals with serum cholesterol levels of 600–2,000 mg/dl, levels far in excess of those found in humans with coronary disease. However, little is known about changes in arterial reactivity associated with moderate elevations of serum cholesterol.

Since vasospasm frequently occurs in vessels without angiographic evidence of overt atherosclerosis and since coronary artery disease occurs in individuals with serum cholesterol levels 3–10-fold lower than used previously in animal studies, it seemed important to investigate the relation between a modest elevation in serum cholesterol and vascular reactivity to vasoconstrictor and vasodilator agents. The aim of our study was to examine alterations in vascular reactivity in rabbits with moderately elevated serum cholesterol. Our results show that small changes in serum cholesterol depress vasorelaxation to (+)-isoproterenol hydrochloride (ISO) and carbachol chloride (CAR) and enhance vasoconstriction to KCl and 5-hydroxytryptamine (5-HT), suggesting that marked elevation in serum cholesterol and overt pathological changes are not required for altered vascular reactivity.

Materials and Methods

Animals

Specific pathogen-free (SPF) male rabbits (Hazelton Laboratories, Richmond, Va.) (n=9) were fed a cholesterol-free diet (Wistar Diet No. 12203, Dyets,
Bethlehem, Pa.) containing (g/kg) casein 246, sucrose 398, corn oil 10, hydrogenated coconut oil 10, and nutrients for 10 weeks. SPF male rabbits of the same strain (n=8) were fed a normal diet of standard lab chow (Purina Mills Inc., St. Louis).

**Materials**

(-)-Arterenol bitartrate, prostaglandin F₂a (PGF₂a), [Asn¹,Val¹]angiotensin II (Ang II), phorbol 12,13-dibutyrate (PDBu), ISO, sodium nitroprusside (SNP), CAR, atropine sulfate, and DL-propranolol hydrochloride were purchased from Sigma Chemical Co., St. Louis. Sodium meclofenamate was a gift from Warner-Lambert, Ann Arbor, Mich.

**Isometric Tension**

Rabbits were anesthetized with 80 mg/kg sodium pentobarbital, and their abdominal aortas were excised quickly and placed into warm (37°C) oxygenated (95% O₂-5% CO₂) Krebs-Henseleit buffer of the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 2.5, and glucose 10.0, at pH 7.4. The vessel was cleaned of fat, blood, and adhering tissue, cut into rings approximately 1–2 mm wide, and mounted in water-jacketed tissue baths on L-shaped hooks between a stainless steel rod and a force transducer (model FT-03, Grass Instrument Co., Quincy, Mass.). Aortic rings were equilibrated for 60 minutes at a resting tension of 1 g, with buffer changes at 5, 10, 15, 30, 45, and 60 minutes. Contractions were evoked by the addition of agonists to the bath in a cumulative fashion. The maximal tension achieved with each agonist was set equal to 100%. For relaxation studies, increasing doses of vasodilator agonist were added to rings that were precontracted to steady-state tension by a specified vasoconstrictor agonist. In all experiments, except with PDBu, each dose remained in the bath for 5 minutes or until a new steady state was reached. Each concentration of PDBu remained in the bath for 30 minutes or until a new steady state was reached.

The effect of ISO was assessed in the presence of 1 mM ascorbic acid in the Krebs-Henseleit buffer. For KCl-induced contractions, the following blockers were added 15 minutes before KCl addition: sodium meclofenamate (1 μM) to inhibit prostaglandin synthesis, propranolol (1 μM) to block β-adrenergic receptors, and atropine (1 μM) to block muscarinic receptors. Results of subsequent studies, however, have shown that the addition of these blockers is unnecessary in experiments with rabbit abdominal aorta.

**Experimental Protocol**

This study was designed to examine vascular reactivity in aortic rings from normal and hypercholesterolemic rabbits using six different contractile agonists: norepinephrine, PGF₂a, Ang II, 5-HT, PDBu, and KCl. In addition, the ability of three different relaxants—the endothelium-dependent vasodilator CAR, the β-adrenergic vasodilator ISO, and the endothelium-independent vasodilator SNP—to relax aortic rings precontracted with a single dose of contractile agonist was assessed. Each tissue was exposed to only one contractile agonist or a combination of one vasoconstrictor and one vasodilator.

All drugs, except PDBu, were prepared fresh daily and dissolved in water. PDBu was dissolved in dimethyl sulfoxide at a concentration of 20 mg/ml and kept frozen. Fresh aliquots were used for each experiment.

**Serum Cholesterol Determination**

Blood (2 ml) was withdrawn from the ear artery immediately before the rabbits were anesthetized. Total cholesterol in the freshly prepared serum was assayed by using a colorimetric assay kit (No. 352, Sigma) according to manufacturer’s instructions. The standard curve was constructed using bovine cholesterol.

**Statistics**

Differences in responses from normal and hypercholesterolemic tissues were tested for statistical significance using Student’s t test. Significance was accepted at the 0.05 level of probability.

**Results**

**Serum Cholesterol Changes**

Based on the serum cholesterol determination after 10 weeks of study, rabbits could be easily separated into two groups: group N, control rabbits with normal serum cholesterol (51±5 mg/dl, n=7); and group H, rabbits with hypercholesterolemia (223±41 mg/dl, n=10). Group N included six rabbits that were fed standard lab chow and one rabbit that did not respond to the cholesterol-free, casein-rich diet and exhibited a serum cholesterol level of 70 mg/dl and vascular reactivity similar to the control group. Group H included eight rabbits that were fed the cholesterol-free, casein-rich diet and two rabbits that were fed standard lab chow and exhibited elevated cholesterol levels of 128 and 114 mg/dl, respectively, and vascular reactivity similar to rabbits fed the special diet.

Serum cholesterol levels in group H were elevated approximately 4.5-fold above levels in group N.

**Vascular Reactivity to Contractile Agonists**

Cumulative doses (1 nM to 0.1 mM) of norepinephrine, PGF₂a, 5-HT, Ang II, PDBu, and KCl (12–80 mM) evoked concentration-dependent contractions in aortic rings from group H and group N (Figure 1). The dose-response curve to 5-HT was shifted significantly to the left in rings from group H compared with group N (EC₅₀ 0.22 μM versus 0.8 μM, p<0.05). Likewise, the dose-response curve to KCl was shifted to the left in group H compared with group N (EC₅₀ 25 versus 30 mM, p<0.05). In comparison, the contractile responses to norepinephrine, PGF₂a, Ang II, and PDBu were similar in rings from group H and group N. Maximal tension development
with the various contractile agonists was similar in vessels from both groups of rabbits; however, tension in response to Ang II was significantly higher in group H (Table 1).

**Vascular Reactivity to Vasodilators**

In aortic rings from group N that were precontracted with norepinephrine (3 μM, EC₉₀), PGF₂α (10 μM, EC₇₅), or 5-HT (10 μM, EC₉₀), CAR-induced vasorelaxation was dose dependent and of comparable magnitude, regardless of the constrictor agonist (Figure 2). In vessels from group H, relaxation by CAR was impaired to different extents, depending on the contractile agonist used. In PGF₂α-contracted rings, CAR relaxation in group H was almost completely abolished (group N, 44±16%; group H, 7±4%; p<0.05) (Figure 2). In 5-HT-contracted tissues, maximal relaxation to CAR in vessels from group H was half as great as in group N (group N, 50±12%; group H, 28±10%; p<0.05).

This was also evident for Ang II (0.1 μM, EC₉₀) (group N, 40±7%; group H, 21±8%; p<0.05). With norepinephrine as a constrictor, there appeared to be no significant difference between the two groups (group N, 36±20%; group H, 22±12%). These data indicate an impaired endothelium-dependent relaxation in vessels from group H. PDBu-contracted rings (30 μM, EC₉₀) from both groups of rabbits were refractory to the vasorelaxant effects of CAR.

The magnitude of relaxation with the β-adrenergic vasodilator ISO was similar among group N rings precontracted with PGF₂α, Ang II, or 5-HT (Figure 3). In group H rings, ISO-induced vasorelaxation was impaired. In PGF₂α-stimulated rings, ISO-induced relaxation in group H was virtually abolished, whereas in the presence of Ang II or 5-HT, vessels relaxed 37±12% and 27±14%, respectively.

In tissues precontracted with PDBu as agonist, ISO was ineffective in relaxing vessels from either group, a phenomenon similarly noted with CAR in the presence of PDBu.

The endothelium-independent vasodilator SNP did not affect relaxation in group H compared with group N for all contractile agonists used, except for KCl. In potassium-depolarized tissues, relaxation was significantly enhanced in group H compared with group N (Figure 4).

**Table 1. Maximal Contractile Force in Rabbit Aortic Rings**

<table>
<thead>
<tr>
<th></th>
<th>Group N</th>
<th>Group H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iso</td>
<td>3.00±0.7</td>
<td>2.45±0.55</td>
</tr>
<tr>
<td>PGE₂α</td>
<td>1.17±0.32</td>
<td>1.58±0.38</td>
</tr>
<tr>
<td>Ang II</td>
<td>0.7±0.15</td>
<td>1.36±0.19</td>
</tr>
<tr>
<td>5-HT</td>
<td>1.6±0.32</td>
<td>1.77±0.48</td>
</tr>
<tr>
<td>KCl</td>
<td>1.8±0.43</td>
<td>2.35±0.61</td>
</tr>
<tr>
<td>PDBu</td>
<td>2.6±1.2</td>
<td>1.6±0.24</td>
</tr>
</tbody>
</table>

Values are derived from cumulative dose-response curves to the respective contractile agonists. Data represent mean±SEM from six to eight rabbits. Group N, control rabbits with normal serum cholesterol; group H, rabbits with hypercholesterolemia; NE, norepinephrine; PGE₂α, prostaglandin F₂α; Ang II, angiotensin II; 5-HT, 5-hydroxytryptamine; PDBu, phorbol 12,13-dibutyrate.

*Significantly different from group N.

**Discussion**

The most striking change in vascular reactivity produced by moderate elevation of serum cholesterol was a 30–90% reduction in receptor-mediated muscarinic (CAR) and β-adrenergic (ISO) vasorelaxation. This cholesterol-induced alteration of vascular responsiveness was maximal in vessels constricted with PGF₂α and Ang II and minimal in the presence of norepinephrine and 5-HT. Although a reduced
endothelium-dependent vasodilation in atherosclerotic vessels has been frequently noted.\(^4\)\(^-\)\(^1\(^1\) the serum cholesterol was threefold to fivefold higher than reported here. Similarly, an altered response to \(\beta\)-adrenergic agonists has been associated with hypercholesterolemia.\(^1\(^4\)\(^,\)\(^1\(^5\)\) However, unlike endothelium-dependent vasorelaxation, reduced \(\beta\)-adrenergic vasodilation has not been a consistent finding.\(^1\(^4\)\(^,\)\(^1\(^5\)\)

This is possibly due to the complexity of the ISO-induced response, which at lower doses stimulates vasorelaxation via \(\beta\)-adrenergic activation in both endothelial and smooth muscle cells and at higher concentrations induces vasoconstriction by activation of \(\alpha\)-adrenergic receptors.\(^1\(^8\)\) Therefore, it is possible that the hypercholesterolemia-dependent reduction in ISO-induced vasorelaxation represents a deficiency in an endothelium-dependent mechanism. An additional effect on \(\beta\)-adrenergic receptor signaling in smooth muscle cannot be ruled out.

Our findings suggest that vasodilator mechanisms, especially mediated through an endothelium-dependent or possibly through a \(\beta\)-adrenergic pathway, are sensitive to relatively small elevations of serum cholesterol.

Additionally, it is emphasized that the opposing vasoconstriction is an important factor in determining the extent of subsequent vasorelaxation. In comparison, an endothelium-independent pathway, mediated by the nitrosodilator SNP, was not affected by hypercholesterolemia. This is in agreement with another report,\(^1\(^1\) in which SNP-induced relaxation was refractory to hypercholesterolemia in the rabbit abdominal aorta.

The mechanism by which modest elevation of cholesterol might inhibit endothelium-dependent effects of CAR and ISO is not clear. In states of severe hypercholesterolemia and in the presence of atherosclerotic plaques, functional alterations of endothelial cells,\(^9\)\(^,\)\(^1\(^2\)\(^,\)\(^1\(^9\)\) a diffusion barrier to EDRF,\(^6\)

and/or intimal trapping of EDRF\(^1\(^8\)\) are postulated for reduced endothelium-dependent vasodilatory responses. Additionally, the high activity of cyclic AMP phosphodiesterase in atheromatous lesions\(^2\(^0\)\) may explain the reduced response to ISO. In states of minimal increase in serum cholesterol and absence of histologically identifiable plaques (personal communication from Dr. Thomas Hodge, Rorer Central Research, Fort Washington, Pa., on similarly treated groups of rabbits), as in this study, the previously postulated mechanisms may still be present, albeit at reduced levels. Alternatively it is suggested that a general, nonspecific mechanism of cholesterol-induced decrease in membrane fluidity may account for changes observed here. An association between increased extracellular cholesterol, decreased membrane fluidity, and decreased \(\beta\)-adrenergic responsiveness has previously been documented in a model system of the erythrocyte membrane.\(^1\(^5\)

Another striking change in hypercholesterolemic rabbits was the increased sensitivity to the vasoconstrictors 5-HT and KCl. Although similar changes in rabbit aortic rings have been reported previously by others,\(^4\)\(^,\)\(^1\(^3\)\(^,\)\(^2\(^0\)\(^,\)\(^2\(^1\)\) serum cholesterol levels in these studies were approximately threefold to sixfold higher than reported here. Thus, our data suggest that increases in sensitivity to membrane depolarization and serotonergic receptor affinity can be induced by minimal changes in serum cholesterol. Increased sensitivity to 5-HT is likely to be due to decreased EDRF-release in hypercholesterolemia and atherosclerosis,\(^2\(^2\)\) whereas increased sensitivity to KCl is thought to be due to changes in the properties of vascular smooth muscle, rather than a consequence of endothelial injury.\(^2\(^1\)\) Changes in membrane properties during hypercholesterolemia might facilitate influx of calcium into the cell, thus allowing higher...
tension development at a given KCl concentration than in control vessels. In comparison, altered sensitivity to other vasoconstrictors, indicating that increased sensitivity is manifest only at substantially higher serum cholesterol levels, has been shown for norepinephrine, clonidine, phenylephrine, and Ang II. An exception is the dog, in which moderate elevations in serum cholesterol sensitized coronary arteries to both the dilator and constrictor effects of norepinephrine. This effect was attributed to a change in the binding characteristics of the α-adrenergic receptor in vascular smooth muscle cells, probably due to a cholesterol-induced increase in the number of membrane α-binding sites.

Activation of protein kinase C by PDBu so far has not been evaluated in hypercholesterolemia. Differences in vascular sensitivity to PDBu with elevated cholesterol might be expected, given the fact that protein kinase C translocates from the cytosol into the membrane, once activated, and that membrane fluidity is influenced by hypercholesterolemic conditions. In our study, however, we did not observe any significant differences in vascular reactivity to PDBu among the two groups. It is possible that the presence of endothelium confounded our results given the observation that 5-HT–induced vasoconstriction was amplified by short-term exposure of arteries to PDBu and CAR-induced vasorelaxation was decreased. In

**Figure 3.** Plots showing vasorelaxant effects of isoproterenol (ISO) on aortic rings from control (●) and hypercholesterolemic (○) rabbits. NE, norepinephrine; PGF2a, prostaglandin F2α; A II, angiotensin II; 5-HT, 5-hydroxytryptamine; PDBu, phorbol 12,13-dibutyrate. Tissues were precontracted with the specified vasoconstrictor, and when tension reached a steady state (100%), cumulative doses of ISO were added to the baths. Values represent mean±SEM for five to seven different rabbits. If no error bar is shown, it is contained within the symbol. Abscissa shows log M concentration for ISO; ordinate shows percent maximal isometric tension. *Significantly different (p<0.05) from control.

**Figure 4.** Plots showing vasorelaxant effects of sodium nitroprusside (SNP) on aortic rings from control (●) and hypercholesterolemic (○) rabbits. NE, norepinephrine; PGF2a, prostaglandin F2α; A II, angiotensin II; 5-HT, 5-hydroxytryptamine; PDBu, phorbol 12,13-dibutyrate. Tissues were precontracted with the specified vasoconstrictor, and when tension reached a steady state (100%), cumulative doses of SNP were added to the baths. Values represent mean±SEM from four to seven different rabbits. If no error bar is shown, it is contained within the symbol. Abscissa shows log M concentration of SNP; ordinate shows percent maximal isometric tension. *Significantly different (p<0.05) from control.
agreement, endothelium-dependent vasorelaxation to both ISO and CAR, but not to SNP, was obliterated in the presence of PDBu. Although release of endothelium-dependent relaxing factors is apparently depressed by protein kinase C activation, the effect of protein kinase C activation on endothelium-dependent contracting factors remains unknown. Therefore, although our results suggest that the net vasoconstrictor effect of protein kinase C activation by PDBu is not influenced by minimal changes in serum cholesterol, protein kinase C activation in denuded aortic rings needs to be examined. Therefore, it is premature to conclude that reactivity of protein kinase C is unaffected by minimal changes in serum cholesterol.

In conclusion, aortic rings from rabbits with modest elevation of serum cholesterol displayed markedly decreased vasorelaxation to CAR and ISO. In addition, increased sensitivity to the vasoconstrictors 5-HT and KCl was observed, suggesting that minimal elevation of serum cholesterol in the absence of overt pathological changes is sufficient for altered vascular reactivity.

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References


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