Mechanisms of Vascular Supersensitivity in Hypercholesterolemia

Thomas A. McCalden, PhD, and Raghu G. Nath, PhD

We have characterized in vitro, for the first time, the phenomenon of acute interaction between hypercholesterolemia and cerebrovascular function. We then used this model to investigate a number of mechanisms for the interaction. Rabbits fed a diet supplemented with 2% cholesterol developed hypercholesterolemia over 4 weeks with no histologically detectable atherosclerosis. This absence of anatomic change was reflected in normal biophysical elastic responses to graded radial stretch and normal optimum tension for responses to exogenous K+ in the selected arteries. However, basilar arteries removed from cholesterol-fed rabbits showed abolished myogenic responses to radial stretch and decreased median effective doses for added norepinephrine. These potentiated constrictor responses to norepinephrine were significantly correlated with increased plasma cholesterol concentration. A mechanism related to the opening of membrane calcium channels may be responsible for the supersensitivity. (Stroke 1989; 20:238-241)

Hypercholesterolemia (HC) induces acute in vivo alterations in vascular sensitivity to a variety of agonists in the coronary, hindlimb, renal, and cerebral circulations. Cerebrovascular studies have shown that an infusion of low density lipoprotein or a short-term high-cholesterol diet potentiated the cerebrovascular response to 5-hydroxytryptamine or norepinephrine (NE) and decreased the cerebrovascular sensitivity to vasodilator CO2. However, there have been no attempts to elucidate the mechanisms responsible for these cerebrovascular alterations.

The in vivo effects are accompanied by alterations in blood vessel function that may be detected in vitro. Coronary or general systemic artery preparations show potentiated constrictor responses to a variety of agents with short-term exposure to cholesterol in various forms. These preparations allow for investigation of the mechanisms of this effect, and preliminary evidence suggests that basilar arteries from rabbits fed a short-term high-cholesterol diet show potentiated constrictor responses to NE.

We performed the present experiments to confirm and further characterize this in vitro model, which was then used to investigate two putative mechanisms for the cholesterol–NE interaction. Since HC was more effective in the basilar artery, we investigated mechanisms that have been described as cerebrovascular characteristics.

First, cholesterol might potentiate the effects of NE by inhibiting potent cerebrovascular NE uptake mechanisms and thereby allow a greater portion of the injected dose to act at the vascular smooth muscle receptors. Thus, we determined whether the potentiating effects of cholesterol on NE could be mimicked by pharmacologic uptake blockade. Second, the incorporation of cholesterol and/or low density lipoprotein into the cell membranes might alter the characteristics of many membrane-based mechanisms. One such important cerebrovascular mechanism is the membrane calcium channels on which the cerebrovascular responses depend. We hypothesize that the cholesterol and/or lipoprotein causes alteration in the permeability of these channels such that occupancy of a given receptor by NE would cause a larger flux of calcium into the cell and, consequently, a larger response. Thus, we investigated the effects of cholesterol feeding on the basilar artery responses to exogenous calcium.

Materials and Methods

Thirty-three age- and weight-matched (2–2.5 kg) male New Zealand White rabbits were housed singly with ad libitum access to food and water. The 16 control (C) rabbits were fed normal chow. The 17 HC rabbits were fed chow supplemented with 2% cholesterol. Blood was sampled weekly for determination of serum cholesterol and triglyceride concentrations (Sigma Chemical Co., St. Louis, Missouri). After 4 weeks the rabbits were killed. The
basilar and ear arteries were rapidly removed into oxygenated Krebs' solution. Samples of each artery, together with a segment of abdominal aorta, were subjected to histologic analysis to determine if structural changes were present. Other 5-mm segments of the basilar and ear arteries were set up in vitro as previously described.14

After equilibration, the vessels were stretched radially by 0.5 mm, and the developed load (grams force) was recorded. The manipulator was returned to the zero position, the tissue was stabilized, and this process was repeated in the presence of 10^-6 M papavarine. The response in the presence of papavarine was used to assess alterations in elastic recoil of the artery, whereas any additional response in the absence of papavarine indicated myogenic activity in the smooth muscle.

After washout of the papavarine, the vessels were again stretched radially and a load that produced optimal responses to the exogenous addition (EDM) (n=7 C and 8 HC).

<table>
<thead>
<tr>
<th>Artery</th>
<th>Group</th>
<th>OPT (g)</th>
<th>E\text{max} (g)</th>
<th>ED\text{50} (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilar</td>
<td>C</td>
<td>0.34±0.10</td>
<td>0.29±0.10</td>
<td>3.50±1.50×10^-6</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>0.38±0.07</td>
<td>0.22±0.10</td>
<td>0.57±0.03×10^-6*</td>
</tr>
<tr>
<td>Ear</td>
<td>C</td>
<td>0.80±0.19</td>
<td>2.07±0.20</td>
<td>13.20±0.96×10^-8</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>0.61±0.09</td>
<td>1.70±0.10</td>
<td>6.28±1.57×10^-8</td>
</tr>
</tbody>
</table>

Values are mean±SEM. OPT, grams force for optimal response to 50 mM K\textsuperscript{+}; E\text{max}, maximum response to cumulative addition of norepinephrine; ED\text{50}, median effective dose of norepinephrine; C, control rabbits fed normal chow; HC, hypercholesterolemic rabbits fed chow supplemented with 2% cholesterol.

*p<0.05 different from C.

**Results**

The samples of artery used for histology showed no detectable intimal or medial atheroma or thickening. However, over the 4 weeks plasma cholesterol concentration in the HC group increased to 2304±206 mg/100 ml while that of the C group stayed at the baseline value of 77.8±7.0 mg/100 ml. No significant alteration in triglyceride concentration was found in either group. Thus, the cholesterol-fed rabbits at 4 weeks showed HC without atherosclerosis.

The ear artery preparation from C rabbits developed 0.44±0.03 g force after 0.5 mm of radial stretch when no papavarine was present in the tissue bath. There was no significant alteration from this value when papavarine was present or when the ear artery was removed from HC rabbits. By contrast, C basilar arteries displayed a response of 0.95±0.14 g, and this value was significantly (paired t test, p<0.05) reduced to 0.53±0.06 g by the presence of papavarine. Basilar arteries from HC rabbits showed no papavarine-sensitive stretch responses (0.53 g both with and without the relaxant).

Table 1 shows that there was no significant difference in OPT or E\text{max} between groups or arteries. However, ED\text{50} for both the ear and basilar arteries were lower in the HC than in the C group; this difference was significant for the basilar artery (p<0.05). Thus, rabbits fed cholesterol for 4 weeks displayed basilar artery supersensitivity to NE. Furthermore, ED\text{50} in the basilar arteries from HC rabbits was negatively correlated with the plasma cholesterol concentrations immediately before the rabbits were killed (r=0.83, p<0.05).

Pharmacologic blockade of neuronal and extraneuronal NE uptake did not produce significant potentiation of HC or C basilar artery sensitivity to NE. Thus, HC could not have exerted its sensitizing effects via this mechanism.

Figure 1 shows the dose–response curve for CaCl\textsubscript{2} in the HC and C basilar arteries. The tissues were depolarized with high K\textsuperscript{+} concentration in a calcium-free medium. No contraction occurred under these circumstances since the activator calcium for K\textsuperscript{+} is largely extracellular. However, in this depolarized state readmission of calcium to the extracellular solution produced graded contraction. The contraction was significantly greater in the HC rabbit basilar arteries at all concentrations of calcium. These data indicate that HC basilar artery has calcium channels that are more easily opened than...
FIGURE 1. Effect of graded readmission of calcium into basilar arteries from control (○, n=5) and cholesterol-fed (●, n=5) rabbits. Each vessel was pretreated with calcium-free medium and 50 mM KCl.

those in C rabbits. No such difference was found in matched segments of ear artery.

Discussion

Despite several in vivo studies showing a dynamic interaction between HC and cerebrovascular function, no systematic in vitro investigation has characterized the phenomenon or investigated the mechanisms. We used such an in vitro model to achieve these aims. Short-term HC produced supersensitivity to NE in the basilar artery but not in a general systemic artery. A significant correlation between the basilar artery ED₉₀ and the final plasma cholesterol concentration was found. Thus, the degree of sensitization was proportional to the degree of HC. There was no evidence that the cholesterol-supplemented diet caused structural alteration of the vessels. The histologic survey showed no intimal thickening, and there was no difference in the elastic recoil developed after graded stretch of the papavarine-treated vessels.

The basilar artery from C rabbits developed significantly greater tension when no papavine was present in the tissue bath. This myogenic response was not present in the ear artery from C rabbits or in the basilar artery from HC rabbits. Such a myogenic response may be at least partly responsible for the in vivo phenomenon of autoregulation, which may be impaired in HC rabbits. One study in atherosclerotic monkeys showed that autoregulation to decreased blood pressure was intact, but the response to increased blood pressure remains to be determined.

Normal cerebral vessels seem to have more avid uptake and degradation mechanisms for NE than other systemic vessels. The cholesterol/lipoprotein may have acted to decrease NE metabolism/uptake to make more added drug available at receptor sites. Our experiments show that the cholesterol potentiation of the basilar artery response to NE could not be mimicked by maximal NE uptake inhibition. Thus, it is clear that this is not the mechanism involved for NE supersensitivity.

It is possible that functional alterations occurred in the cholesterol-treated endothelial cells and that removal of an endothelium-derived smooth muscle relaxant factor caused the potentiated effects. The inhibition of the myogenic response to stretch would support this hypothesis since the cerebrovascular myogenic responses are endothelium dependent.

It may be that HC interacted in some way with any part of the receptor-coupling–contraction mechanism to potentiate the response to NE. Some interaction of cholesterol with the plasma membrane and potentiation via altered calcium channel permeability seems likely since several studies show that calcium channel blockers may reverse the potentiating action of HC in systemic vessels. Similar conclusions have been reached for the interaction of cholesterol and/or low density lipoprotein with the coronary artery. Rosendorff et al showed that the dog coronary circulation normally produces a vasodilator response to infused NE. However, in a group of animals that were fed a high-cholesterol diet, this dilator response was reversed to a vasoconstrictor one. In common with cerebral arteries, the coronary vessels depend for vasoconstriction on calcium flux across the cell membrane. Indeed, low density lipoprotein caused an acute contraction of the coronary artery that was reversed by a calcium channel blocker, and cholesterol increased the sensitivity to exogenous calcium. These results, as well as ours, support the general hypothesis that the synergistic interaction between cholesterol and some vasoconstrictors are early features of HC and that this interaction could produce inappropriate reduction in blood flow to the affected organ before any structural atherosclerosis had occurred. In addition, vascular beds that depend heavily on the influx of extracellular calcium for vasoconstriction (coronary and cerebral circulations) may be most at risk.

The potential clinical significance of these observations is extensive. Although cholesterol-fed rabbits show serum cholesterol concentrations that are many times higher than those found in humans, the animal is well accepted as a model for HC and atherosclerosis. There is no doubt that ischemia of the heart and brain are correlated with alterations in circulating cholesterol concentrations. Previous studies have focused on the structural insult producing vascular lumen occlusion. However, ischemia may also result from vascular spasm incidental to the HC-induced potentiation of vasomotor responses to normal circulating substances.

References


**KEY WORDS** • basilar artery • cholesterol • norepinephrine • rabbits
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