Sensitization of Isolated Canine Coronary Arteries to Calcium Ions after Exposure to Cholesterol

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SUMMARY The abundance of membrane cholesterol is an important determinant of the functional properties of biomembranes. To determine whether arterial smooth muscle acquires altered contractile properties in a high cholesterol environment, isolated canine coronary arteries were exposed to cholesterol in stable aqueous solution. Cholesterol, $10^{-11}$ to $10^{-16}$ M, was an efficacious vasoconstrictor, as maximum contractions equaled those obtained with 15 mM KCl. Antiadrenergic interventions, including chemical sympathectomy in vivo with 6-hydroxydopamine and a- and $\beta$-adrenergic blockade with phentolamine and L-propranolol (both $10^{-7}$ M), did not significantly attenuate the contractions. However, responses to cholesterol were abolished completely by (±)-verapamil ($10^{-7}$ M). Cholesterol in picomolar concentration enhanced the constrictor effects of CaCl$_2$ and KCl, both in the presence and absence of a- and $\beta$-adrenergic blockade. Increases in tone in response to graded elevations in the CaCl$_2$ concentration (0-2 mM) were augmented up to 1.5-fold by $10^{-10}$ M cholesterol ($P < 0.01$). Results indicate that cholesterol sensitizes isolated coronary arteries to external Ca$^{2+}$ by a nonadrenergic mechanism. The findings are consistent with the hypothesis that acquisition of membrane cholesterol may alter the contractile properties of coronary arterial smooth muscle, a phenomenon that could play a role in the pathophysiology of atherosclerotic heart disease. Circ Res 45: 479-486, 1979

STUDIES on the deposition of lipids in atherosclerotic lesions suggest that accumulation of cholesterol in cell membranes may be one of the earliest biochemical changes of atherosclerosis (Small and Shipley, 1974). Although changes in membrane-bound cholesterol alter the fluidity and permeability of lipid membranes (Poznansky et al., 1973; Papahadjopoulos et al., 1973; Inoue, 1974; Jain and White, 1977) and activation of excitable cells critically depends on the functional characteristics of the surface membrane, the possibility that changes in membrane function may alter the contractile activity of smooth muscle in atherosclerotic arteries has received relatively little attention.

In the present study, we have examined the vasoactive effects of exogenous cholesterol on isolated canine coronary arteries. Results demonstrate that cholesterol in stable aqueous solution ($\leq 10^{-10}$ M) exerts unexpectedly potent constrictor effects and sensitizes the artery to calcium and potassium ions. The findings are consistent with the hypothesis that acquisition of membrane cholesterol may alter the contractile properties of arterial smooth muscle.

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Methods

Animals

Mongrel dogs weighing 17-23 kg were anesthetized with pentobarbital, 25 mg/kg, iv, intubated, ventilated with a piston respirator, and subjected to left thoracotomy. The heart was excised quickly and placed in standard buffer (see below) at room temperature for immediate dissection of the coronary vessels. In some experiments, coronary arteries were obtained from dogs treated with 6-hydroxydopamine (50 mg/kg, iv) as previously described (Borda et al., 1977). After treatment with 6-hydroxydopamine, the constrictor response to tyramine ($10^{-6}$ M) in isolated coronary arteries was abolished (Borda et al., 1977).

Coronary Artery Ring Preparation

The left anterior descending coronary artery was dissected, placed in oxygenated standard buffer, and cleaned of surrounding tissue. Segments measuring 4 mm in length and 2.0-2.5 mm in outer diameter were cut from the artery. Each segment was cannulated with two stainless steel tubes (0.46 mm o.d.), which were used to mount the artery in an organ bath essentially as described by Bevan and Osher (1972). One tube was immobilized in a horizontal position by attaching its ends to the bottom of the organ bath. The other was suspended parallel to the stationary tube with 5-0 silk to a Statham UC2 force transducer, which was mounted on a micropositioner. By moving the tubes apart, a hoop stress (preload) was applied to the vascular...
ring. Signals from the transducer were amplified with a Honeywell bridge amplifier (Accudata 143) and recorded with a Brush recorder (model 220). The organ bath was filled with 100 ml of standard buffer containing (mM): NaCl, 118; KCl, 4.0; CaCl₂, 1.5; NaH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; and glucose, 5. After equilibration at 37°C with a 95% O₂:5% CO₂ gas mixture, the pH was approximately 7.38. An initial preload of 1.5 g was applied to the vascular ring. After an equilibration period of 1 hour, during which the preparation underwent a stress-relaxation of approximately 0.5 g, preload was adjusted to 1 g.

Chemicals

Cholesterol (Steraloids Laboratories) was recrystallized three times from hot 95% (vol/vol) ethanol. Its purity was evaluated by two-dimensional, thin layer, silica-gel chromatography [Polygram Sil G, 250 μm thick, Brinkmann Instruments; 250 μg spot; first run: benzene:diethyl ether:ethanol:glacial acetic acid, 50:40:2.0:2.0 (by vol); second run: cyclohexane:diethyl ether:glacial acetic acid, 80:10:10 (by vol)]. The chromatographic system separated a number of sterols of potential physiological importance, including 7-keto-, 25-hydroxy-, 26-hydroxy-, 20α-hydroxy-, 7α-hydroxy-, 7β-hydroxy-, 4β-hydroxy-, 6-keto-, and 5,6-epoxy-cholesterol. Purity was assessed further by hot stage microscopy and differential scanning calorimetry. Results indicated a purity of >99%. Storage for up to 4 weeks in the dark under a nitrogen atmosphere at 4°C did not measurably affect purity, and the sterol remained in its anhydrous form by laser Raman spectroscopy. (1,2-3H(N))-Cholesterol (specific activity 40 Ci/mmol), and (4-14C)-cholesterol (54 mCi/mmol) in benzene were obtained from New England Nuclear. All other sterols used in this study were purchased from Steraloids with a nominal chemical purity of >99%. L-tyramine-HCl and 6-hydroxydopamine (99% pure) from chemical companies: L-propranolol-HCl (Ayerst), phentolamine (Ciba), and racemic verapamil (Knoll).

Dispersion of Cholesterol in Standard Buffer

Because the cholesterol concentrations used in the present experiments were below the subnanomolar range, it was not practical to dissolve directly the sterol in the buffer. Instead, samples of chromatographically pure cholesterol in benzene, 10⁻⁸ M, were evaporated under a nitrogen stream at room temperature in a rotary evaporator. Appropriate amounts of standard buffer then were added to the flasks to make up nominal cholesterol concentrations ranging between 10⁻⁹ and 10⁻¹³ M. After addition of the buffer, the flasks were rotated for 30 minutes to effect mixing. Commercial solutions of radioactive cholesterol in benzene (1,2-³H-cholesterol, 1.18 to 2.25 × 10⁻⁴ m) were treated similarly. Control buffers were prepared by exactly the same procedure, except that no cholesterol was added to the benzene. When samples of 10⁻¹⁰ M aqueous dispersions of radioactive cholesterol were transferred with polyethylene pipets from the evaporator flasks directly into scintillation counter vials containing 10 ml of Aquafluor (New England Nuclear), recovery of radioactivity in the aqueous phase averaged 97 ± 1% (SE; n = 16 dispersions). After transfer of samples of 10⁻¹⁰ M dispersions into polyyallomer ultracentrifuge tubes (Beckman) and centrifugation at 150,000 g for 12 hours at 25°C, recovery of radioactivity in the upper and lower third of the tubes averaged 97 ± 2% (n = 16) and 89 ± 2% (n = 16), respectively. Dispersions containing less than 10⁻¹⁰ M cholesterol exhibited practically identical percent recoveries. However, 10⁻⁶ M dispersions showed a 77 ± 3% (n = 12) recovery after direct transfer; after transfer plus ultracentrifugation, recovery in the upper and lower third of the tubes was only 58 ± 4% (n = 12) and 41 ± 4% (n = 12), respectively. Results of these experiments indicate that dispersions up to a concentration of 10⁻¹⁰ M were fairly stable for a period of 12 hours, but that more concentrated dispersions were unstable. Although the present experiments were not designed to study the solubility and physical state of cholesterol in water, our observations suggest that the solubility of cholesterol in buffer at room temperature does not exceed 10⁻¹⁰ M, an estimate that is consistent with that obtained with a fluorescent label technique (L. C. Smith, personal communication; Kao et al., 1977; Charlton et al., 1978; Charlton et al., 1976) but is 10²-10⁴ times lower than previously published values (Gemant, 1962; Haberland and Reynolds, 1973).

Experimental Procedure

Pilot experiments revealed that cholesterol produced slow, progressive rises in arterial tone that attained a plateau only after approximately 1 hour. In addition, it was noted that removal of cholesterol produced only partial relaxations. Accordingly, for the study of the concentration-response relation, it was practical to test only a single cholesterol concentration in each preparation. To expose the artery to a selected cholesterol concentration, the organ bath was replaced by the overflow technique (Furchgott and Bhadrakom, 1953). Replacement was 99% complete, as assessed by the washout of buffers labeled with radioactive markers. Each cholesterol concentration was tested in six preparations. Plots of cholesterol concentration vs. mean increases in arterial tone, measured 1 hour after addition of cholesterol, were obtained. Buffers containing radioactive cholesterol were recovered quantitatively 70 minutes after their addition to the organ bath. The radioactive buffers (n = 5) were extracted twice with equal volumes of benzene, and the combined organic phase was evaporated under a nitrogen atmosphere. In other experiments, the buffers (n = 5) were nitrogenated, lyophilized in the
presence of 1 mM EDTA (disodium ethylenediamine tetraacetic acid), the residue extracted twice with 100 ml of ethanol, and the ethanol evaporated. The residues were dissolved in benzene and analyzed by thin layer chromatography as described above. In all instances, there was a single radioactive spot in the cholesterol position, and only background radioactivity was detected in the positions of the co-developed oxygenated sterols enumerated above (see Chemicals). Thus, in the present series of experiments, appreciable autoxidation of cholesterol was not demonstrable. This finding is consistent with studies showing that quantitative autoxidation of cholesterol in aqueous dispersions requires hours of incubation and elevated temperatures (Weiner et al., 1973; Kimura et al., 1976). To measure radioactivity bound to tissue, the arteries were digested in 1 ml of Protosol (New England Nuclear). In samples from arteries exposed to $10^{-10}$ M nonradioactive ($n = 6$) and radioactive ($n = 6$) cholesterol, total arterial radioactivity averaged $73 \pm 6$ and $64 \pm 6$ counts/min, respectively, values which were not significantly different ($P > 0.05$; t-test for unpaired samples). Thus, the small amounts of radioactivity bound to the artery precluded analysis of the radioactive sterols in the tissue. To ascertain whether cholesterol-induced increases in arterial tone partly reflected inhibition of the extraneural uptake of endogenous catecholamine (Salt and Iversen, 1972), ring preparations from normal dogs and from dogs chemically sympathectomized with 6-hydroxydopamine were stimulated with cholesterol-containing buffers, in the presence and absence of adrenergic receptor blockade, with $10^{-6}$ M phentolamine and/or $10^{-6}$ M L-propranolol. In other experiments, responses to cholesterol-containing buffers were elicited in the presence of $10^{-6}$ M (±)-verapamil. To study the effects of cholesterol on the vasomotor response to changes in the external calcium and potassium ion concentration, concentration-response relations for CaCl$_2$ and KCl in the presence and absence of $10^{-12}$ M cholesterol were obtained. Paired experiments were performed with two arterial rings cut from contiguous portions of the vessel. One ring was used to define the concentration-response relation before and after a 1-hour incubation in buffer containing $10^{-12}$ M cholesterol. The other ring was used to assess the reproducibility of the dose-response without cholesterol before and after 1 hour of incubation in standard buffer. The ion concentration-response relations were determined by the cumulative addition of 0.5 M CaCl$_2$ to standard buffer containing initially no added CaCl$_2$. Potassium ion concentration-response relations were determined by the cumulative addition of 3 mM KCl to standard buffer containing an initial concentration of 4 mM KCl.

### Statistical Analysis

All results expressed are means ± 1 SEM. The significance of the difference between group means was evaluated by the t-test for unpaired samples. The t-test for paired samples was applied when sequential mean values in the same group were compared.

### Results

#### Effect of Cholesterol on Arterial Tone

Figure 1 is a representative tracing of an artery suddenly exposed to buffer containing $10^{-10}$ M cholesterol in the absence (Fig. 1A) and presence of $(\pm)$-verapamil, $10^{-6}$ M (Fig. 1B). Note that replacement of the fluid in the bath with control buffer (see Methods) prior to addition of cholesterol did not affect resting tone. After the addition of cholesterol, arterial tone transiently decreased, then increased progressively, and reached a plateau after approximately 1 hour (Fig. 1A). Verapamil produced a stable relaxation and abolished the response to cholesterol (Fig. 1B). Figure 2 summarizes the results of experiments performed as described in Figure 1. It can be seen that cholesterol produced concentration-dependent increases in tone and that verapamil, $10^{-6}$ M, consistently inhibited the evoked contractions.

#### Effect of Antiadrenergic Interventions on Cholesterol-Induced Contractions

We have considered the possibility that cholesterol increases arterial tone by promoting the release and/or inhibiting the disposal of catecholamines. In particular, cholesterol, like other compounds with steroid structure, may act by inhibiting the extraneural uptake of catecholamines (Uptake 2) (Salt and Iversen, 1972). Representative tracings

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Effect of cholesterol in aqueous solution ($10^{-10}$ M) on resting tone. A: no drug. B: after treatment with $10^{-6}$ M (±)-verapamil. Arrows with asterisks denote the exchange of the bath fluid with control buffer (see Methods). Note that these exchanges do not affect resting tone.
Effect of Cholesterol on the Calcium-Force Relation

Having demonstrated that cholesterol-induced contractions were not mediated predominantly by an adrenergic mechanism and knowing that the abundance of membrane cholesterol may affect the permeability of biomembranes for strong electrolytes, we wondered whether the responses to cholesterol partly reflected an increased permeability for calcium and/or potassium ions, cations that may contract arterial smooth muscle. To demonstrate the reproducibility of the concentration-responses in the same preparation, we sequentially exposed arteries to graded increases in the calcium or potassium ion concentration. After the usual 1-hour equilibration, arteries were exposed to the initial conditions of the concentration-response experiment. Arterial tone was allowed to equilibrate fully at each dose level. After equilibration at the highest concentration, the bath fluid was replaced with standard buffer and the artery incubated for 1 hour. Thereafter, the concentration-response was repeated under identical conditions. Table 2 demonstrates that corresponding values of sequential concentration-responses were very similar and statistically not significantly different.

Representative tracings of concentration-responses for calcium chloride obtained in the same preparation before and after addition of cholesterol, $10^{-10}$ M, are shown in Figure 4. After exposure to cholesterol in aqueous solution ($10^{-10}$ M) on resting arterial tone. A: intact artery. B: intact artery after addition of phentolamine plus $l$-propranolol (both $10^{-6}$ M). C: denervated artery (see Methods). Arrows with asterisks denote exchanges of the bath fluid with control buffer. Note the unresponsiveness of the denervated artery to tyramine, $10^{-6}$ M (C).
cholesterol, increases in the calcium ion concentration produced greater augmentation in arterial tone. Experiments, as depicted in Figure 4, are summarized in Figure 5A. With each concentration tested, steady arterial tone was significantly increased after addition of cholesterol. Results of concentration-responses obtained in the presence of adrenergic blockade with phenolamine and L-propranolol (both $10^{-6}$ M) are depicted in Figure 5B. Although the combined $\alpha$- and $\beta$-blockade tended to depress developed force in response to increases in the calcium ion concentration, values after addition of cholesterol again were significantly elevated compared to those obtained under control conditions, confirming that cholesterol did not act predominantly by an adrenergic mechanism.

**Effect of Cholesterol on the Potassium-Force Relation**

As demonstrated in Table 2, sequential concentration-responses for potassium chloride in the presence and absence of adrenergic blockade were reproducible. Results of the experiments with potassium chloride in the absence and presence of cholesterol are illustrated in Figure 6. Cholesterol significantly increased arterial tone at high KCl concentrations, both without (Fig. 6A) and with adrenergic blockade (Fig. 6B).

### Discussion

In the present study, cholesterol in stable aqueous solution was used to investigate possible effects of cholesterol on arterial vasomotion. Results demonstrate that picomolar concentrations of cholesterol increased the response of isolated canine coronary arteries to the constrictor effects of calcium and potassium ions.

#### Table 1: Increases in Arterial Tone Induced by Exposure to Cholesterol ($10^{-12}$ M) for 1 Hour in the Absence and Presence of Antiadrenergic Interventions

<table>
<thead>
<tr>
<th>Group</th>
<th>Developed force (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control ($n = 15$)</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>II. 6-OH-Dopamine* ($n = 5$)</td>
<td>68 ± 9</td>
</tr>
<tr>
<td>III. Phenolamine† + propranolol† ($n = 5$)</td>
<td>67 ± 10</td>
</tr>
<tr>
<td>IV. 6-OH-Dopamine* + phenolamine† + propranolol† ($n = 5$)</td>
<td>65 ± 7</td>
</tr>
</tbody>
</table>

Resting tone before cholesterol in groups I, II, III, and IV was 1000 mg (= preload), 1000 mg (= preload), 997 ± 2 mg (SE), and 995 ± 3 mg, respectively. Values in groups II, III, and IV did not differ significantly from the control value (group I) ($P > 0.05$; $t$-test for unpaired samples).

* Chemical sympathectomy in vivo with 6-hydroxydopamine.
† Drug concentration = $10^{-6}$ M.

#### Table 2: Concentration-Response Relations for CaCl$_2$ and KCl

<table>
<thead>
<tr>
<th>CaCl$_2$ concentration (mM)</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blockers ($n = 5$)</td>
<td>31 ± 8</td>
<td>56 ± 12</td>
<td>60 ± 11</td>
<td>68 ± 14</td>
</tr>
<tr>
<td>Phenolamine + propranolol ($n = 4$)</td>
<td>27 ± 9</td>
<td>50 ± 11</td>
<td>62 ± 11</td>
<td>69 ± 13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KCl concentration (mM)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blockers ($n = 6$)</td>
<td>26 ± 2</td>
<td>159 ± 23</td>
<td>378 ± 51</td>
<td>533 ± 98</td>
</tr>
<tr>
<td>Phenolamine + propranolol ($n = 5$)</td>
<td>23 ± 1</td>
<td>157 ± 26</td>
<td>387 ± 52</td>
<td>542 ± 81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KCl concentration (mM)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blockers ($n = 5$)</td>
<td>24 ± 4</td>
<td>134 ± 20</td>
<td>367 ± 51</td>
<td>525 ± 99</td>
</tr>
<tr>
<td>Phenolamine + propranolol ($n = 5$)</td>
<td>26 ± 3</td>
<td>124 ± 16</td>
<td>346 ± 47</td>
<td>519 ± 91</td>
</tr>
</tbody>
</table>

Numbers express mean increases ± SE in force (mg) above resting tone measured at the beginning of each dose-response experiment. In the CaCl$_2$-response experiments, resting tone after stabilization in Ca$^{2+}$-free buffer (see Methods) ranged between 940 and 902 mg. In the KCl-response experiments, resting tone did not significantly differ from the initially imposed preload of 1000 mg. Upper and lower numbers for each experimental condition indicate corresponding values from sequential concentration-responses obtained before and after 1 hour of equilibration in standard buffer. None of the sequential values was significantly different ($P > 0.05$; $t$-test for paired samples).
The concentration of unesterified cholesterol in the aqueous phase of extracellular fluids in vivo is not known. However, knowledge of the physical state of cholesterol in biological fluids is of crucial importance for the understanding of the transfer of cholesterol from a lipoprotein to another lipoprotein or a cell membrane. It has been demonstrated clearly that specific lipoprotein receptors on cell surfaces may facilitate and regulate the formation of collisional complexes (Goldstein and Brown, 1977). On the other hand, there is increasing evidence that receptor-independent transfer of lipids may, in part, involve the diffusion of lipid into the aqueous phase (Charlton et al., 1976; Charlton et al., 1978; Kao et al., 1977; Backer and Dawidowicz, 1978). In the present experiments, cholesterol in concentrations up to $10^{-10}$ M appeared to be stable in the aqueous phase, suggesting that saturation was reached at approximately that concentration. This estimate of the solubility of cholesterol in aqueous solvent is in basic agreement with that obtained using a fluorescent label technique (L. C. Smith, personal communication; Kao et al., 1977; Charlton et al., 1978). Recent studies indicate that human low density lipoprotein, the lipoprotein richest in cholesterol, accumulates in arterial walls in proportion to its concentration in plasma (Hoff et al., 1977) and appears in peripheral lymph in concentrations that are approximately one-tenth that in plasma (Reichl et al., 1978). Assuming that the lipid composition of lipoproteins in interstitial fluids is not substantially different from that of plasma lipoproteins, only approximately 1 millionth the concentration of bound cholesterol would be required to saturate the aqueous phase with cholesterol. This emphasizes the importance of precisely defining the physical characteristics of lipid carriers in physiological fluids.

By inhibiting the extraneural disposal of catecholamines, cholesterol may promote the accumulation of the neural transmitter at postsynaptic receptor sites and enhance constrictor tone (Salt and Iversen, 1972). The contractions elicited by cholesterol in arteries from dogs denervated with 6-hydroxydopamine or in arteries exposed to high concentrations of phentolamine plus propranolol (both $10^{-6}$ M) demonstrate clearly that the responses were not mediated predominantly by an adrenergic mechanism.

Physiological studies have indicated that cholesterol plays an important role in regulating the fluidity and permeability of cell membranes (Poznansky et al., 1973; Papahadjopoulos et al., 1973; Inoue, 1974; Jain and White, 1977). Accordingly, contractions evoked by cholesterol in arteries from dogs denervated with 6-hydroxydopamine or in arteries exposed to high concentrations of phentolamine plus propranolol (both $10^{-6}$ M) demonstrate clearly that the responses were not mediated predominantly by an adrenergic mechanism.
tington-Coleman and Carrier, 1970), and pharmacological inhibition of the deposition of calcium minimizes arterial injury without, however, reducing the hypercholesterolemia (Kramsch and Chan, 1978; Chan et al., 1978). This suggests that atherosclerotic injury may be mediated partly by calcium, a phenomenon that could be explained on the basis of an increased membrane permeability for calcium ions.

Studies with red cell membranes indicate that modulation of membrane cholesterol may affect active monovalent cation transport (Poznansky et al., 1973; Wiley and Cooper, 1975). It has been demonstrated that cholesterol inhibits the activity of reconstituted preparations of (Na⁺ + K⁺)-dependent ATPase (Kimmelberg and Pappahadjopoulos, 1974). These findings suggest that acquisition of membrane cholesterol could act by inhibiting (Na⁺ + K⁺)-dependent ATPase and exerting an ouabain-like effect on vascular smooth muscle.

Cholesterol dispersed in aqueous solvents may undergo autoxidation (Weiner et al., 1973; Kimura et al., 1976). In addition, cholesterol may have affinity for highly reactive oxygen species that may be generated at membrane sites (Suwa et al., 1977). Autoxidation products of cholesterol, such as 7-ketocholesterol or 25-hydroxycholesterol, have proved to be potent inhibitors of the biosynthesis of cholesterol (Goldstein and Brown, 1977). Thus, vasoactive effects observed in this study could be attributed not to cholesterol itself but to one of its potent oxygenated derivatives. With our chromatographic techniques, we were unable to demonstrate oxygenated sterols in the buffers recovered at the end of the experiments. However, the possibility that oxygenated sterols play a role cannot be ruled out easily, as oxygenated species could be generated at the bath fluid. However, in ongoing experiments, we have demonstrated that among many oxygenated sterols, only 7-ketocholesterol exerted weak constrictor effects on isolated coronary arteries (Yokoyama et al., 1979). Thus, exogenously produced oxygenated sterols are not the cause of the mechanical responses of isolated arteries exposed to cholesterol dispersed in oxygenated buffer.

In summary, low concentrations of cholesterol exert constrictor effects on isolated canine coronary arteries which do not appear to be mediated by an adrenergic mechanism. Cholesterol appears to sensitize the artery to calcium ions; verapamil, an agent that acts probably by inhibiting the inward movement of calcium into cells (Fleckenstein, 1977), is a potent inhibitor of the sterol-induced contractions. Recently, we have demonstrated that similar alterations in arterial reactivity can be induced by stimulating the biosynthesis of cholesterol in vitro with mevalonate (Fishier et al., 1979). This observation is consistent with the hypothesis that arterial smooth muscle cells in a high cholesterol environment may acquire altered contractile properties.

**Acknowledgments**

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Brain Adenosine Production in the Rat during 60 Seconds of Ischemia

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SUMMARY In rats, cerebral perfusion pressure was altered abruptly by aortic transection to determine the production by ischemic brain of adenosine and its metabolites, inosine and hypoxanthine. Brain samples were obtained after 0, 5, 10, 15, 30, and 60 seconds of ischemia. Also measured were ATP, ADP, AMP, phosphocreatine (PCr), lactate, and pyruvate. Blood pressure was monitored continuously, and arterial Po2, Pco2, and pH were measured just prior to induction of ischemia. Adenosine was elevated to 2.30 ± 0.31 (SE) nmol/g at 5 seconds from a control value of 0.96 ± 0.07. A significant elevation of adenosine continued to 60 seconds (5.50 ± 1.24). Furthermore, inosine showed a progressive upward trend during the entire 60 seconds of ischemia, whereas no change in hypoxanthine occurred between the moment of transection (31.81 ± 2.01 nmol/g) and 60 seconds of ischemia (34.72 ± 2.93). PCr decreased by 1.24 μmol/g within the first 5 seconds. After the onset of hypotension, significant changes did not occur in AMP and ADP until 30 seconds, and in ATP and pyruvate until 60 seconds after aortic transection; lactate was elevated by 10 seconds. The rapid rise of cerebral adenosine within 5 seconds after the onset of ischemia supports a role for adenosine in the regulation of cerebral blood flow.

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THE mechanism whereby the brain regulates its own blood flow during changes in perfusion pressure is unclear. Neurogenic (James et al., 1969), myogenic (Bayliss, 1902), and metabolic (Roy and Sherrington, 1890) theories have been suggested as playing a role in the autoregulation of cerebral blood flow (CBF). The metabolic theory proposes that a chemical factor couples blood flow to metabolism, and candidates previously suggested for this chemical linkage include hydrogen ion (Lassen, 1968; Betz et al., 1973; Kontos et al., 1977a), carbon dioxide (Severinghaus and Lassen, 1967; Kontos et al., 1977b), oxygen (Courtice, 1941), potassium (Kuschinsky et al., 1972), and lactate (Siesjo and Zwetnow, 1970).

Recently, Berne et al. (1974) found that cerebral adenosine levels were elevated following 1 minute of total ischemia and that topically applied adenosine dilated pial vessels; the latter observation was confirmed by Wahl and Kuschinsky (1976). However, no dilation of pial vessels occurred when adenosine was administered intra-arterially (Buyniski and Rapela, 1969; Berne et al., 1974). In addition, Rubio et al. (1975) reported an increase in brain adenosine concentrations with hypotension, hypoxia, hypocarbia, and brain excitation by electrical stimulation in rats. However, production of adenosine by brain within seconds of the onset of hypotension has not been determined. If adenosine is a mediator of autoregulation of CBF, changes in adenosine levels should be observed within seconds of alteration of perfusion pressure, since CBF...
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