Effect of hypercholesterolemia on Ca$^{2+}$-dependent K$^+$ channel-mediated vasodilatation in vivo

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Jeremy, Richmond W., and Hugh McCarron. Effect of hypercholesterolemia on Ca$^{2+}$-dependent K$^+$ channel-mediated vasodilatation in vivo. Am J Physiol Heart Circ Physiol 279: H1600–H1608, 2000.—Nitric oxide (NO)-mediated and NO-independent mechanisms of endothelium-dependent vasodilatation involve Ca$^{2+}$-dependent K$^+$ (K$_{Ca}$) channels. We examined the role in vivo of K$_{Ca}$ channels in NO-independent vasodilatation in hypercholesterolemia. Hindlimb vascular conductance was measured at rest and after aortic injection of ACh, bradykinin (BK), and sodium nitroprusside in anesthetized control and cholesterol-fed rabbits. Conductances were measured before and after treatment with the NO synthase antagonist N$^\omega$-nitro-l-arginine methyl ester (l-NAME, 10 mg/kg) or K$_{Ca}$ blockers tetraethylammonium (30 mg/kg), charybdoxotoxin (10 μg/kg), and apamin (50 μg/kg). The contribution of NO to basal conductance was greater in control than in cholesterol-fed rabbits (2.2 ± 0.4 vs. 1.1 ± 0.3 [SE] ml·min$^{-1}$·kg$^{-1}$·100 mmHg$^{-1}$, P < 0.05), but the NO-independent K$_{Ca}$ channel-mediated component was greater in the cholesterol-fed than in the control group (1.1 ± 0.4 vs. 0.3 ± 0.1 ml·min$^{-1}$·kg$^{-1}$·100 mmHg$^{-1}$, P < 0.05). Maximum conductance response to ACh and BK was less in cholesterol-fed than in control rabbits, and the difference persisted after l-NAME (ACh: 7.7 ± 0.7 vs. 10.1 ± 0.5 ml·min$^{-1}$·kg$^{-1}$·100 mmHg$^{-1}$, P < 0.005). Blockade of K$_{Ca}$ channels with tetraethylammonium or charybdoxotoxin + apamin almost completely abolished l-NAME-resistant vasodilatation after ACh or BK. The magnitude of K$_{Ca}$-mediated vasodilatation after ACh or BK was impaired in hypercholesterolemic rabbits. Vasodilator responses to nitroprusside did not differ between groups. In vivo, hypercholesterolemia is associated with an altered balance between NO-mediated and NO-independent K$_{Ca}$ channel contributions to resting vasomotor tone and impairment of both mechanisms of endothelium-dependent vasodilatation.

cholesterol; endothelium; microcirculation; endothelium-derived factors

MEDIATORS OF ENDOTHELIUM-DEPENDENT vasodilatation include nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). NO causes relaxation of smooth muscle cells via a cGMP-dependent mechanism, leading to accelerated Ca$^{2+}$ uptake into the sarcoplasmic reticulum (14, 15). Endothelium-dependent vasodilatation and hyperpolarization of vascular smooth muscle cells, persisting in the presence of inhibitors of cyclooxygenase and NO synthase, are attributed to EDHF (11, 13, 31). Although the identity of EDHF is uncertain (21, 25), some evidence suggests that a P-450 epoxygenase metabolite of arachidonic acid may be involved (23, 30, 35). Present evidence indicates that it mediates vasorelaxation via large- and small-conductance Ca$^{2+}$-dependent K$^+$ channels (K$_{Ca}$ channels) (5, 16, 28, 30).

Recent evidence shows that NO can also activate K$_{Ca}$ channels in smooth muscle cells, directly and via cGMP (4), causing hyperpolarization and inhibiting entry of Ca$^{2+}$ via voltage-dependent Ca$^{2+}$ channels (1, 4). Furthermore, K$_{Ca}$ channels appear to be important in maintaining increased intracellular Ca$^{2+}$ in endothelial cells after stimulation with agonists such as ACh (10, 14, 19). The K$_{Ca}$ channels therefore play a role in NO-mediated and NO-independent vasodilator responses.

The NO-independent mechanisms of vasodilatation appear to be more important in smaller arteries (6, 25, 36), and there may be “cross talk” between NO-mediated and NO-independent mechanisms of vasodilatation, with NO-mediated effects predominating under physiological conditions (2, 30). Although there is evidence that tonic NO release contributes to basal arterial vasomotor tone (26), there is less information about the independent roles of K$_{Ca}$ channels as regulators of basal arterial tone (5, 10). The first aim of this study was to examine the relative contributions of NO-mediated and NO-independent K$_{Ca}$ channel activity to basal arterial tone in vivo.

Endothelium-dependent vasodilatation is abnormal in hypercholesterolemia (9, 18, 27, 39), possibly because of deficiency of l-arginine substrate for NO synthesis (18, 20) or scavenging of NO by oxidized lipoproteins or superoxide radicals (7, 24). The effector mechanisms by which NO induces vasodilatation are also altered by hypercholesterolemia, with impaired cGMP-mediated vasodilatation and an increased contribution of K$_{Ca}$ channels to the vasodilator response to NO (32, 33).

The effects of hypercholesterolemia on NO-independent vasodilator responses are less well characterized. Inasmuch as changes in membrane cholesterol content...
can alter the probability of opening of KCa channels (3), the vasodilator responses mediated by these channels may also be abnormal. A recent human study does suggest that NO-independent vasodilator responses are impaired by age and hypercholesterolemia (38). The second aim of this study was therefore to determine whether NO-independent KCa channel-mediated vasodilatation is impaired by hypercholesterolemia.

The apparent interaction between NO-mediated and NO-independent KCa channel-mediated vasodilatation in vitro (2) raises the possibility that upregulation of KCa channel activity may compensate for impaired NO-mediated vasodilatation in hypercholesterolemia (30, 32, 33). The third aim of this study was, therefore, to determine whether KCa channel-mediated vasodilatation compensates for impaired NO-mediated vasodilatation associated with hypercholesterolemia in vivo.

**METHODS**

**Study groups.** Adult male New Zealand White rabbits (2.5–3.5 kg) fed standard chow and water ad libitum were randomized to a normal diet or dietary supplementation with 0.5% cholesterol for 16 wk. The experimental protocol was in accord with National Health and Medical Research Council of Australia guidelines for experimental studies and was approved by the institutional Ethics Committee for Animal Research.

**Experimental preparation.** Rabbits were anesthetized with pentobarbital sodium (45 mg/kg) via an ear vein cannula and allowed to breathe spontaneously with supplemental oxygen via a mask. Anesthesia was maintained with supplemental pentobarbital sodium (15 mg/kg iv each 60 min). Arterial blood gases were monitored to ensure that arterial oxygen saturation was >95% and that acid-base status was physiological. Rabbits were placed on a heating pad warmed to 38°C. The right femoral artery was ligated, and a double-lumen polyethylene catheter was advanced retrograde to the distal abdominal aorta for drug injection and arterial pressure measurement (model P23 dB, Statham). A calibrated electromagnetic flow probe (Carolina Instruments) was placed around the left femoral artery. Absolute zero reference was established by transient occlusion of the artery, and a brisk hyperemic response confirmed that there was no occlusion or kinking of the vessel. Arterial pressure and left femoral artery flow were recorded continuously on chart paper (model 7D, Grass Instruments).

**Experimental protocols.** After instrumentation, each rabbit received heparin (2,500 IU iv). Possible confounding effects of activation of the sympathetic nervous system, associated with anesthesia or systemic administration of ACh (34), were blocked with pentolamine (0.3 mg/kg iv). The initial phenolamine dose typically resulted in a 10–15 mmHg fall in mean arterial pressure and a 10–20% increase in mean hindlimb blood flow. Supplemental doses of phenolamine (0.1 mg/kg) were given every 60 min during the study. No hemodynamic data were recorded for 15 min after supplemental pentobarbital sodium or phenolamine.

The first series of experiments examined the effects of N^+^-nitro-l-arginine methyl ester (l-NAME, inhibition of endothelial NO synthase) and tetraethylammonium (TEA, blockade of KCa channels) on agonist-stimulated vasodilatation. Four control rabbits received cumulative doses of l-NAME (5, 10, and 25 mg/kg over 10 min), and vasodilator responses to intra-aortic injection of ACh (250, 1,250, 2,500, and 12,500 ng, equivalent to 1.5, 7, 15, and 70 nmol) were recorded after each l-NAME dose. Another three rabbits received cumulative doses of TEA (15, 30, and 60 mg/kg over 10 min), and vasodilator responses to ACh were recorded after each TEA dose. Ten minutes were allowed after l-NAME or TEA treatment before ACh dose-response studies were performed. Immediately after each ACh injection, femoral blood flow increased, and the magnitude of the peak flow response was recorded. An interval of ≈2 min was allowed between doses to ensure that hindlimb conductance had returned to baseline, and flow response to each drug dose was measured in duplicate. The order of drug doses was varied at random. After the ACh studies, sodium nitroprusside (125 and 250 μg) was administered to document vasodilator responses to exogenous NO in the presence of l-NAME and TEA. Blank injections of 0.9% saline solution were used to exclude flow artifacts. The maximal blocking effect of l-NAME was achieved with a dose of 10 mg/kg, and TEA at 30 mg/kg was effective in blocking vasodilator responses to ACh. These doses were used in the subsequent experiments. Blockade of endothelial NO synthase by l-NAME is prolonged by blockade of KCa channels by TEA (30 mg/kg over 10 min), so infusion of TEA was continued at 1 mg·kg⁻¹·min⁻¹ after the loading dose. Vasodilator dose-response studies were completed within 30–40 min after the TEA loading dose.

The second series of experiments further examined the types of Ca²⁺ channels involved in the NO-independent vasodilator responses. Another seven control rabbits were studied after initial treatment with indomethacin (20 mg/kg) and l-NAME (10 mg/kg). Vasodilator responses to ACh were compared before and after treatment with charybotoxin (CTX, 10 μg/kg, blocking of large-conductance KCa channels) and repeated after addition of apamin (50 μg/kg, blocking of small-conductance KCa channels). Subsequently, TEA (30 mg/kg) was administered, and dose responses to ACh were repeated.

The third series of experiments compared vasodilator responses to ACh (250, 1,250, 2,500, and 12,500 ng) between control (n = 14) and cholesterol-fed (n = 13) rabbits. After the initial drug dose-response studies, rabbits received l-NAME (10 mg/kg), and resting femoral blood flow was monitored until a steady-state reduction in flow was observed (~10 min) before the vasodilator studies were repeated. Rabbits then received TEA (30 mg/kg over 10 min, then 1 mg·kg⁻¹·min⁻¹) before the vasodilator studies were repeated a third time. Finally, sodium nitroprusside (125 and 250 μg) was injected into the aorta to document endothelium-independent vasodilator responses.

The fourth series of experiments compared vasodilator responses to bradykinin (BK; 6.25, 12.5, 62.5, 125, and 625 ng, equivalent to 6, 12, 60, 120, and 600 pmol, respectively) in control (n = 7) and cholesterol-fed (n = 9) rabbits. Vasodilator responses were studied before and after treatment with l-NAME and then TEA, as described above.

**Drugs.** ACh, BK, indomethacin, l-NAME, TEA, CTX, and apamin were purchased from Sigma Chemical, pentobarbital sodium from Boehringer, sodium nitroprusside from David Bull Laboratories, and phenolamine from Ciba-Geigy. Cholesterol was supplied by ICN Biomedicals. All drug solutions were freshly prepared on the day of experiment and kept at 4°C until required. Before hemodynamic studies, a venous blood sample was withdrawn from an ear vein for measurement of cholesterol and arginine levels, as previously described (29).

**Data analysis.** Hindlimb conductance (ml·min⁻¹·kg⁻¹·100 mmHg⁻¹) was calculated as the quotient of mean femoral flow and mean arterial pressure and corrected for body weight. The resting conductance may include an NO-mediated con-
component (defined as reduction in conductance by l-NAME) and a NO-independent component mediated by K_{Ca} channels (defined as reduction in conductance by TEA or CTX + apamin, in the presence of l-NAME). Similarly, the increase in conductance in response to ACh or BK may include NO-mediated and K_{Ca} channel-mediated components. Hemodynamic variables in controls were compared with those in cholesterol-fed animals by ANOVA, with comparison of group means by t-test with Bonferroni correction where there was a significant difference within or between groups. The dose-response relations for changes in conductance after ACh or BK were compared between control and cholesterol-fed groups by two-way ANOVA for repeated measures, with means comparison by t-test with Bonferroni correction (37). Values are means ± SE, and a two-tailed P < 0.05 is described as statistically significant.

RESULTS

Plasma cholesterol was 35 ± 7 and 3.4 ± 1.2 mmol/l in cholesterol-fed and control rabbits, respectively (P < 0.01). There was no significant difference in plasma arginine levels between control and cholesterol-fed animals (135 ± 18 and 114 ± 29 μmol/l, respectively). Body weights were similar for cholesterol-fed and control rabbits (3.4 ± 0.2 kg, respectively). The effects of l-NAME and TEA on vasodilator responses to ACh are illustrated in Fig. 1. Before l-NAME, peak conductance after 12,500 ng of ACh was 15.9 ± 1.6 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹. After l-NAME at 5 mg/kg, conductance was reduced to 11.7 ± 0.8 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ (P < 0.05), and after l-NAME at 10 mg/kg, conductance was 10.9 ± 2.6 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹. Increase in l-NAME to 25 mg/kg did not further reduce the vasodilator response to ACh. Subsequently, l-NAME at 10 mg/kg was used to block endothelial NO synthesis. Treatment with TEA at 30 mg/kg reduced the vasodilator response to all doses of ACh, so that peak conductance after 12,500 ng of ACh was 4.6 ± 0.8 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ after l-NAME only but 5.1 ± 0.8 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ after addition of CTX (P < 0.05). The addition of apamin markedly reduced the vasodilator response to all doses of ACh, so that peak conductance after 12,500 ng of ACh was 2.5 ± 0.4 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ after l-NAME only, with a mean increase above baseline of 3.1 ± 0.6 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ (P < 0.005 vs l-NAME). There were no significant differences in the vasodilator responses to ACh between rabbits treated with l-NAME + TEA and those treated with l-NAME + CTX + apamin. Addition of TEA after treatment with CTX and apamin did not further significantly reduce the vasodilator response to ACh (mean increase in conductance after 12,500 ng of ACh = 2.5 ± 0.4 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹). In controls treated with l-NAME, resting conductance was 2.3 ± 0.1 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹.

![Fig. 1. Pilot studies documenting effects of different doses of nitro-L-arginine methyl ester (l-NAME, A; n = 4) and tetraethylammonium (TEA, B; n = 3) on vasodilator responses to ACh (hindlimb conductance, ml·min⁻¹·kg⁻¹·100 mmHg⁻¹) to 12,500 ng of ACh. Increase in l-NAME above 10 mg/kg and increase in TEA above 30 mg/kg did not further reduce the vasodilator response to ACh, and these doses were used in subsequent experiments. *P < 0.05 vs no drug; **P < 0.05 vs TEA at 15 mg/kg. C: increases in conductance in response to sodium nitroprusside before and after TEA at 30 mg/kg. *P < 0.05; **P < 0.01 vs basal before TEA (n = 3).](http://ajpheart.physiology.org/DownloadedFrom)
which was reduced to 1.5 ± 0.2 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ by CTX (P < 0.01), with a minimal further reduction to 1.4 ± 0.2 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ after addition of apamin, suggesting that KCa channels contribute to resting vasomotor tone. Hemodynamic data are compared for control and cholesterol-fed groups in Table 1. At commencement, there were no significant differences in mean arterial pressure or resting conductance between groups. After L-NAME, mean arterial pressure increased in controls (P < 0.005), and conductance was reduced (P < 0.005 vs. before L-NAME). In cholesterol-fed rabbits, there was an insignificant increase in arterial pressure after L-NAME, but mean conductance was mildly reduced (P < 0.005 vs. before L-NAME). The magnitude of the NO-mediated component of resting conductance was greater in control than in cholesterol-fed rabbits (2.2 ± 0.4 vs. 1.1 ± 0.3 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹, P < 0.05). Treatment with TEA did not significantly alter resting mean arterial pressure (which remained increased compared with that before L-NAME) or conductance in the control group, but mean conductance was further reduced in the cholesterol-fed group (P < 0.05). The KCa channel-mediated component of resting conductance was greater in cholesterol-fed than in control rabbits (1.1 ± 0.4 vs. 0.3 ± 0.1 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹, P < 0.05).

The vasodilator responses to ACh and BK, in the absence of NO synthase or KCa channel blockade, are compared for control and cholesterol-fed rabbits in Fig. 3. Data are shown for peak hindlimb conductance and increase in conductance above resting levels after each drug dose. Peak conductance after 12,500 ng of ACh was 16.6 ± 1.0 and 6.1 ± 0.7 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ in control and cholesterol-fed rabbits, respectively (P < 0.005). The mean increases in conductance above resting levels after 12,500 ng of ACh for control and cholesterol-fed groups were 10.7 ± 1.0 and 0.4 ± 0.1 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹, respectively (P < 0.005). Similarly, peak conductance after 625 ng of BK was 16.0 ± 1.6 and 9.7 ± 1.0 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹ in control and cholesterol-fed rabbits, respectively (P < 0.05). The mean increases in conductance above resting levels for control and cholesterol-fed groups were 10.3 ± 1.4 and 5.4 ± 0.9 ml·min⁻¹·kg⁻¹·100 mmHg⁻¹, respectively (P < 0.05).

Table 1. Resting hemodynamics in control and hypercholesterolemic groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline Mean Pao (mmHg)</th>
<th>Conductance (ml·min⁻¹·kg⁻¹·100 mmHg⁻¹)</th>
<th>L-NAME Mean Pao (mmHg)</th>
<th>Conductance (ml·min⁻¹·kg⁻¹·100 mmHg⁻¹)</th>
<th>TEA Mean Pao (mmHg)</th>
<th>Conductance (ml·min⁻¹·kg⁻¹·100 mmHg⁻¹)</th>
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<tbody>
<tr>
<td>Control</td>
<td>99 ± 2</td>
<td>6.0 ± 0.6</td>
<td>107 ± 2*</td>
<td>4.0 ± 0.4‡</td>
<td>107 ± 3*</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>96 ± 3</td>
<td>5.2 ± 0.5</td>
<td>99 ± 4</td>
<td>4.0 ± 0.6‡</td>
<td>98 ± 4‡</td>
<td>2.8 ± 0.3§</td>
</tr>
</tbody>
</table>

Values are means ± SE. L-NAME, Nω-nitro-L-arginine methyl ester; TEA, tetraethylammonium; mean Pao, mean aortic pressure (mmHg); conductance, mean hindlimb conductance (ml·min⁻¹·kg⁻¹·100 mmHg⁻¹). *P < 0.005; †P < 0.0005 vs. baseline. ‡P < 0.05 vs. control. §P < 0.05 vs. L-NAME.
The vasodilator responses to ACh and BK, after L-NAME treatment, are compared for control and cholesterol-fed rabbits in Fig. 4. The vasodilator responses to ACh remained impaired in the cholesterol-fed group. After L-NAME, conductance after 12,500 ng of ACh was reduced to $10.1 \pm 0.5$ ml/min kg$^{-1}$100 mmHg$^{-1}$ in controls ($P < 0.0005$ vs. before L-NAME) and $7.7 \pm 0.7$ ml/min kg$^{-1}$100 mmHg$^{-1}$ ($P < 0.005$ vs. control) in cholesterol-fed rabbits. The mean increase in conductance above resting levels in response to ACh was approximately halved in the cholesterol-fed rabbits. *$P < 0.05$; **$P < 0.005$ vs. controls. Similarly, vasodilator responses to BK remained significantly impaired in the cholesterol-fed group. The mean increase in conductance above baseline in response to ACh was $4.0 \pm 0.5$ vs. $6.4 \pm 0.3$ ml/min kg$^{-1}$100 mmHg$^{-1}$ ($P < 0.0005$). Similarly, vasodilator responses to BK were also $50\%$ less in the cholesterol-fed rabbits. *$P < 0.05$ vs. controls.
The vasodilator responses to ACh and BK, after addition of TEA to L-NAME, are compared for control and cholesterol-fed rabbits in Fig. 5. Peak conductance after 12,500 ng of ACh was reduced to $6.7 \pm 0.7 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \cdot 100 \text{ mmHg}^{-1}$ in controls ($P < 0.005$ vs. L-NAME) and $5.0 \pm 0.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \cdot 100 \text{ mmHg}^{-1}$ in the cholesterol-fed group ($P < 0.005$ vs. L-NAME, not significant vs. controls). The mean increase in conductance above resting levels after 12,500 ng of ACh did not differ significantly between control and cholesterol-fed groups ($3.2 \pm 0.5$ and $2.2 \pm 0.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \cdot 100 \text{ mmHg}^{-1}$, respectively). Treatment with TEA also further reduced the vasodilator response to BK in both groups, but there was no significant difference in conductance responses between control and cholesterol-fed groups. The mean increase in conductance after BK treatment was slightly greater in the controls than in the cholesterol-fed group, but the difference was significant only at the lowest doses of BK.

The relative magnitudes of the L-NAME- and TEA-sensitive components of the vasodilator responses to ACh and BK are compared in control and cholesterol-fed rabbits in Fig. 6. The L-NAME-sensitive component in controls increased with dose of ACh but was independent of dose of BK. The cholesterol-fed group had a smaller L-NAME-sensitive contribution to the vasodilator response to ACh and BK. The TEA-sensitive component of the vasodilator response was independent of dose of ACh but appeared to increase with dose of BK. The cholesterol-fed group had significantly smaller TEA-sensitive components of the vasodilator response to ACh and tended to a smaller response to BK, but the difference between groups did not achieve statistical significance ($P = 0.08$).

The vasodilator responses to nitroprusside are compared for control and cholesterol-fed rabbits in Fig. 7. There were no significant differences between the dose-response curves for the two groups. Peak conductance after 250 $\mu$g of nitroprusside for controls did not significantly differ from that for cholesterol-fed animals ($11.4 \pm 1.0$ and $10.1 \pm 1.0 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \cdot 100 \text{ mmHg}^{-1}$, respectively).

**DISCUSSION**

This study investigated the effects of hypercholesterolemia on NO-independent K$_{Ca}$ channel-mediated vasomotor tone and endothelium-dependent vasodilatation. The role of K$_{Ca}$ channels was investigated using blocking drugs in control and hypercholesterolemic rabbits. TEA blocks large- and small-conductance K$_{Ca}$ channels at $<5 \text{ mmol/l}$ and other K$^+$ channels at higher concentrations (5). We used a dose comparable to that previously employed in the cat hindlimb in vivo (10). Our observations with CTX and apamin indicate that large- and small-conductance K$_{Ca}$ channels contribute to the NO-independent vasodilator responses. It is unlikely that TEA is acting to any significant degree via blockade of other K$^+$ channels, inasmuch as the degree of inhibition of NO-independent vasodilatation by CTX + apamin was comparable to that observed with TEA, and addition of TEA to CTX + apamin did not further reduce vasodilator responses. Another consideration is the site of K$_{Ca}$ channel blockade. The data of Demir et al. (19) suggest that TEA

![Fig. 5. Comparison of vasodilator responses to ACh and BK between control and cholesterol-fed rabbits after treatment with L-NAME and TEA. A: peak hindlimb conductance after each dose of ACh did not significantly differ between the cholesterol-fed group and controls. B: peak hindlimb conductance after each dose of BK did not significantly differ between the cholesterol-fed group and controls. C: increase in conductance above resting levels in response to ACh was slightly less in the cholesterol-fed rabbits, but differences between groups were not significant. D: increase in conductance above resting levels in response to BK was similar in the control and cholesterol-fed rabbits, except for a small difference at the lowest doses of BK. $*P < 0.05$ vs. controls.](http://ajpheart.physiology.org/)
predominantly blocks endothelial $K_{Ca}$ channels, with little effect on vascular smooth muscle cells, but some antagonism of ACh-induced vasorelaxation is observed when TEA is applied directly to vascular smooth muscle (19), and our present data show that TEA antagonizes the vasodilator effects of exogenous sodium nitroprusside in vivo. These observations suggest that TEA also blocks smooth muscle $K_{Ca}$ channels, albeit to a lesser degree than in the endothelium.

Inasmuch as $K_{Ca}$ channels may play a role in the stimulus to NO release from the endothelium and mediation of the vasodilator effect of NO in the smooth muscle cell (19, 30, 32, 33), examination of the independent role of the $K_{Ca}$ channels was undertaken in the presence of blockade of NO synthesis. Although we cannot examine the role of $K_{Ca}$ channels in the NO-mediated vasodilator responses in this study, previous data show that the $K_{Ca}$ channels may compensate for an abnormal cGMP-dependent component of the NO-mediated vasodilator response (32, 33). The residual $K_{Ca}$ channel-dependent vasodilatation we observed after L-NAME most likely represents EDHF-mediated vasodilatation. It is unlikely that the $K_{Ca}$ channel-dependent vasodilatation was in response to prostacyclin, inasmuch as the vasodilator responses and effects of $K_{Ca}$ channel blockers were similar in rabbits treated with and without indomethacin.

**Resting hindlimb conductance.** Inhibition of NO synthesis is associated with an increase in resting vasomotor tone (10, 31), as was observed in the present control group, and manifest as a reduction in resting vascular conductance and an increase in systemic arterial pressure. Blockade of $K_{Ca}$ channels had little effect on resting hindlimb conductance in the controls, indicating that NO-independent $K_{Ca}$ channel activity contributed little toward resting vasomotor tone in this group. Similar findings have been reported in the hindlimb of the cat by Champion and Kadowitz (10) and in anesthetized pigs by Zanzinger et al. (41). We observed different responses in the hypercholesterolemic rabbits, in which the reduction in resting conductance after L-NAME was approximately one-half that of the
controls. In contrast to the controls, $K_{Ca}$ channel blockade caused a significant further reduction in resting conductance in the cholesterol-fed group. This finding suggests that hypercholesterolemia is associated with a change in the balance between NO-mediated and NO-independent $K_{Ca}$ channel-mediated contributions to resting vasomotor tone.

One possible explanation for these observations is that impaired NO-mediated vasodilatation in the hypercholesterolemic rabbits results in a compensatory increase in activity of the NO-independent mechanisms. There is some experimental evidence to support such an interaction between NO-mediated and NO-independent mechanisms in the regulation of vascular tone in vivo. In rabbit carotid and porcine coronary arteries, an NO donor has been shown to reduce the magnitude of NO-independent vasodilator responses (2). Similarly, in the rabbit hindlimb, Cohen and co-workers (12) found that NO appears to inhibit EDHF-mediated vasodilatation, which may depend on activation of $K_{Ca}$ channels (21). Support for the concept of a compensatory increase in activity of other endothelium-dependent vasodilator mechanisms is also provided by studies of chronic inhibition of NO synthesis in rabbits (40). After an initial increase, systemic vascular resistance returns toward control levels, despite continued inhibition of NO synthesis.

Another possible mechanism of the increased contribution of NO-independent $K_{Ca}$ channel activity to resting vasomotor tone in the hypercholesterolemic group may be independent of any specific EDHF-mediated effect. When intracellular Ca$^{2+}$ is increased, the opening probability of the $K_{Ca}$ channels is increased, and the smooth muscle cell becomes hyperpolarized (5). This mechanism may serve as a negative feedback, opposing excessive myogenic tone. An increase in the cholesterol content of the cell membrane is associated with increased Ca$^{2+}$ uptake into arterial smooth muscle cells, and therefore the activity of the $K_{Ca}$ channels in these cells may be increased. Against this hypothesis are observations that increased membrane cholesterol content is associated with reduced open time of the $K_{Ca}$ channels (3).

**Endothelium-dependent vasodilator responses.** NO-mediated and NO-independent mechanisms contribute to the vasodilator responses to pharmacological agonists such as ACh and BK. Treatment with N-monomethyl-$\text{L}$-arginine does not eliminate vasodilator responses to ACh or substance P in the intact circulation (6, 31, 40). The present observations are in accord with these earlier findings. Inadequate blockade of NO synthesis by $\text{N}$-NAME is unlikely, given the findings in our initial studies and the fact that the present dose of $\text{N}$-NAME is similar to that employed in other in vivo studies of inhibition of NO synthesis (8). The residual endothelium-dependent vasodilator responses observed after $\text{N}$-NAME treatment were largely abolished by $K_{Ca}$ channel blockers TEA or CTX + apamin. A small residual vasodilator response was observed after TEA treatment, which could represent incomplete blockade of $K_{Ca}$ channels or the effect of other vasodilator substances such as prostacyclin. A similar residual vasodilatation after TEA treatment has been observed in the cat hindlimb (10). Incomplete blockade of the $K_{Ca}$ channels is a possibility, perhaps due to impaired penetration of blockers to the smooth muscle cells in the arterial wall. An effect of prostacyclin is less likely, inasmuch as similar residual vasodilatation was observed in rabbits treated with indomethacin.

Endothelium-dependent vasodilatation in large conduit arteries is severely impaired by hypercholesterolemia (9, 18, 39), possibly as a result of inadequate synthesis or accelerated scavenging of NO. In the coronary circulation, hypercholesterolemia is associated with abnormal endothelium-dependent vasodilatation of the microvasculature (20), and we previously found that endothelium-dependent vasodilator responses are reduced in the hindlimbs of hypercholesterolemic rabbits (29). The present data show that NO-mediated and NO-independent $K_{Ca}$ channel-mediated vasodilator responses to ACh and BK are impaired in hypercholesterolemia. These observations are consistent with in vitro observations of changes in large-conductance $K_{Ca}$ channel kinetics in the presence of cholesterol loading of the cell membrane (3). These findings are also consistent with in vitro studies showing that lysophosphatidylcholine inhibits NO-independent vasodilatation of aorta and carotid artery rings (17, 22). Although there appeared to be some increase in the relative contribution of $K_{Ca}$ channels to resting hindlimb conductance in hypercholesterolemic rabbits, we found no evidence of a compensatory increase in the $K_{Ca}$ channel-mediated responses to vasodilator agonists in the hypercholesterolemic rabbits.

**Conclusions.** Hypercholesterolemia is associated with a reduced contribution of NO and increased contribution of NO-independent $K_{Ca}$ channel activity to basal arterial vasomotor tone. Agonist-stimulated endothelium-dependent vasodilatation includes NO-mediated and NO-independent $K_{Ca}$ channel-mediated contributions. Hypercholesterolemia is associated with impairment of NO-mediated and NO-independent $K_{Ca}$ channel-mediated vasodilatation.

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**REFERENCES**


