BK$_{Ca}$ channels compensate for loss of NOS-dependent coronary artery relaxation in cardiomyopathy

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Clark, Shawn G., and Leslie C. Fuchs. BK$_{Ca}$ channels compensate for loss of NOS-dependent coronary artery relaxation in cardiomyopathy. Am J Physiol Heart Circ Physiol 279: H2598–H2603, 2000.—Previously, we showed that development of myocardial necrotic lesions is associated with impaired endothelium-dependent coronary artery relaxation in young cardiomyopathic hamsters. Since active necrosis declines with aging, this study was designed to determine whether coronary artery endothelium-dependent relaxation to ACh is restored and to identify the mechanisms mediating this effect. Intraluminal diameter was recorded in coronary arteries (150–250 μm) from control (C, 297 ± 5 days old) and cardiomyopathic (M, 296 ± 4 days old) hamsters. Relaxation to ACh ($10^{-6}$–$3 	imes 10^{-5}$ M) was similar in vessels from C and M hamsters. However, mechanisms mediating relaxation to ACh were altered. Inhibition of nitric oxide synthase (NOS) activity with N-nitro-L-arginine (1 mM) had a greater inhibitory effect in vessels from C hamsters, indicating a reduction in NOS-dependent relaxation in vessels from M hamsters. Conversely, inhibition of large Ca$^{2+}$-dependent K$^+$ (BK$_{Ca}$) channels with charybdotoxin (CTX, 0.1 μM) had a greater inhibitory effect in vessels from M hamsters. In the presence of both N-nitro-L-arginine and CTX, relaxation to ACh was abolished in both groups. CTX (0.1 μM) produced a 50 ± 4 and 30 ± 3% contraction of vessels from M and C hamsters, respectively, indicating an enhanced role for BK$_{Ca}$ channels in regulation of coronary artery tone in M hamsters. Finally, vasodilatory cyclooxygenase products contributed to ACh-induced relaxation in vessels from M, but not C, hamsters. In conclusion, NOS-dependent relaxation of coronary small arteries is reduced in the late stage of cardiomyopathy. An increase in relaxation mediated by BK$_{Ca}$ channels and vasodilatory cyclooxygenase products compensates for this effect.

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releases several vasodilatory substances, including NO, PGI₂, and EDHF, was used to elicit endothelium-dependent relaxation in these studies (8, 18).

METHODS

General procedures. Male Golden Syrian control and cardiomyopathic hamsters (Bio 14.6) were obtained from Bio- breeders (Fitchburg, MA) and housed individually in the Medical College of Georgia animal care facilities. Hamsters were weighed and anesthetized with pentobarbital sodium (60 mg/kg ip). Heparin (100 units) was administered into the left ventricle. A midline thoracotomy was performed, and the heart was removed, weighed, and placed in chilled oxygenated (20% O₂, 6% CO₂, 5% O₂, balance N₂) Krebs-Ringer bicarbonate solution (composition in mM: 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, and 11.1 dextrose). With the aid of a dissecting scope (model SZ30, Olympus), a second-order branch of the left main coronary artery was dissected free of myocardial tissue. All vessels were 1–2 mm long and 150–250 μm in intraluminal diameter. Isolated small arteries were transferred to a vessel bath containing oxygenated Krebs solution (Medical Instruments, University of Iowa, Iowa City, IA). Vessels were mounted between two glass micropipettes (100-μm-diameter tip) with 10-0 ophthalmic suture. The vessel bath was transferred to the stage of an inverted light microscope (model CK2, Olympus). The lumen of the vessel was filled with Krebs-Ringer solution through the micropipettes and held at a constant pressure of 40 mmHg. Intraluminal diameter was measured in micrometers with a video dimension analyzer (Living Systems Instrumentation, Burlington, VT) and recorded continuously on a Grass polygraph.

Protocol. Oxygenated Krebs-Ringer solution was maintained at 37°C and continuously circulated through the tissue bath. All vessels were allowed to equilibrate for ≥30 min. Coronary small arteries from control and cardiomyopathic hamsters were preconstricted to 35–55% of resting diameter with the thromboxane A₂ analog U-46619 and allowed to stabilize. Endothelium-dependent relaxation was assessed by performing a concentration-response curve to ACh (10⁻⁹–3 × 10⁻⁵ M). To determine the contribution of vasoactive prostanooids to ACh-induced relaxation, vessels were pretreated with indomethacin (Indo, 10 μM), an inhibitor of cyclooxygenase, for 20 min before a concentration-response curve to ACh was performed. To determine the role of NO synthase (NOS) in the response to ACh, vessels were pretreated with N-nitro-L-arginine (L-NNA, 1 mM), an inhibitor of NOS activity, for 20 min before the concentration-response curve to ACh was performed. The reactivity of the vascular smooth muscle to exogenous NO was also examined by performing a concentration-response curve to sodium nitroprusside (NP, 10⁻⁵–3 × 10⁻⁴ M). To determine the contribution of K⁺-channels to the NOS-independent relaxation induced by ACh, vessels were preconstricted with L-NNA (1 mM) and then preconstricted to 35–55% of resting diameter with KCl (25–50 mM) before a concentration-response curve to ACh was performed. This concentration of KCl effectively blocks K⁺ efflux and prevents relaxation mediated by opening of K⁺ channels. Additionally, the role of large Ca²⁺-dependent K⁺ (BKCa) channels in mediating NOS-independent relaxation to ACh was determined in vessels pretreated with a selective antagonist, charybdotoxin (CTX, 0.1 μM). Finally, a concentration-response curve to ACh was performed after pretreatment of vessels with CTX (0.1 μM) and L-NNA (1 mM). In all vessels pretreated with inhibitors, baseline diameters were recorded before and 20 min after the inhibitor was added to the vessel bath to determine the effect of inhibitors on basal vascular tone. When possible, more than one vessel was obtained from the same hamster, but each experiment was performed only once per hamster. Additionally, only one concentration-response curve was performed per vessel.

Chemicals. All chemicals were obtained from Sigma Chemical (St. Louis, MO). U-46619 was dissolved in 10% ethanol. Indo was dissolved in nanopure water in the presence of 94 mM NaCO₃. L-NNA was dissolved in acidic nanopure water and adjusted to pH 7.4 with 0.1 N NaOH. Other agents were dissolved in nanopure water. All agents were diluted with Krebs solution.

Data analysis. Data obtained from coronary arteries were expressed as intraluminal diameter in micrometers. Vasodilatory and vasoconstrictor responses were expressed as percent relaxation and percent contraction, respectively. EC₅₀ values were calculated using Prism GraphPad version 2.0. Values are means ± SE. Statistical differences were determined by ANOVA for repeated measures followed by the Student’s modified t-test with Bonferroni correction for multiple comparisons. The criterion for significance was P < 0.05.

RESULTS

The heart weight-to-body weight ratios were 7.9 ± 0.6 and 4 ± 0.1 mg/g in cardiomyopathic (297 ± 5 days old) and control (296 ± 4 days old) hamsters, respectively. The significant increase in heart weight-to-body weight ratio suggests myocardial hypertrophy in late-stage cardiomyopathic hamsters. Concentration-response curves to ACh in the absence and presence of Indo in vessels from control and cardiomyopathic hamsters are shown in Fig. 1. Indo had no significant effect on basal coronary vascular tone in vessels from control and cardiomyopathic hamsters. Relaxation to ACh and EC₅₀ values were unaffected by the presence of Indo in vessels from control hamsters. However, Indo significantly reduced relaxation to ACh (10⁻⁷ M) and increased the EC₅₀ value in vessels from cardiomyopathic hamsters (Fig. 1, Table 1).

Fig. 1. Concentration-response curves to ACh in the presence and absence of indomethacin (Indo, 10 μM). Coronary small arteries were isolated from control (C) and cardiomyopathic (M) hamsters and preconstricted with the thromboxane A₂ analog U-46619. Baseline intraluminal diameter was 172 ± 6, 209 ± 17, 184 ± 15, and 208 ± 19 μm for the C, M, C-Indo, and M-Indo groups, respectively. Percent contraction after U-46619 was 43 ± 2, 42 ± 2, 39 ± 2, and 37 ± 2 for the C, M, C-Indo, and M-Indo groups, respectively. Values are means ± SE. *P < 0.05 vs. respective untreated group.
Table 1. EC<sub>50</sub> values for ACh in control and cardiomyopathic hamsters

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<thead>
<tr>
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<th>Control</th>
<th>Cardiomyopathic</th>
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<tr>
<td>Untreated</td>
<td>20 ± 3</td>
<td>11 ± 5</td>
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<tr>
<td>Indo</td>
<td>17 ± 2</td>
<td>27 ± 6&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-NNA</td>
<td>73 ± 21&lt;sup&gt;s&lt;/sup&gt;</td>
<td>96 ± 15&lt;sup&gt;s&lt;/sup&gt;</td>
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Values are means ± SE expressed as ×10<sup>-6</sup> M. Indo, indomethacin (10 μM); L-NNA, N-nitro-L-arginine (1 mM). *P < 0.05 vs. respective untreated group; †P < 0.05 vs. control.

Concentration-response curves to ACh in the presence and absence of L-NNA in vessels isolated from control and cardiomyopathic hamsters are shown in Fig. 2. L-NNA had no significant effect on basal vascular tone in vessels from control or cardiomyopathic hamsters. L-NNA significantly inhibited relaxation to ACh and increased the EC<sub>50</sub> (Table 1) in vessels from control and cardiomyopathic hamsters compared with respective untreated groups. In the presence of L-NNA, relaxation to ACh (1 × 10<sup>-6</sup>–3 × 10<sup>-5</sup> M) was significantly less in control than in cardiomyopathic vessels. Maximum relaxation to ACh was not altered by L-NNA in vessels from cardiomyopathic hamsters but was significantly reduced from 97 ± 1 to 50 ± 9% in coronary small arteries from control hamsters. The addition of NP (10<sup>-4</sup> M) after the last concentration of ACh in L-NNA-pretreated vessels from control hamsters produced a 96 ± 4% relaxation, indicating functional integrity of the vascular smooth muscle. To determine whether vascular smooth muscle responsiveness to exogenous NO was altered in vessels from cardiomyopathic hamsters, concentration-response curves to NP were performed. NP produced similar relaxation in vessels from control and cardiomyopathic hamsters (Fig. 3).

To determine the role of K<sup>+</sup> channels in the relaxation to ACh that remained after inhibition of NOS, vessels were preconstricted with KCl in the presence of L-NNA before the concentration-response curve to ACh was performed. The degree of preconstriction produced by KCl in the presence of L-NNA was 42 ± 3 and 42 ± 5% in vessels from control and cardiomyopathic hamsters, respectively. This was similar to the preconstriction produced by U-46619 in the presence of L-NNA (41 ± 3 and 39 ± 2% in vessels from control and cardiomyopathic hamsters, respectively). The relaxation that remained after inhibition of NOS in vessels from control and cardiomyopathic hamsters was completely abolished in the presence of a high level of extracellular K<sup>+</sup>, indicating that this component of relaxation was mediated by K<sup>+</sup> channels (data not shown).

To assess the role of BK<sub>Ca</sub> channels in the NOS-independent component of relaxation, concentration-response curves to ACh were performed in the presence of CTX + L-NNA. CTX alone produced contraction of coronary small arteries that was significantly greater in vessels from cardiomyopathic than from control hamsters, indicating that control of basal vascular tone by BK<sub>Ca</sub> channels is enhanced in cardiomyopathy (Fig. 4).

![Fig. 2. Concentration-response curves to ACh in the absence and presence of N-nitro-l-arginine (l-NNA, 1 mM).](image1)

![Fig. 3. Concentration-response curves to sodium nitroprusside (NP) in coronary small arteries isolated from C and M hamsters and preconstricted with the thromboxane A<sub>2</sub> analog U-46619. Baseline intraluminal diameter was 200 ± 14 and 216 ± 20 μm for the C and M groups, respectively. Percent contraction after U-46619 was 40 ± 2 and 44 ± 4 for the C and M groups, respectively. Values are means ± SE. There were no significant differences.](image2)

![Fig. 4. Contractile response to charybdotoxin (CTX, 0.1 μM) in coronary small arteries isolated from C and M hamsters. Baseline intraluminal diameters were 184 ± 7 and 216 ± 19 μm for the C and M groups, respectively. Values are means ± SE. *P < 0.05 vs. C.](image3)
to $10^{-7}$ M, followed by contraction at higher concentrations of ACh in vessels from control hamsters (Fig. 5). Conversely, in vessels from cardiomyopathic hamsters, relaxation to ACh was abolished in the presence of CTX. The addition of pinacidil ($10^{-5}$ M), an ATP-activated K\(^+\) channel opener, resulted in 98 ± 2 and 94 ± 5% relaxation of vessels from control and cardiomyopathic hamsters, respectively, indicating that ATP-activated K\(^+\) channel relaxation remained intact. As shown in Fig. 6, L-NNA abolished relaxation to ACh that remained after inhibition of BK\(_{Ca}\) activity with CTX in coronary small arteries from control hamsters. The addition of NP ($10^{-4}$ M) after the concentration-response curve to ACh in the presence of CTX and L-NNA caused 71 ± 8 and 65 ± 10% relaxation of vessels from control and cardiomyopathic hamsters, respectively, demonstrating the ability of the vessels to relax under these conditions. The finding that these vessels did not relax 100% in response to NP is likely due to the ability of CTX to reduce NO-mediated opening of BK\(_{Ca}\) channels, as we previously reported (4).

**DISCUSSION**

The mechanisms mediating the development of cardiomyopathy are unknown. Abnormal intracellular Ca\(^{2+}\) handling appears to contribute to the onset of cardiomyopathy (6, 34). A role for inadequate coronary blood flow in producing necrosis has been suggested. Administration of verapamil, a Ca\(^{2+}\) channel blocker, to young cardiomyopathic hamsters has been shown to abolish microvascular hyperreactivity and to prevent myocytolytic lesions (17). Transient spasms in the coronary microcirculation occur during the necrotic phase of cardiomyopathy (7). Additionally, in preneecrotic hamsters, sections of the myocardium that did not show capillary filling were found to correlate with sections of the myocardium that became necrotic as hamsters aged (22). In a previous study, we demonstrated that superoxide anions contributed to impaired endothelium-dependent relaxation of coronary arteries from cardiomyopathic hamsters in the necrotic phase (10). Therefore, evidence supports a role for coronary vasospasm in the development of myocardial lesions observed in the cardiomyopathic hamster.

The present study was performed in hamsters in the late phase of cardiomyopathy, in which hypertrophy is typically present but necrotic lesions are not actively developing. In this stage of cardiomyopathy, coronary small artery endothelium-dependent relaxation was not altered quantitatively. However, the mechanisms mediating endothelium-dependent relaxation in vessels preconstricted with U-46619 were altered. A shift in the relative contribution of NO and EDHF to endothelium-dependent relaxation has also been reported in mesenteric arteries of transgenic hypertensive rats (25). In our study, the contribution of NO to ACh-induced relaxation of coronary small arteries was reduced in vessels from cardiomyopathic hamsters. Studies by others on vascular endothelial production of NO in heart failure are controversial. ACh-induced release of NO was lowered in human and canine coronary arteries from failing hearts (16, 35). Similar experiments performed in dogs in heart failure demonstrated enhanced NO production (24). Elevated levels of free radicals have been observed in cardiomyopathic hamster hearts compared with control (11). Free radicals are produced by the endothelium and are known to scavenge NO (26). Our finding that sensitivity of the vascular smooth muscle to NP was not reduced in cardiomyopathic hamster vessels suggest that mechanisms of NO formation in the endothelial cell and/or diffusion of biologically active NO from the endothelial cell to the vascular smooth muscle cell are impaired in the advanced stage of cardiomyopathy.

Despite a loss of NOS-dependent relaxation, endothelium-dependent relaxation to ACh remained intact in coronary arteries from hamsters in the late stage of cardiomyopathy. This appears to be due to the activa-
tion of compensatory vasodilator pathways. Coronary small arteries from cardiomyopathic, but not control, hamsters were partially dependent on vasodilatory prostanooids to produce relaxation to ACh. A role for vasodilatory cyclooxygenase products in the coronary circulation of cardiomyopathic hamsters was suggested by the finding that cyclooxygenase inhibition reduced the dilator response to ACh in the coronary circulation of isolated hearts from cardiomyopathic hamsters (33). These findings indicate that production of PGI2 in response to ACh is enhanced in the coronary circulation of cardiomyopathic hamsters.

In the present study, NOS-independent relaxation was enhanced in vessels from cardiomyopathic hamsters. This component of ACh-induced relaxation was mediated by opening of BKCa channels. In coronary small arteries from cardiomyopathic hamsters, ACh-induced relaxation was completely dependent on opening of BKCa channels. However, in vessels from control hamsters, an NOS-mediated relaxation was present that was not dependent on BKCa channels. In addition to having an enhanced role in ACh-induced relaxation, BKCa channels also had an enhanced role in regulation of tone of isolated coronary arteries from cardiomyopathic hamsters. BKCa channels are important regulators of coronary arterioles from humans as well (21).

Opening of smooth muscle cell BKCa channels produces relaxation via hyperpolarization, leading to closing of voltage-sensitive Ca2+ channels and reduction in intracellular Ca2+.

The increase in BKCa channel-mediated relaxation of coronary arteries from cardiomyopathic hamsters may be due to several factors. One possibility is an increase in release of EDHF. An EDHF that is distinct from NO has been described and may be an arachidonic acid metabolite of the cytochrome P-450 pathway (3). Recently, cytochrome P-450 2C has been reported to be an EDHF synthase in coronary arteries, and a role for EDHF in regulation of coronary arteriolar tone has been shown in vivo in dogs (9, 23). EDHF contributes to shear-stress-induced endothelium-dependent relaxation in rat mesenteric arteries, an effect that was partially mediated by Ca2+-dependent K+ channels (32).

NO has been shown to inhibit release of EDHF (1). We may speculate that if production of NO is reduced in coronary arteries of cardiomyopathic hamsters, the inhibitory effect of NO on EDHF release would be reduced, leading to an increase in EDHF production. Several studies indicate a reciprocal relationship between NO and EDHF that is altered in a variety of pathological conditions. An upregulation of EDHF-mediated relaxation was observed in rat and dog models of ischemia-reperfusion (5, 19). EDHF, but not NO or PGI2, release was resistant to oxidative stress in the bovine coronary artery (15). Irradiation of human cerebral arteries impaired NO- and PGI2-mediated, but not EDHF-mediated, relaxation (31). Hypoxia and alkalization inhibited NO-mediated, but not EDHF-mediated, responses in the porcine coronary artery (27).

Another explanation for the increase in BKCa channel-mediated relaxation of coronary arteries from cardiomyopathic hamsters would be an increase in the number or activity of BKCa channels. This effect was seen in spontaneously hypertensive rats, in which the membrane potential and activity of Ca2+-dependent K+ channels were increased in interlobular arteries (20). In our study, it is also possible that BKCa channels, if present in endothelial cells, could alter production of endothelium-derived relaxing factors. However, this would not be supported by the finding of others: that CTX did not affect the increase in intracellular Ca2+ or hyperpolarization produced by ACh in the endothelium of second-order branches of the Golden Syrian hamster femoral artery (2).

In summary, this study has shown that BKCa channels and vasodilatory cyclooxygenase products compensate for a decrease in NOS-dependent relaxation of coronary small arteries in the late stage of cardiomyopathy. This is in contrast to cardiomyopathic hamsters in the necrotic phase of cardiomyopathy, in which endothelium-dependent relaxation of coronary small arteries is impaired and myocardial necrotic lesions develop (10).

These studies were supported by an American Heart Association Established Investigator Grant to L. C. Fuchs.

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