Constriction to hypoxia-reoxygenation in isolated mouse coronary arteries: role of endothelium and superoxide

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Liu, Qiang. Constriction to hypoxia-reoxygenation in isolated mouse coronary arteries: role of endothelium and superoxide. J. Appl. Physiol. 87(4): 1392–1396, 1999.—The aim of the present study was to determine the role of endothelium and superoxide in the responses of isolated mouse coronary arteries to hypoxia-reoxygenation. Isolated mouse coronary artery was cannulated, pressurized at 60 mmHg, and constantly superfused with recirculating Krebs-Ringer bicarbonate solution for continuous measurement of intraluminal diameter (ID) by video microscopy. Under a no-flow condition, hypoxia (0% O2, 30 min) caused vasconstriction. Reoxygenation caused a further vasconstriction (ID change from 111.4 ± 11.1 to 91 ± 16.5 µm) that was significantly reduced by removal of endothelium (ID change from 105.4 ± 27 to 109.9 ± 23.4 µm). Cu/Zn superoxide dismutase (150 U/ml) did not alter the hypoxic vasconstrictor but abolished the reoxygenation-caused endothelium-dependent vasconstriction. Hypoxia-reoxygenation markedly enhanced the generation of superoxide that was significantly reduced by either removing the endothelium or treated these endothelium-intact vessels with superoxide dismutase. These results suggest that, in isolated mouse coronary arteries, hypoxia causes vasconstriction that is independent of endothelium, whereas reoxygenation causes vasconstriction that is mediated by enhanced generation of superoxide from endothelium.

METHODS

Preparation of isolated mouse coronary artery. The experimental model and protocols in this study were approved by the Animal Care and Use Committees at The Johns Hopkins Medical Institutions. Adult male mice (20–25 g, B6SJL G93A background) were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained for a week in the approved animal facility in the Asthma and Allergy Building, under the care of a licensed veterinarian at The Johns Hopkins Medical Institutions. On the day of the experiment, mice were killed by cervical dislocation. The heart was removed rapidly and placed in cold Krebs-Ringer bicarbonate solution containing (in mM) 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 25.0 NaHCO3, and 11.1 glucose. Mouse coronary artery (without pressure), ~70–90 µm in diameter and 1 mm long, was isolated from the left main coronary artery under a dissection microscope. Isolated coronary artery was placed in a microvascular chamber, cannulated at one end with a glass micropipette, and secured with a 12–20 nylon monofilament suture. Krebs-Ringer bicarbonate solution was infused slowly through the cannula, until the vessel was completely filled. The other end of the vessel was then cannulated with a second micropipette filled with Krebs-Ringer bicarbonate solution. Both cannulas were connected to reservoirs filled with Krebs-Ringer bicarbonate solution that could be raised or lowered to control the transmural pressure (Ptm). Upstream and downstream Ptm was measured continuously with two pressure transducers positioned at the level of the vascular lumen. In the microvascular chamber, the vessels were constantly superfused with recirculating Krebs-Ringer bicarbonate solution (total volume = 100 ml) gassed with 16% O2-5% CO2-balance N2 (pH 7.35–7.45), and maintained at 37°C. A custom-built Plexiglas cover was placed over the chamber to control O2 tension over the superfusate. An O2 electrode (Microelectrode, Bedford, NH) was passed through a port in the cover into the superfusate and positioned near the vessel to provide continuous measurement of O2 tension. The vessel was placed on the stage of an inverted microscope (Nikon TMS-F, Japan) connected to a video camera (Panasonic, CCTV camera, Japan). The vascular image was projected onto a video monitor, and intraluminal diameter (ID) was measured continuously by a video-dimension analyzer (Living Systems Instrumentation, Burlington, VT). Vascular ID, Ptm, and O2 tensions were continuously recorded by using a four-channel recorder (Gould, Cleveland, OH). In some vessels, endothelial cells were disrupted by gently rubbing the intraluminal surface with a steel wire. These vessels were then perfused with 2-ml air bubbles followed by 2-ml Krebs-Ringer bicarbonate solution.

Experimental protocol. The measurements of ID were started immediately after the vessel was mounted and continued throughout the experiment. Initially, isolated mouse coronary artery was allowed to equilibrate in the microvascular chamber for 30 min at a Ptm of 10 mmHg. Ptm was increased to 60 mmHg in 10-mmHg steps at 5- to 7-min intervals. Vascular ID, Ptm, and O2 tensions were monitored continuously. The vessel was then exposed to hypoxia (0% O2, 30 min). The vessel was reperfused with Krebs-Ringer bicarbonate solution for 5 min and then reexposed to hypoxia. Finally, the vessel was reperfused with Krebs-Ringer bicarbonate solution for 5 min.

RECENT STUDIES in isolated coronary arteries have shown that hypoxia-reoxygenation (H/R) causes vasoconstriction (13, 20, 26). The precise mechanism(s) of H/R-induced vascular constriction remains unclear. Some studies have demonstrated that H/R increases the generation of superoxide in various tissues as well as in endothelium and that it could be prevented by superoxide dismutase (SOD) (15, 29, 30). Because superoxide radicals are known to exert diverse effects on vascular functions by direct activation of vascular smooth muscle cells and modulation of the release of endothelium-derived relaxing and constricting factors (7, 14, 21, 24, 25), it is possible that the H/R-induced vasconstriction is mediated via the generation of superoxide radicals. The aim of present study was to determine the roles of endothelium and superoxide anions in isolated mouse coronary artery response to H/R. Furthermore, the development of isolated mouse coronary artery preparation in evaluation of vascular reactivity in response to H/R would be useful for studying the isolated human coronary circulation; superoxide anions

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RESULTS

Drug concentrations are expressed as final molar concentrations. Stock solutions of drugs were prepared fresh each day in deionized water and stored at 4°C during the experiment. All drug concentrations are expressed as final molar concentration (mol/l) in the chamber superfusate.

Data analysis. Vascular responses to physiological (pressure, H/R) and pharmacological (U-46619, ACh, and papaverine) stimulations were expressed as ID (µm). The measurements of superoxide anions (chemiluminescence signal) were expressed as relative light units (REL) per second.

Drugs. ACh chloride, Cu/Zn-SOD, lucigenin, and papaverine were obtained from Sigma Chemical (St. Louis, MO). U-46619 was purchased from Cayman Chemical (Ann Arbor, MI). Stock solutions of drugs were prepared fresh each day in deionized water and stored at 4°C during the experiment. All drug concentrations are expressed as final molar concentration (mol/l) in the chamber superfusate.

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tion (Fig. 3) in coronary arteries with endothelium (ID change from 114.4 ± 11.1 to 91 ± 16.5 µm, n = 7), which was abolished in coronary arteries without endothelium (ID change from 105.4 ± 27 to 109.9 ± 23.4 µm, n = 7). There was a significant difference between the two groups (P < 0.01). Extraluminal administration of Cu/Zn-SOD (150 U/ml) did not alter the coronary arteries with endothelium in response to U-46619, ACh, and papaverine (Fig. 2) during normoxia, and the vasoconstriction in response to hypoxia (ID change from 147 ± 12.6 to 128.8 ± 5.8 µm, n = 5, Fig. 4). However, SOD abolished the reoxygenation-caused further vasoconstriction (ID change from 123.8 ± 5.8 to 132.2 ± 6.4 µm, n = 5; two groups compared, P < 0.01) in vessels with intact endothelium. Indeed, in coronary arteries with endothelium pretreated with SOD, reoxygenation caused vasodilatation (Fig. 4). Extraluminal administration of Cu/Zn-SOD (150 U/ml) did not alter the coronary arteries without endothelium in response to H/R (Fig. 4).

**DISCUSSION**

The present study indicated that isolated mouse coronary arteries have similar reactivity as those in other animal species (2, 12, 13, 21–23) in response to physiological (pressure, O₂) and pharmacological (U-46619, ACh, and papaverine) stimulation (Figs. 1–3). Isolated mouse coronary arteries with endothelium exhibited a progressive vasodilatation to increases of Ptm that was similar to that observed by Ku et al. (11), whereas it differed markedly from the coronary arteries without endothelium, which developed a myogenic response. This myogenic tone in coronary arteries without endothelium is more likely to result from endothelium removal than from vascular smooth muscle.

Measurements of superoxide anions. In isolated mouse coronary arteries with endothelium under a normoxic conditions, the measurements of superoxide anions were 6.5 ± 0.5 (SE) RLU/s (n = 4). After hypoxia (0% O₂, 30 min), reoxygenation (16% O₂) significantly enhanced the generation of superoxide anions (mean ± RLU/s = 19.5 ± 3.3, n = 5, P < 0.01, compared with normoxic coronary arteries with endothelium). In coronary arteries without endothelium, reoxygenation-enhanced generation of superoxide was markedly reduced (mean ± SE of RLU/s = 12.8 ± 1.3, n = 5, P < 0.05, compared with coronary arteries with endothelium), but still significantly higher than in the normoxic coronary arteries with endothelium. In isolated mouse coronary arteries with endothelium, in the presence of Cu/Zn SOD (150 U/ml), the reoxygenation-enhanced generation of superoxide was significantly limited (mean ± SE of RLU/s = 11.7 ± 1.7, n = 5, P < 0.05, compared with coronary arteries without SOD group) (Fig. 5).
damage due to the denudation procedure, because the vessels display similar constriction to U-46619 and dilatation to papaverine than do endothelium-intact coronary arteries, indicating normal smooth muscle function (Fig. 2). The myogenic responses in systemic arteries have been studied for many years. Some studies suggested that myogenic constriction was caused by endothelium-independent mechanisms (2, 12, 19), whereas others concluded that endothelial cells played a major role (10, 22, 23). Stretch could activate the smooth muscle ion channels to induce a depolarization and calcium influx (8, 16). However, stretch could also act on endothelium, inducing release of endothelium-derived relaxing factor, nitric oxide (NO), which might modulate the myogenic constriction (5).

Hypoxia (0% O₂, 30 min) caused vasoconstriction in isolated mouse coronary arteries both with and without endothelium, suggesting that endothelium-independent mechanism might play a major role in this hypoxic vasoconstriction. Toda et al. (26) have previously observed similar results in isolated human, monkey, and dog coronary arteries. However, in most systemic arteries, hypoxia caused vasodilatation that was thought to be mediated by activation of K_ATP channels (4, 6, 13). The explanation for these conflicts is unknown.

The reoxygenation after hypoxia caused endothelium-dependent vasoconstriction. Our previous studies in isolated porcine small coronary arteries have indicated that H/R-induced vasoconstriction was not altered by the inhibition of NO or prostacyclin production (13), suggesting that the H/R-induced vasoconstriction was not mediated by decreases of activity of endothelial NO or prostacyclin. However, reoxygenation-induced vasoconstriction was prevented by SOD (150 U/ml), suggesting that reoxygenation, after hypoxia, enhances the generation of superoxide anions. The enhanced generation of superoxide anions was directly measured by using a lucigenin-enhanced chemiluminescence technique. Furthermore, removal of endothelium from these coronary arteries or treatment of the endothelium-intact coronary arteries with Cu/Zn-SOD significantly reduced the generation of superoxide anions during reoxygenation. This evidence supports that the generations of superoxide anions from endothelium cause coronary arterial constriction during reoxygenation. The endothelium-derived superoxide anions have been found in isolated pig coronary arteries under basal conditions (1), and H/R markedly enhanced the generation of superoxide anions in human aortic endothelial cells (29). It has been suggested that superoxide anion is an endothelium-derived contracting factor (14, 27). Superoxide anions can modulate vasoconstriction through different mechanisms. They can directly cause contraction (7, 14, 15, 23, 27) of vascular smooth muscle cells; can rapidly destroy endothelium-derived relaxing factor-NO (7, 14, 15, 23, 27); and they may also affect the release of other endothelium-derived contracting factors, such as endothelin (9, 28). SOD is well known to be a scavenger of superoxide anions, and it catalyzes the rapid dismutation of superoxide anions to hydrogen peroxides (3, 18, 29). Because SOD would not have been expected to rapidly traverse the endothelium and myocyte membranes due to its 32-kDa molecular mass (29), it suggests that superoxide generation occurs at the cell surface of endothelium and/or within the vascular lumen adjacent to the endothelium.

H/R-induced vasoconstriction was abolished after removal of endothelial (Fig. 3). In these endothelium-denuded vessels during reoxygenation, the generation of superoxide was markedly reduced (Fig. 5). Extraluminal administration of Cu/Zn-SOD (150 U/ml) did not alter the coronary arteries without endothelium in response to H/R. These results suggest that endothelial cells are a major source of superoxide anion generation. The fact that the generation of superoxide anions in these endothelium-denuded vessels after exposure to reoxygenation was still significantly higher than in normoxic vessels with endothelium suggests that endothelium may not be the only source of superoxide radicals and that vascular smooth muscle could also generate superoxide anions (15, 17). The reason why reoxygenation-caused vasoconstriction have not been seen in these endothelium-denuded vessels is that the generation of superoxide anions from vascular smooth muscle during reoxygenation might be not high enough to cause vasoconstriction. It has been proposed that the enzyme xanthine oxidase may be a central mechanism of superoxide free radical generation in a variety of postischemic cells and tissues (15). In human aortic endothelial cells subject to anoxia and reoxygenation, superoxide free radical generation has been thought to react with iron to form the reactive hydroxyl radicals (29).

In summary, the present study demonstrated that in isolated mouse coronary arteries hypoxia caused an endothelium-independent vasoconstriction, whereas reoxygenation caused a further vasoconstriction that was mediated by enhanced generation of superoxide anions from endothelium.

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