Coronary Arteriolar Myogenic Response Is Independent of Endothelium

Lih Kuo, William M. Chilian, and Michael J. Davis

The purpose of this study was to investigate if myogenic responses of isolated coronary arterioles were dependent on an intact, functional endothelium. Arterioles were located in situ by intracoronary perfusion with India ink-gelatin solution and then dissected and cannulated at both ends with glass micropipettes. Intraluminal pressure was initially set at 60 cm H₂O; then the pressure was altered in steps of 20 cm H₂O over a range of 20–140 cm H₂O. Arterioles developed spontaneous tone and exhibited a significant myogenic response in physiological saline solution (36°±3° C). Arteriolar dilation and constriction were observed at lower (20–60 cm H₂O) and higher (60–140 cm H₂O) pressures, respectively. The presence of a functional and anatomically intact endothelium was confirmed by relaxation to the endothelium-dependent vasodilator bradykinin and by transmission electron microscopy, respectively. After mechanical denudation of the endothelium with a specially designed abrasive micropipette, spontaneous tone and myogenic responses were preserved. Denudation of the endothelium was verified functionally (no response to bradykinin) and with transmission electron microscopy. Moreover, the mechanical denudation technique did not deleteriously affect smooth muscle because vasoconstrictor and vasodilator responses to nonendothelial-dependent drugs were the same before and after denudation. In summary, the present study demonstrates that pressure-dependent responses occur in isolated coronary arterioles and that this response is not dependent on the endothelium. Therefore, pressure-induced changes in coronary arteriolar tone are a true myogenic response in that they originate from smooth muscle. (Circulation Research 1990;66:860–866)

In 1902 Baylis1 reported that arterial smooth muscle contracted in response to augmented intraluminal pressure. Previous evidence has shown that this response, the myogenic response, is not mediated by nerves, vasoactive metabolites, or circulating vasoactive substances.2–5 Recently, the myogenic concept, that is, pressure-induced contraction originating in vascular smooth muscle, has been challenged by studies that show the response to be dependent on an intact, functional endothelium.6–9

Since over half of coronary resistance resides in arterioles less than 100 μm in diameter,10 it is important to understand the fundamental regulatory mechanisms that control resistance in these small arteriolar vessels. We have recently reported myogenic activity of small (80–100-μm) isolated coronary arterioles.11 Thus, the possibility exists that myogenic mechanisms in small coronary arterioles may contribute to the regulation of coronary blood flow. The primary goal of this study was to determine if the coronary myogenic response is dependent on the endothelium.

Materials and Methods

General Preparation and Cannulation

Pigs (4–8 weeks old of either sex, n=26) were sedated with ketamine (2.5 mg/kg i.m.) and Rompun (2.25 mg/kg i.m.; Haver-Lockhart, Cutter Laboratories, Shawnee, Kansas), anesthetized with pentobarbital sodium (20 mg/kg i.v.), intubated, and ventilated with room air. After a left thoracotomy, heparin (1,000 units/kg) was administered into the left atrium, and the heart was electrically fibrillated, excised, and immediately placed in cold (4° C) saline solution.

The techniques for identification and dissection of coronary arterioles were reported previously.11 In brief, India ink–gelatin physiological saline solution (PSS) was perfused into the left anterior descending artery and the circumflex artery (0.35 and 0.3 ml,
respectively) to visualize the coronary microvessels. At 4°C, arterioles from the left anterior descending or circumflex artery (1.0–1.5 mm in length and 40–70 μm i.d.) were selected and dissected quickly from the surrounding cardiac tissue and transferred for further dissection to a dish (4°C) containing filtered PSS-albumin (1 g/100 ml bovine serum albumin) solution at pH 7.4. After careful removal of any remaining cardiac tissue, the arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin solution equilibrated with room air at ambient temperature. One end of the arteriole was cannulated with a glass micropipette (40 μm in tip diameter and filled with filtered PSS-albumin solution), and the outside of the arteriole was securely tied to the pipette with 11-0 ophthalmic suture. The ink-gelatin column inside the vessel was flushed out at low perfusion pressure (<20 cm H₂O). Any small branches were tied off; then the other end of the vessel was cannulated with a second micropipette and secured with suture.

**Experimental Procedures and Protocol**

After the vessel was cannulated, one micropipette was connected to a pressure reservoir, and the other was connected to a low-volume displacement strain gauge transducer (Statham P23 Db, Gould, Cleveland, Ohio) for intraluminal pressure measurements. Under these conditions, the isolated arteriole could be pressurized without flow by adjusting the height of the reservoir. Leaks were detected by differences between reservoir pressure and luminal pressure. Leaky vessels were excluded from the data analysis. For internal diameter measurements, the preparation was then transferred to the stage of an inverted microscope (model IM35, Carl Zeiss, Thornwood, New York) coupled to a TV camera (67M Newvicon, Dage-MTI, Michigan City, Indiana), video micrometer, and video recorder (Panasonic). Lumen diameters were measured continuously throughout the experiments by videomicroscopic techniques.

The cannulated vessel bathed in PSS-albumin solution in the chamber was maintained at 36°C–37°C C by an external heat exchanger. The vessel was set to its in situ length and allowed to develop spontaneous tone at 60 cm H₂O luminal pressure. After the vessel developed spontaneous tone, the relation between intraluminal pressure and vessel diameter (myogenic response) was examined. Initially, vessel diameter was measured at the control pressure; then pressure was increased in steps of 20 cm H₂O to 140 cm H₂O, then reduced in the same steps to 20 cm H₂O, and finally returned to control. At each step, the pressure was maintained until a stable lumen diameter was obtained (2–3 minutes). After completion of this protocol, acetylcholine chloride (ACh), an endothelium-independent vasoconstrictor in porcine coronary artery, was applied to test the contractile response of the vessel at 60 cm H₂O luminal pressure. ACh was administered in cumulative doses until the vessel constricted approximately 60% (range, 55–70%) of its initial diameter with tone. Bradykinin (10⁻⁸ M), an endothelium-dependent vasodilator of porcine coronary artery, was then added to the preconstricted vessel to verify the functional integrity of endothelial cells. All drugs used in this study were obtained from Sigma Chemical, St. Louis, Missouri.

**Endothelium Denudation Procedures**

Three methods of endothelial denudation were attempted in this study: 1) The endothelium was chemically removed by intraluminal perfusion with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; 0.2% or 0.5%) in PSS for 90 seconds. After this procedure, the vessel segment was perfused (washed) with PSS-albumin for 30 minutes. Vessels treated in this manner behaved passively with intraluminal pressure changes and had altered contractile responses to ACh. This indicated that the vascular smooth muscle was damaged. 2) The endothelial cells were removed by intraluminal injection of an air bolus, which has proven to be a satisfactory procedure in isolated small arteries (approximately 400 μm i.d.). However, we had to apply very high pressure to the perfusion system (in excess of 240 cm H₂O) to push the air bubble through the lumen of our small pipettes. Pressures in this range have been found to produce vascular injury, and vessels in our study lost their spontaneous tone and myogenic responses after this procedure (two of two). 3) Mechanical removal of the endothelium was accomplished with a concentric glass abrasive micropipette with a long shank (5 mm) and an irregular tip (20 μm in diameter). This micropipette was advanced into the lumen of the cannulated vessel. A negative intraluminal pressure (−20 cm H₂O) was produced to collapse the vessel and to allow the abrasive pipette to closely contact the endothelial cells. The endothelial cells were disrupted by passing the abrasive pipette back and forth several times. After mechanical denudation of the endothelium, the cellular debris inside the lumen were washed out by perfusion (40 cm H₂O) with warm (37°C) PSS-albumin solution for 5 minutes. The vessels were allowed to equilibrate at 60 cm H₂O intraluminal pressure for 40 minutes to regain vessel tone. The myogenic response and smooth muscle and endothelial cell viability were reexamined as previously described. If endothelial functional responses persisted (i.e., the vessel relaxed to bradykinin), the denudation procedure was repeated until the endothelium-dependent relaxation was abolished. Finally, the vessels were maximally relaxed with sodium nitroprusside (10⁻⁴ M). The series of pressure changes described previously was then performed to obtain the passive pressure-diameter relation. Adequacy of endothelial denudation with intact smooth muscle cells was confirmed by transmission electron microscopy.
Table I. Internal Diameter of Subepicardial Arterioles in Physiological Saline-Albumin Solution and Response to Pressure (Myogenic Response), Acetylcholine, and Bradykinin at 60 cm H₂O Transmural Pressure Before (Control) and After Endothelial Denudation

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<th>CHAPS denudation</th>
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<tr>
<td>Control</td>
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<tr>
<td>Excessive abrasion</td>
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Values are mean±SEM, n, number of arterioles; PSS, physiological saline solution; ACh, 5×10⁻⁷ M acetylcholine; BK, 10⁻⁸ M bradykinin. Excessive abrasion was defined as that which eliminated resting tone and attenuated ACh-induced constrictions in addition to abolishing the BK-induced dilations. For statistical purposes, data from vessels treated with CHAPS (0.2% and 0.5%) were combined. Note that either perfusion with CHAPS or excessive abrasion (four of 16 mechanical denudation vessels) resulted in abolition of spontaneous tone and myogenic responses. The reactivity of these vessels to ACh was also attenuated.

*Significantly different from ACh (p<0.05) based on paired t test.
†Significantly different from control (p<0.05) based on paired t test.

Electron Microscopy

After determination of the pressure-diameter relation, four randomly selected arterioles were prepared for transmission electron microscopy. These vessels were fixed in glutaraldehyde (2.5%) and sodium cacodylate trihydrate (0.1 M) for 1 hour at room temperature and 60 cm H₂O intraluminal pressure and then stored in 0.1 M cacodylate buffer at 4°C. The vessels were postfixed in 1.0% OsO₄ and subjected to a standard series of dehydration steps in ethanol before being embedded in Epoxy resin (Ted Pella, Tustin, California) with acetone as a diluent. Thin sections were taken of the vessels in a longitudinal plane and viewed with a transmission electron microscope (model 420, Philips Electronic Instruments, Mahwah, New Jersey) after lead staining.

Data Analysis

A single vessel was studied from each pig for statistical analysis (23 reactive vessels) with the exception of one animal in which two vessels were used. All internal vessel diameters were normalized to the diameter at 60 cm H₂O luminal pressure in the presence of nitroprusside (i.e., passive state). Normalized diameters were averaged at each pressure step. All data are reported as mean±SEM. Statistical comparisons between groups (with and without endothelium) and within groups were made with factorial or repeated-measures analysis of variance tests with Fisher least significant difference multiple-range tests when appropriate. Differences in diameters between control (PSS-albumin) and in the presence of drugs (e.g., ACh, bradykinin, and nitroprusside) before and after endothelial denudation were detected with paired (two-tailed) t tests. Significance was defined as p≤0.05.

Results

In our preparations, about 80% of the cannulated subepicardial arterioles developed spontaneous tone within 40 minutes at 60 cm H₂O intraluminal pressure and 37°C bath temperature. The results of pressure-diameter relations from five reactive subepicardial arterioles with intact endothelium (control) were averaged and are summarized in Figure 1. In PSS-albumin solution at 60 cm H₂O intraluminal pressure, the vessels spontaneously constricted to 76.4±3.3% of their maximum diameters (i.e., compared with their diameter in nitroprusside solution). In all vessels, increasing intraluminal pressure caused a significant decrease in diameter (16% decrease in diameter when intraluminal pressure was increased to 140 cm H₂O) rather than the increase observed in the presence of nitroprusside (10⁻⁴ M) (Figure 1). At the lower pressure range (<60 cm H₂O), vessel dilation was observed in four of five vessels. In contrast, all vessels behaved passively after perfusion with either concentration of CHAPS (Figure 1).

The average absolute diameters of arterioles in the PSS-albumin solution and their responses to ACh and bradykinin at 60 cm H₂O before and after endothelial denudation are presented in Table 1. With the endothelium intact, ACh (5×10⁻⁷ M) reduced diameter by about 60%, and these ACh-preconstricted vessels relaxed in response to bradykinin. We used three criteria to determine the efficacy of selective endothelium removal without
affecting vascular smooth muscle function: 1) preservation of spontaneous tone, 2) constriction to ACh, and 3) lack of dilation of preconstricted vessels to bradykinin. After 0.2% or 0.5% CHAPS perfusion, the contractile response of vessels to ACh was attenuated or completely abolished, respectively, and these vessels failed to demonstrate myogenic responses. After mechanical denudation, the above three criteria were satisfied in 12 of 16 vessels. Endothelial removal in these 12 vessels did not affect the active pressure-diameter relations (Figure 2). All 12 vessels constricted and diluted at high (>60 cm H2O) and low (<60 cm H2O) pressures, respectively, and none of these vessels dilated in response to bradykinin. In four of 16 vessels, however, the myogenic response was abolished after mechanical denudation, spontaneous tone was eliminated, and their contractile responses to ACh were significantly reduced (Table 1). This suggests that excessive abrasion could produce vascular smooth muscle damage. After administration of nitroprusside, all vessels dilated and behaved passively during changes in intraluminal pressure (Figure 2).

Successful disruption of endothelium after mechanical abrasion was additionally verified with electron microscopy. Figure 3 (top panel) shows a transmission electron micrograph from a control vessel with intact endothelium and vascular smooth muscle. The bottom panel illustrates a mechanically denuded vessel with the endothelial layer removed.

**Discussion**

The major finding of this study is that the myogenic response of small coronary arterioles does not depend on the presence of an intact, functional endothelium. This is an important observation, albeit a negative one, with regard to the accumulating evidence describing the complex interactions between the endothelium and vascular smooth muscle. Specifically, the endothelium has been shown to modulate the vasoactivity of several autacoids,18 was reported to mediate flow-induced vasodilation of large arteries,19 and has been implicated in stretch-induced and pressure-dependent vascular constrictor responses.6-9 To provide a perspective for our results, we will discuss some critical aspects of our study, which include methodological considerations such as the viability of the preparations, the efficacy of the denudation procedure, and the relation of our findings to other studies.

**Methodological Considerations**

According to previous studies,11,17 three major criteria for reactive isolated arterioles are 1) development of spontaneous tone at 37°C, 2) myogenic responses, and 3) sensitivity to vasoactive agents. In the present experiments, 23 of 27 control coronary arterioles met these criteria. Introduction of the air bolus under the necessary high pressure or perfusion with CHAPS abolished spontaneous tone of the arterioles. Also, we were unable to find a concentration of CHAPS that blocked the arteriolar response to endothelial-dependent vasodilators without altering smooth muscle function (Table 1). Although
CHAPS-treated vessels still responded to ACh, the threshold for contraction increased fivefold. After mechanical denudation, four of 16 vessels showed the same characteristics as those perfused with CHAPS; this finding suggested that excessive abrasion could produce damage to vascular smooth muscle. With more moderate abrasion, however, the endothelium appeared to be selectively denuded without detectable changes in smooth muscle function. There are several lines of evidence to support this contention. First, during luminal abrasion under brightfield illumination, we observed that endothelial cells were disrupted, as shown by membrane remnants attached to the lumen wall and cellular debris floating in the lumen. Second, bradykinin did not dilate the vessels after this procedure. Third, transmission electron microscopic examination indicated that endothelial cells were disrupted while vascular smooth muscle remained intact (Figure 3). Fourth, after abrasion, we observed no alterations in spontaneous tone, constriction to ACh, or myogenic responses. Collectively, these results verified that endothelial cells were selectively removed and that smooth muscle was unaffected by the micropipette abrasion technique.

Consideration of Related Studies

The role of the endothelium in pressure-dependent responses is quite controversial. Mechanical denudation of rabbit ear arteries and small pulmonary arteries was reported not to affect myogenic responses; thus, direct response of vascular smooth muscle to stretch was implied. Our results showing endothelial independence of coronary myogenic responses extend this concept to the microcirculation. Four laboratories, however, have provided evidence that stretch-induced and pressure-dependent vascular constriction required an intact endothelium. Three of those studies were performed on cerebral arteries, and the other was performed on the carotid artery. Although the reasons for these apparently contradictory observations regarding the role of the endothelium in pressure-induced constrictions are not understood, some possible explanations include tissue and species variations, methodological procedures, and differences in vessel size.

With regard to methodological differences, studies using enzymatic or chemical denudation techniques cannot unequivocally eliminate the possibility of nonspecific damage to vascular smooth muscle, because small artery preparations lose resting vasomotor tone after that procedure. It was argued that nonspecific damage did not occur as a result of enzymatic treatment, because such vessels responded to vasoactive substances similarly to control (untreated) vessels. When damage occurs in isolated arterioles during isolation procedures, basal tone and myogenic responses are compromised first, before the vascular sensitivity to pharmacological agents is affected; therefore, the possibility exists that enzymatically treated vessels may be partially damaged.

Small vessels may be more susceptible than large vessels to damage during denudation. Larger vessels have multiple layers of vascular smooth muscle, while vessels 40–70 μm in diameter have only two or three layers (Figure 3). Enzymatic digestion with collagenase and chemical lysis with detergents could also damage vascular smooth muscle by disrupting myoendothelial junctions. It is noteworthy that these junctions are reported to occur more frequently in arterioles than in arteries, so that chemical treatments might produce greater vascular smooth muscle damage in microvessels than in large vessels. This observation, in part, could account for smooth muscle damage by CHAPS in our microvessel preparation, whereas in arteries CHAPS was found to not substantially alter pharmacological vascular reactivity.

Our studies do not preclude the possibility that the endothelium may be releasing contractile factors in response to stretch in organ systems other than the heart. For example, Katusic et al reported that development of active tension after stretch of isolated canine basilar arteries was dependent on a prostanooid released from the vascular endothelium and that cyclooxygenase inhibition (indomethacin) or endothelial denudation blocked this response. It is noteworthy to indicate that the development of active tension in their preparations was not sustained, because the isolated vessels relaxed after 2 minutes of peak tension development (refer to Figure 1A of Katusic et al). In our preparations, as well as during myogenic autoregulatory mechanisms, the response in vessel diameter is maintained throughout the pressure step; thus, the data of Katusic et al do not completely fit with documented observations of myogenic responses. In contrast, Rubanyi reported that pressure-induced contractions of isolated canine carotid arteries were not due to a prostanooid metabolite but, rather, to a depression of the synthesis and/or release of endothelium-derived relaxing factor. Recently, Harder et al found that pressure-induced contraction of isolated cerebral arteries was mediated by release of a contractile substance from the vascular endothelium and not by inhibition of endothelium-derived relaxing factor release. Collectively, there does not seem to be any consensus regarding the role of the endothelium-derived factors in pressure-induced contractile responses: the process might involve inhibition of endothelium-derived relaxing factor release or production of contractile substance.

Physiological Implications

Our results indicate that vascular smooth muscle cells can respond directly to changes in wall stretch. In support of this idea, the membrane potential of vascular smooth muscle has been shown to vary directly with vascular constriction, and stretch-activated cationic channels have recently been found in both visceral and vascular smooth muscle cell membranes. Moreover, visceral smooth muscle
FIGURE 3. Top panel: Transmission electron micrograph (×6,960) of control porcine subepicardial arteriole (56 μm i.d.). The control vessel has been cannulated in a tissue bath containing physiological saline–albumin solution and subjected to myogenic tests. Intact endothelial and vascular smooth muscle layers can be seen. Bottom panel: Transmission electron micrograph (×6,970) of mechanically denuded porcine subepicardial arteriole (60 μm i.d. in physiological saline–albumin solution). The endothelial layer was removed after mechanical abrasion, and the two layers of vascular smooth muscle appear normal.
responds actively to stretch,27,28 and isolated coronary smooth muscle cells contract when mechanically perturbed.29 It should be mentioned that the degree or method of cell stretching in many of the above studies may not have been physiological, and thus, the findings cannot be extrapolated directly to the vascular myogenic response. Nevertheless, such studies indicate that stretch of smooth muscle cell can cause changes in ion flux and membrane potential that are appropriate to explain the alterations in microvessel tone observed in response to luminal pressure changes.

It is important to highlight the physiological significance of the myogenic response. Myogenic mechanisms have been shown to contribute to autoregulatory adjustments in many different organ systems3,22,23,30 and have been implicated in the establishment of baseline vasomotor tone.2 In the heart, myogenic responses may contribute to the maintenance of basal vasomotor tone and play a role in the regulation of coronary blood flow.11 Our results demonstrate that the myogenic response of coronary arteries is not dependent on an intact, functional endothelium.

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References


Key Words • porcine coronary artery • intraluminal pressure • isolated arterioles • coronary microcirculation • vascular smooth muscle • autoregulation
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