Extravascular pressure modulates responses of isolated rat coronary arteries to vasodilator, but not vasoconstrictor, stimuli

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Azzawi, May, and Clare Austin. Extravascular pressure modulates responses of isolated rat coronary arteries to vasodilator, but not vasoconstrictor, stimuli. Am J Physiol Heart Circ Physiol 290: H1151–H1156, 2006. First published October 21, 2005; doi:10.1152/ajpheart.00307.2005.—The aims of the study were to investigate whether elevated extravascular pressure modulates responses of isolated rat coronary arteries to constrictor and dilator stimuli. Isolated segments of rat coronary artery were mounted in a modified pressure myograph system that allowed independent modulation of both intra- and extravascular pressures. The influence of elevated extravascular pressure on stable levels of myogenic tone and on responses to vasoconstrictor and vasodilator stimuli was investigated at constant overall transmural pressures. Stable levels of myogenic tone were independent of the relative levels of intra- and extravascular pressure, as were responses to depolarization and to addition of the thromboxane agonist U-46619. Elevating extravascular pressure, however, significantly reduced dilatory responses to introduction of intraluminal flow and to addition of endothelium-dependent and endothelium-independent vasodilatory agonists. These results support the notion that elevated extravascular pressure may attenuate responses of coronary arteries to a variety of dilatory stimuli. This finding may be of relevance to cardiac disorders associated with elevated ventricular pressures.

contractile function; coronary circulation; vasoconstriction/dilation

AN UNDERSTANDING OF THE FACTORS that determine coronary vascular resistance is paramount to our understanding of the regulation of blood flow to the heart in both health and disease. Although metabolic control is clearly a major determinant of coronary perfusion, physical factors such as changes in blood flow and intraluminal pressure also contribute to the regulation of small arterial tone, and thus resistance, via shear stress and myogenic mechanisms, respectively (6, 7, 9). These factors are highly interactive (7, 15). In addition to these intraluminal physical stimuli, coronary arteries also experience extravascular compressive forces due to the contraction of the myocardial muscle itself. We have previously shown, using a novel in vitro system, that isolated coronary arteries exhibit myogenic regulatory responses to elevations in extravascular pressure; although a rise of external pressure results in an initial anticipated compression of the vessel, this is followed by an active dilatory response such that initial diameter is maintained (at least over certain pressures) (2). Thus, in addition to intraluminal physical forces, local autoregulation of resistance artery diameter, and thus blood flow in the heart, is likely to be influenced by extravascular pressures. The interactions between elevated extravascular pressure and responses to internal physical stimuli (pressure and flow) are unknown.

In the coronary circulation, therefore, it is clear that small arteries exhibit myogenic regulatory responses to changes in both intra- and extravascular pressure. At any time, therefore, the transmural pressure across the arterial walls is determined by both the intra- and extravascular pressures. As such, similar transmural pressures may be attained by different combinations of intra- and extravascular pressures. Although it is generally assumed that stable levels of myogenic tone are dependent only on transmural pressure, this has not previously been directly proven. It is now recognized that changes in cyclic strain and/or endothelial cell deformation may alter the release and/or synthesis of various endothelial factors, including nitric oxide (1, 13). Because the influence of intra- and extravascular pressures on these endothelial stimuli may vary, and because the endothelium is known to modulate stable levels of coronary artery myogenic tone (e.g., Refs. 4, 14), the possibility exists that the relative contributions of these pressures to the transmural pressure does influence tone. As such, it is essential that this is fully characterized.

Although changes in the extravascular forces experienced by the coronary arteries obviously will be experienced during the normal cardiac cycle, elevated pressures also are evident during disease states associated with elevated ventricular pressures such as congestive heart failure. Although impaired endothelium-dependent responses of the coronary vasculature have been reported in such conditions (e.g., Refs. 17, 19), others have found that if correction is made for the elevated left ventricular diastolic pressure, the responses are similar to normal (16). This finding has led to the suggestion that increased extravascular forces may act to limit endothelium-dependent dilatory responses; however, this possibility has not been directly examined. It also is unknown whether elevated external pressures modulate responses to vasoconstrictor stimuli.

Therefore, the aims of this study were to directly examine the effects of elevated extravascular pressure on responses of isolated coronary arteries to vasoconstrictor and vasodilator stimuli. We examined its influence on myogenic tone, on responses to constrictor stimuli (agonist and depolarization), and on responses to both endothelium-dependent (flow, acetylcholine) and endothelium-independent (isoprenaline) vasodilatory stimuli.

MATERIALS AND METHODS

General Preparation and Cannulation

Male Wistar rats (150–250 g; Charles River) were humanely killed by stunning and cervical dislocation. All procedures were performed in accordance with our institutional guidelines and the United King-
dom Animals (Scientific Procedures) Act of 1986. Experiments were performed with the approval of the Review Board of the University of Manchester and the Home Office. The heart was removed and placed in ice-cold physiological salt solution (PSS) of the following composition (in mM): 119 NaCl, 4.7 KCl, 1.2 MgSO4, 7H2O, 25 NaHCO3, 1.17 KHPO4, 0.03 K2EDTA, 5.5 glucose, and 1.6 CaCl2·2H2O (pH 7.4). First-order septal arteries (200–250 μm initial diameter, 1–2 mm in length) were dissected out and mounted on a modified pressure myograph (Living Systems Instrumentation, Burlington, VT). Arteries were initially pressurized to an intraluminal pressure of 60 mmHg by using a pressure servocontrol unit and were checked for leaks. A tightly fitting lid was then placed over the myograph chamber. Extravascular pressure was altered by introduction of gas (95% air–5% CO2) from a standard gas cylinder) from a luer connection in the top part of the chamber above the vessel, as we have described previously (1). Inflow of gas, and thus extravascular pressure, was controlled via a pneumatic valve and recorded with a mercury manometer. All vessels were constantly superfused with PSS at 37°C, pH controlled via a pneumatic valve and recorded with a mercury manometer. The top part of the chamber above the vessel, as we have described previously (2). Inflow of gas, and thus extravascular pressure, was controlled via a pneumatic valve and recorded with a mercury manometer. All vessels were constantly superfused with PSS at 37°C, pH 7.4, and gassed with 95% air–5% CO2, with the superfuse entering and leaving the chamber via luer connections in the side of the chamber, pH was continuously monitored using a flow-through pH probe (Living Systems Instrumentation) placed immediately after the luer fitting through which the solution left the chamber. We could detect no change in pH with the elevations in extravascular pressure used in this study. Lumen diameters were constantly measured using a video dimension analyzer, with data recorded on computer and a chart recorder for later analysis. Where appropriate, arteries were superfused with calcium-free PSS containing 2 mM EGTA for at least 30 min to obtain passive diameters.

Experimental Protocols

Do relative contributions of intra- and extravascular pressure to transmural pressure influence stable levels of myogenic tone? Arteries were initially pressurized to an intravascular pressure of 60 mmHg and allowed to develop spontaneous myogenic tone. All those arteries that did not develop tone within 90 min or that did not develop tone of at least 25% of original diameter were excluded from the study. We wanted to determine whether stable levels of myogenic tone were influenced by the relative levels of intra- and extravascular pressure or were purely dependent on the overall transmural pressure. To investigate this, we examined the effects on tone of similar transmural pressures produced by differing combinations of intra- and extravascular pressures. For example, transmural pressures of 40 mmHg were obtained by the following combinations of intra- and extravascular pressures: intravascular pressure 60 mmHg/extravascular pressure 0 mmHg; intravascular pressure 40 mmHg/extravascular pressure 0 mmHg; and intravascular pressure 100 mmHg/extravascular pressure 60 mmHg (see Fig. 1 for other combinations used). In all cases, zero additional extravascular pressure is defined as atmospheric pressure. The ways in which these stable combinations of extra- and intravascular pressure were attained were varied.

To investigate the involvement of the endothelium, we carried out the above protocols before and after endothelial denudation. Vessels were denuded by the introduction of four to five air bubbles through the lumen of vessels, with functional denudation confirmed by abolition of the dilatory response to acetylcholine (ACH; 50 μM). Any vessels that permanently lost myogenic tone following denudation were excluded from the study. The influence of inhibition of nitric oxide synthase (NOS) and cyclooxygenase also were investigated by performing experiments in the presence of N-nitro-L-arginine (L-NNA; 50 μM) and indomethacin (10 μM), respectively.

Does changing the relative contributions of intra- and extravascular pressures to overall transmural pressure modulate constrictor responses to agonist addition or depolarization? Constrictor responses to addition of the thromboxane analog U-46619 (0.25 μM) or by depolarization (40 mM KCl) were investigated at a transmural pressure of 60 mmHg, obtained using the following combinations of intra- and extravascular pressure: intravascular pressure 60 mmHg/extravascular pressure 0 mmHg; intravascular pressure 100 mmHg/extravascular pressure 40 mmHg; and intravascular pressure 120 mmHg/extravascular pressure 60 mmHg. At an intravascular pressure of 60 mmHg, these stimuli produced submaximal levels of constriction.

Does changing the relative contributions of intra- and extravascular pressure to overall transmural pressure modulate responses to vasodilatory stimuli? Intraluminal Flow. We have previously shown that isolated rat coronary arteries possessing myogenic tone (at intravascular pressure of 60 mmHg/extravascular pressure 0 mmHg) exhibit vasodilatory responses to introduction of intraluminal flow. Responses are maximal and repeatable at flow rates of ~5 μl/min (maximal dilations = 35 ± 6, 25 ± 4, and 25 ± 4% of tone with 3 successive exposures to 5 μl/min flow, n = 3). As such, we investigated the influence of differing combinations of intra- and extravascular pressure (see above), to give a combined transmural pressure of 60 mmHg, on responses of isolated rat coronary arteries to this flow rate. Vessels were initially allowed to develop myogenic tone at an intravascular pressure of 60 mmHg (additional extravascular pressure = 0 mmHg). We have previously shown that responses of isolated rat coronary arteries to intraluminal flow are entirely dependent on the presence of a functionally intact endothelium (unpublished observations).

DILATOR AGONISTS. In a separate series of experiments, the influence of the different pressure combinations (intravascular pressure 60 mmHg/extravascular pressure 0 mmHg; intravascular pressure 120 mmHg/extravascular pressure 60 mmHg), designed to maintain transmural pressure at 60 mmHg, on dilatory responses to the endothelium-dependent agonist ACh (1 μM) were examined. The influence on dilatory responses to the β-adrenergic agonist isoprenaline (0.5 μM) also were studied. In coronary arteries, isoprenaline has been shown to produce dilatory responses that are independent of the endothelium (20); these responses are readily reversible.

Data Analysis

All results are presented as means ± SE, with n representing the number of animals. One artery was studied from each animal. Statistically significant differences between groups were assessed using ANOVA or t-tests as appropriate. Myogenic tone was calculated as the difference between active (in calcium-containing PSS) and passive (in calcium-free PSS) diameters at each transmural pressure. To allow direct comparison between the different experimental protocols, we normalized diameters to the stable diameter at an intravascular pressure of 60 mmHg (extravascular pressure 0 mmHg) as appropriate. Transmural pressure was calculated as the difference between intra- and extravascular pressures.

Drugs and Chemicals

All drugs and chemicals were obtained from Sigma and were dissolved in PSS.

RESULTS

Do the Relative Contributions of Intra- and Extravascular Pressure to Transmural Pressure Influence Stable Levels of Myogenic Tone?

Arteries used in these studies had an initial mean diameter of 237 ± 5 μm (n = 52). All arteries began to develop myogenic tone within 60 min of mounting and attained mean stable active diameters of 172 ± 8 μm. All arteries rapidly diluted when placed in calcium-free PSS.

Transmural pressures of 20–60 mmHg resulted in stable diameters that were not significantly different from one
another, confirming our previous findings that there is active myogenic regulation over this pressure range (2). The results of the present study, however, show that stable levels of tone were independent of the relative contributions of intra- and extravascular pressure to overall transmural pressure (Fig. 1). Although endothelial denudation did significantly reduce active diameter (Fig. 1), stable levels of myogenic tone once again appeared to be dependent only on the transmural pressure (Fig. 1); this also was observed in the presence of NOS and cyclooxygenase inhibition (Fig. 1). In calcium-free solution, arteries dilated when transmural pressure was increased; diameters were again independent of the relative contributions of intra- and extravascular pressures to overall pressure (Fig. 1).

Does the Changing Relative Contributions of Intra- and Extravascular Pressures to Overall Transmural Pressure Modulate Constrictor Responses to Agonist Addition or Depolarization?

In a separate set of experiments, the effects of different intra- and extravascular pressure combinations on constrictor responses to addition of the thromboxane analog U-46619 and depolarization were examined. Responses to addition of U-46619 were unaffected by modulation of the intra- and extravascular pressure ratio at a transmural pressure of 60 mmHg (n = 4). Similarly, responses to high-K⁺ solution were unchanged (Fig. 2).

Does Elevated Extravascular Pressure Modulate Responses to Dilator Stimuli?

Intraluminal flow. Introduction of intraluminal flow (5 μl/min) resulted in a dilation of all vessels by 55 ± 13% of developed tone (maximal dilation). At transmural pressures of 60 mmHg, produced by elevating both extra- and intravascular pressure (to 40 and 100 mmHg, respectively), however, dilation to flow was significantly reduced (P < 0.04) and was virtually abolished when extra- and extravascular pressures were elevated further to 60 and 120 mmHg, respectively (P < 0.03). Responses to flow were restored (to 86 ± 17% of original) when any extravascular pressure was removed and intravascular pressure was returned to 60 mmHg. As above, myogenic tone was independent of the relative contributions of intra- and extravascular pressure to it (Fig. 3).

Fig. 1. Normalized mean active diameters of isolated rat coronary arteries at transmural pressures (TmP) of 20–80 mmHg, produced by different combinations of intra- (IvP) and extravascular pressures (EvP). Diameters are shown in endothelium-intact arteries (A) and functionally endothelium-denuded arteries (B) and in the presence of N-nitro-L-arginine and indomethacin (Indo/L-NNA; C) and in calcium-free solution (D). Bar graphs show that 1) the diameters are independent of the relative contributions of IvP and EvP to the overall TmP in all conditions and that 2) in calcium-containing solution, there are no significant differences in mean stable diameters in this range, indicating active myogenic regulation.

Fig. 2. Influence of different IvP and EvP combinations on constrictor responses of isolated rat coronary arteries to addition of 0.25 μM U-46619 (A) and 40 mM KCl (B) at a maintained transmural pressure of 60 mmHg.
Dilator agonists. At a transmural pressure of 60 mmHg, produced by an intravascular pressure of 60 mmHg and extravascular pressure of 0 mmHg, addition of isoprenaline resulted in a rapid dilation of U-46619-preconstricted tissues to 51 ± 18% constriction. Sequentially increasing both intra- and extravascular pressures significantly reduced the magnitude of this dilation (Fig. 4). Initial responses were restored (to 104 ± 5% of original) when intravascular pressure was returned to 60 mmHg (extravascular pressure 0 mmHg). Similar effects were observed with ACh in preparations constricted by KCl; although elevating intravascular pressure to 120 mmHg and extravascular pressure to 60 mmHg had no significant effect on the magnitude of the stable constriction (see above), it did significantly reduce the magnitude of the dilation to ACh (n = 5) (Fig. 4). Again, responses were returned to control values (>100%, n = 2) when extravascular pressure was removed and intravascular pressure was returned to 60 mmHg once again.

Responses to ACh were similarly reduced by elevating intra- and extravascular pressure in tissues precontracted with U-46619. Elevating intravascular pressure alone did not reduce dilations to ACh [e.g., at an intravascular pressure of 60 mmHg, dilations were 64 ± 15 μm (n = 7), and at an intravascular pressure of 100 mmHg, dilations were 73 ± 16 μm; extravascular pressure was maintained at 0 mmHg throughout].

**DISCUSSION**

Thus we have provided the first direct evidence that 1) constrictor responses to depolarization and addition of U-46619 are independent of the relative levels of intra- and extravascular pressure at a constant transmural pressure and 2) dilatory responses to introduction of intraluminal flow and agonist addition (both endothelium dependent and independent) are influenced by the relative contributions of the two pressures. We also have shown that stable levels of myogenic tone are dependent only on the overall transmural pressure (irrespective of intra- and extravascular pressures) in both endothelium-intact and endothelium-denuded arteries.

Although it has generally been assumed that myogenic tone is governed by the absolute level of transmural pressure, it had previously only been demonstrated in isolated pressurized coronary arteries in responses to changes in intravascular pressure (5, 9, 8). Although this may be valid for many vascular beds, in those embedded in contracting muscle, as discussed previously, internal pressures will be opposed by external compressive forces. We are the first to have shown that isolated coronary arteries also exhibit regulatory myogenic responses to elevations of external pressure (2); as such, extravascular pressure will contribute to stable levels of tone in these vessels. In the present study we have demonstrated that stable levels of myogenic tone are dependent only on the absolute value of transmural pressure, irrespective of the relative values of intra- and extravascular pressure, at least over the range of transmural pressures of 20–80 mmHg. This is in support of our earlier findings over a smaller pressure range (2). Interestingly, although we observed active regulatory responses to changes in intra- and extravascular pressure over this pressure range, stable diameters are maintained, rather than reduced, as we have found in other vascular beds (10, 11). This is in accordance with our previous findings and those of others in our group (Ref. 2; unpublished observations). The reasons for this are unclear but may reflect differences in their physiological roles and perhaps the relative importance of, and interactions with, other local regulatory mechanisms. Differences in arterial size/anatomical location and, indeed, species also may be important. In support of this, porcine subepicardial arteries have been shown to exhibit more pronounced myo-
extravascular pressures may limit responses in coronary vessels. The results of the present study are consistent with this idea and further show that responses to more physiological stimuli of endothelial factor release (i.e., flow) and to endothelium-dependent dilatory agonists are reduced as external pressure is elevated. We cannot totally discount the possibility that these effects may be related to the concurrent increase in intravascular pressure (to maintain transmural pressure), although we think this is unlikely. This is difficult to test, however, because increasing intravascular pressure to 100 and 120 mmHg alone will change diameter (2); this itself will influence responses to flow and other stimuli (7).

We have, however, shown that elevating intravascular pressure alone (at zero extravascular pressure) does not inhibit dilatory responses to ACh, making it unlikely to be responsible for the reduced dilatory responses we observed when extravascular pressure also is elevated. A direct inhibitory effect of extravascular pressure on dilator responses will have important implications for our understanding of the underlying mechanisms, and indeed the treatment options, associated with conditions such as heart failure.

Thus we have shown, for the first time, that modulating the relative combinations of intra- and extravascular pressures at a constant transmural pressure has no effect on stable levels of myogenic tone or on responses of isolated rat isobarically mounted coronary arteries to constrictor agonist addition or to depolarization. Conversely, the relative contribution of internal and external pressures does influence responses to vasodilatory stimuli when transmural pressure remains constant. In agreement with a previous in vivo study (16), we suggest that these effects are due to the elevated extravascular pressure opposing dilation. We do, of course, recognize that our simple experimental procedures do not directly represent the more complex role played by elevated extravascular pressure in modulating coronary arterial diameter in vivo, where differences in the absolute and relative values of intra- and extravascular pressures, and indeed transmural pressures, will be observed throughout the cardiac cycle. Similarly, the magnitude of the external pressures required to oppose dilation will likely depend on many factors, including compression of the venular system (and thus elevation of intravascular pressure) and possibly different forces the vessels themselves may have when embedded in intact muscle (e.g., a compressive force of the adventitia). Although these clearly warrant further study, our observation will nevertheless have important implications for our understanding of coronary blood flow in disease states such as congestive heart failure associated with elevated left ventricular pressure.

GRANTS

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REFERENCES


