Requisite Role of Cardiac Myocytes in Coronary α_1-Adrenergic Constriction

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Background—α1-Adrenergic activation in vivo causes constriction of coronary arterioles, but, paradoxically, in vitro these microvessels do not contract to this stimulus. We hypothesized that cardiac myocytes have a requisite role in α1-adrenergic coronary arteriolar constriction through the release of myocyte-derived contractile factor(s).

Methods and Results—Administration of the α1-adrenergic agonist phenylephrine did not constrict isolated coronary arterioles, but constriction was observed to supernatant obtained from phenylephrine-treated cardiac myocytes. Constriction to the supernatant was blocked by administration of an endothelin-A antagonist to the microvessel preparation or an α-adrenergic antagonist to the myocytes and was augmented after administration of an adenosine antagonist. Administration of phenylephrine to the myocytes increased endothelin-1 levels in the supernatant, but only to subthreshold concentrations.

Conclusions—Cardiac myocytes have a requisite role in constriction of coronary resistance vessels to α1-adrenergic stimuli, which may be mediated by endothelin-1 and other unidentified myocyte-derived vasoconstrictors. (Circulation. 1998;98:9-12.)

Key Words: microcirculation ■ endothelin ■ phenylephrine ■ dogs

Numerous laboratories, including ours, reported that coronary resistance vessels constrict to α-adrenergic activation,1-5 but we and another group made the contrasting observation that isolated coronary arterioles are refractory to α-adrenergic agonists.6,7 This is puzzling, because isolated arterioles from a variety of organ systems,6,8,9 and in vitro coronary venules6 and large coronary arteries (≥500 μm in diameter)10-12 constrict to α1- and/or α2-adrenergic stimulation. A reconciliation between in vivo and in vitro coronary arteriolar responses to α1-adrenergic activation would occur if arteriolar vasoconstriction in vivo is produced indirectly, ie, activation of α-adrenergic receptors on parenchymal cells provokes the release of a factor that induces coronary vasoconstriction. Because our microvessel preparations are devoid of cardiac myocytes, we hypothesized that these cells have a requisite role in modulating coronary arteriolar constriction to α1-adrenergic activation. We tested this by examining the vasoactive responses of isolated arterioles to graded doses of the α1-adrenergic agonist phenylephrine and by reexamining the arteriolar responses to aliquots of fluid obtained from isolated myocytes treated with phenylephrine. Autoradiographic studies have detected a substantially higher density of α1-adrenergic receptors on cardiac myocytes than on coronary arterioles,13 which adds substance to our argument that cardiac myocytes are “targeted” by α1-adrenergic agonists. We also proposed that in vivo, α1-adrenergic agonists induce coronary arteriolar constriction by stimulating cardiac myocytes to release endothelin-1, which constricts coronary resistance vessels. To test this, we assessed vasoactive responses during antagonism of ETA receptors on arterioles and α1-adrenergic receptors on cardiac myocytes and measured endothelin-1 production by cardiac myocytes during α1-adrenergic activation. Although cardiac myocytes possess α1-adrenergic receptors14 and produce endothelin-1,15 a functional link between these characteristics is not established.

Methods

The use of animals was in accordance with Medical College of Wisconsin and National Institutes of Health guidelines, and protocols were approved by the institutional laboratory animal care committee. Coronary arterioles (50 to 80 μm in intraluminal diameter) were isolated from the canine hearts (n=11), cannulated with micropipettes at both ends, bathed in buffered albumin-PSS (pH 7.4, 37±1°C), and studied with videomicroscopy.16 The arterioles developed spontaneous tone during equilibration (60 cm H2O).

Cardiac myocytes were enzymatically isolated and purified.17 The left coronary artery of a dog heart was perfused with collagenase-buffer mix (pH 7.4) for 30 minutes at 37°C. Then the left ventricle was minced into small, 0.25- to 0.5-g pieces in 100 mL of the collagenase-buffer solution and further digested in an orbital shaker (240 min-1) for 20 to 40 minutes (37°C). This crude fraction was filtered through surgical gauze and resuspended in buffer. Aliquots of this suspension were placed in 50-mL conical tubes, and the myocytes were allowed to settle for 10 minutes. Floating cells and debris were aspirated, and the myocyte fraction was resuspended, then allowed to settle for 5 minutes. This procedure was repeated,
and myocytes were resuspended in buffer with 1% BSA. CaCl₂ was added incrementally in five 5-minute steps to a final [Ca²⁺] of 1 mmol/L. Myocytes were concentrated to 1.5×10⁷ to 2.0×10⁷/mL by rinsing the suspension through cheesecloth and collecting the unfiltered fraction, then pipetting it into centrifuge tubes. Myocytes were maintained at 37°C in a humidified incubator (20% O₂, 5% CO₂, 75% N₂) in buffer containing the following additional compounds (mmol/L): taurine 60, creatine 20, and CaCl₂ 1, plus 1.5% BSA. The purity of the suspension was verified by microscopic analyses. Viability was evaluated by trypan blue exclusion and a roddlike configuration after videomicroscopic images were digitized and the total areas of all and of dead (nonsviable) myocytes planimetered. The area of dead cells was expressed as a percentage of the total area; thus, 1–% dead cells = % viable myocytes. The average viability of cells was 91% (range, 82% to 96%) of 14 images from 4 different experiments.

Endothelin-1 levels were assessed in the myocyte-derived supernatant under basal conditions and during stimulation by phenylephrine with a radioimmunoassay (Amersham). Chemicals and drugs were obtained from the following sources: buffers, salts, taurine, creatine, and adenosine, Sigma Chemical Co; albumin (for microvessel preparations) (crystallized bovine, >98% purity), US Biochemical; albumin (for myocyte isolation), fraction V, Calbiochem; phenylephrine hydrochloride, S(-)-propranolol hydrochloride, prazosin hydrochloride, serotonin hydrochloride, sodium nitroprusside, 8-((p-sulfophenyl)theophylline, and endothelin-1 (peptide free base), Research Biochemicals International; collagenase (type 2), Worthington; and endothelin-A receptor peptide antagonist FR 139317, Abbott Laboratories. All drugs, except prazosin, used for the studies of vasoactivity were dissolved in PSS without albumin to make stock solutions (1 to 10 mmol/L), then divided into aliquots and stored at −20°C. Aliquots were used once and kept on ice during the experiments. Prazosin was initially dissolved in absolute ethanol to make a 10 mmol/L stock, then and kept on ice during the experiments. Prazosin was initially dissolved in absolute ethanol to make a 10 mmol/L stock, then diluted 2-fold in PSS. When the prazosin-ethanol was added to the organ chamber, the final concentration of ethanol in the microvascular organ chamber was 0.1%, which when added as a vehicle did not affect tone.

Vasoactive reactions were assessed in coronary arterioles (n=6 to 9) by addition of the drugs directly to the microvessel organ chamber (2-mL volume) or to the myocyte suspensions, which were apportioned into 1-mL volumes (1.5×10⁷ to 2.0×10⁷ cells). After 20 minutes of incubation with the drug, the myocytes were “pelleted” with gentle centrifugation (500 rpm), and the supernatant was collected and administered to the isolated arterioles (20-μL aliquots).

All results are expressed as mean±SEM. Percent dilation was calculated as (diameter during an intervention minus baseline diameter)/(diameter during maximal dilation with 10⁻⁶ mol/L adenosine minus baseline diameter). Vasoconstriction was calculated as the percent decrease in diameter from baseline and is expressed as −%. The average baseline diameter of all vessels was 58±3 μm, and the maximal dilation was 70±4 μm. Resolution with the ×20 objective was 1 μm. Results were analyzed by 1- or 2-way repeated-measures ANOVA and are presented as mean±SEM. A value of P<0.05 was accepted for statistical significance.

Results

The α₁-adrenergic agonist phenylephrine administered directly to the microvessel did not elicit any change in diameter in either the absence or presence of β-adrenergic blockade (propranolol 10⁻⁶ mol/L). During β-adrenergic blockade, phenylephrine at 10⁻⁶, 10⁻⁵, and 5×10⁻⁵ mol/L produced relaxation of 4±1%, 9±1%, and 10±1%, respectively (n=8). The vessels constricted (20% to 40% decrease in diameter) to endothelin-1 (10⁻¹⁰ mol/L) and dilated near maximally (20% to 30% increase in diameter) to endothelin-independent (adenosine, 10⁻⁶ mol/L; nitroprusside, 10⁻⁵ mol/L) and endothelin-dependent (serotonin, 10⁻⁵ mol/L) agonists, indicating that they were responsive to several agonists. Responses to ET-1 were not completed in every vessel because the vasoconstriction often lasted for 90 to 120 minutes, but we evaluated constriction in 5 vessels to 10⁻¹⁰ mol/L endothelin (-29±4%) and completed a full dose-response relationship to endothelin-1 (n=3; 10⁻¹² mol/L, -6%; 10⁻¹¹ mol/L, -18%; 10⁻¹⁰ mol/L, -29%; 10⁻⁹ mol/L, -48%; and 10⁻⁸ mol/L, -65%).

Administration of the M-D S/N to the microvessel produced vasodilation, which was attenuated by the adenosine antagonist 8-PSPT (50 μmol/L) (Figure 1). This dose of 8-PSPT shifted the dose-response curve to adenosine by 2 log orders (data not shown). These results suggest that the isolated cardiac myocytes are producing vasodilatory quantities of adenosine.

Supernatant (20 μL) from phenylephrine-treated myocyte suspensions (10⁻⁶, 10⁻⁵, and 5×10⁻⁵ mol/L) produced less vasodilation as the dose of phenylephrine was increased (Figure 2). Administration of 8-PSPT to the microvessel preparation unmasked dose-dependent vasoconstriction of the supernatant from the phenylephrine-treated myocytes (Figure 2). The constriction lasted for >90 minutes. The constricted microvessels dilated maximally to serotonin (10⁻⁵ mol/L) or nitroprusside (10⁻⁵ mol/L), demonstrating that the progressive constriction was not due to deterioration of the preparations.

Administration of the α₁-adrenergic antagonist prazosin (10⁻⁶ mol/L) to the myocyte suspension before administration of phenylephrine inhibited the production of contractile

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**Selected Abbreviations and Acronyms**

- ETₐ = endothelin-A receptors
- M-D S/N = myocyte-derived supernatant
- 8-PSPT = 8-(p-sulfophenyl)theophylline
- PSS = physiological salt solution

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**Figure 1.** Effects of myocyte-derived supernatant obtained under control conditions (control; n=8) on arteriolar dilation and administered after adenosine antagonism with 50 μmol/L 8-PSPT (n=8), which was added to microvessel chamber (refer to inset showing route of administration of substance; this format will be used in subsequent figures). Dilation to myocyte-derived supernatant was significantly attenuated by 8-PSPT. Baseline diameters were 58±3 μm (control) and 58±5 μm (8-PSPT).
factor(s) by the cardiac myocytes (Figure 3). Prazosin given directly to the microvessels did not alter the vasoconstrictive properties of the supernatant (n=6). The ETα antagonist FR 139317, 10 μmol/L, given to the arteriole blocked the vasoconstrictive properties of the supernatant from phenylephrine-treated cardiac myocytes (Figure 3). This dose of antagonist blocked vasoconstriction to 100 pmol/L endothelin-1 but did not alter baseline tone or affect vasodilation to adenosine or serotonin, suggesting antagonist specificity at this dose.

The control level of ET-1 in the supernatant was 66±10 pmol/L, and it increased to 102±18, 104±12, and 118±12* pmol/L (**P<0.05 versus control) during stimulation with 10−6, 10−5, and 5×10−5 mol/L phenylephrine, respectively.

### Discussion

We have found that cardiac myocytes exert a requisite role in α1-adrenergic coronary arteriolar constriction. The results also suggest that the coronary constriction is mediated, in part, by endothelin(s), because the ETα antagonist blocked the constrictor properties of the supernatant. However, we cannot make unequivocal assertions about the role of endothelin-1, because its concentration in the supernatant from phenylephrine-treated myocytes would be below threshold after the dilution of the supernatant in the bath (1:100) is considered. It is important to highlight some considerations that are pertinent to our results and conclusions.

Can we eliminate the possibility that our in vitro microvessel (arteriole) preparations have been injured, rendering them unresponsive to α1-adrenergic activation? The failure of isolated arterioles is not related to a more general problem with receptor coupling. Agonists signaling through G protein–coupled receptors include α1-adrenergic agonists,18 angiotensin II,19 endothelin-1,20 and U44619 (thromboxane mimetic),21 and coronary arterioles directly respond to all of these factors except α1-adrenergic agonists. Furthermore, isolated coronary venules and skeletal muscle arterioles constrict during α1- or α1-adrenergic activation, demonstrating that our experimental procedures do not eliminate these adrenergic responses.8 We believe that our preparations have not produced an artifact but rather that isolated coronary arterioles do not respond directly to α1-adrenergic activation.

What is the physiological significance of endothelin-1 production by cardiac myocytes? This is a puzzling aspect of our results. We found that phenylephrine treatment increased endothelin-1 levels in the myocyte-derived supernatant to >100 pmol/L. Because the supernatant was diluted 100-fold in the organ chamber, the final concentration of endothelin would be below threshold to elicit vasoconstriction. Yet, we found that the constriction induced by the supernatant of phenylephrine-treated myocytes was blocked by the ETα antagonist. Our best reconciliation between these seemingly disparate results is to hypothesize that during α1-adrenergic activation, cardiac myocytes are producing constrictors other than endothelin-1. It is possible that other endothelins that would be blocked by ETα antagonist are produced by the myocytes. Alternatively, endothelin-1 could have a "permissive" effect on or synergize the action of another vasoconstrictor. Even though our dissimilar results preclude an unequivocal conclusion about the role of endothelin-1 in mediating the constriction, we believe that our most important observation is that cardiac myocytes have a requisite role in mediating α1-adrenergic coronary arteriolar vasoconstriction.

The present findings also corroborate a preliminary observation we made; specifically, phenylephrine-induced coro-
Cardiac Myocyte–Arteriolar Interaction

Coronary vasoconstriction of epicardial coronary arterioles in situ was inhibited by an ET\(_\alpha\) antagonist.\(^2\) This observation provides compelling evidence that our in vitro observations extend to the intact coronary circulation. Although we cannot state unequivocally that endothelin-1 is the single causal factor, our results imply that activation of ET\(_\alpha\) receptors may be involved as physiological effectors of coronary arteriolar constriction during \(\alpha_1\)-adrenergic activation of cardiac myocytes. We conclude with conviction that cardiac myocytes exert a requisite role in \(\alpha_1\)-adrenergic constriction of coronary resistance vessels.

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References

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