Regulation of coronary vascular tone via redox modulation in the α1-adrenergic-angiotensin-endothelin axis of the myocardium

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Yamaguchi O. Kaneshiro T. Saitoh S. Ishibashi T. Maruyama Y. Takeishi Y. Regulation of coronary vascular tone via redox modulation in the α1-adrenergic-angiotensin-endothelin axis of the myocardium. Am J Physiol Heart Circ Physiol 296: H226–H232, 2009. First published November 21, 2008; doi:10.1152/ajpheart.00480.2008.—We hypothesized that α1-adrenoceptor stimulation of cardiac myocytes results in the production of an endothelin (ET)-releasing factor that stimulates the coronary vasculature to release ET and, by manipulating the redox state of cardiac and vascular cells, may influence the extent of α1-adrenergic-ET-1 vasoconstriction. Dihydroethidium (DHE) and dichlorodihydrofluorescein (DCF) intensities were increased by phenylephrine stimulation in isolated rat cardiac myocytes, which were enhanced by the mitochondrial electron transport chain complex I inhibitor rotenone (DHE: 20.4 ± 1.2-fold and DCF: 25.2 ± 0.9-fold, n = 8, P < 0.01, respectively) but not by the NADPH oxidase inhibitor apocynin. Olmesartan, an angiotensin II type 1 receptor antagonist, and enalaprilate did not change DHE and DCF intensities by phenylephrine. Next, we measured the vasoconstriction of isolated, pressurized rat coronary arterioles (diameter: 74 ± 8 μm) in response to supernatant collected from isolated cardiac myocytes. The addition of supernatant from phenylephrine-stimulated myocytes to a 2-mL vessel bath (n = 8 each) caused volume-dependent vasoconstriction (500 μL: −14.8 ± 2.2%; Olmesartan and TA2021, an ET type A receptor antagonist, converted vasoconstriction into vasodilation (8.5 ± 1.2% and 10.5 ± 0.5%, P < 0.01, respectively) in response to supernatant from phenylephrine-stimulated myocytes, which was eliminated with catalase. Vasoconstriction was weakened using supernatant from phenylephrine with rotenone-treated myocytes. Treatment of arterioles with apocynin to myocyte supernatant converted vasoconstriction into vasodilation (7.8 ± 0.8%, P < 0.01). These results suggest that α1-adrenergic stimulation in cardiac myocytes produces angiotensin I and H2O2 and that angiotensin releases ET-1 through NADPH oxidase in coronary arterioles. Thus, coronary vascular constriction via the α1-adrenergic-angiotensin-ET axis appears to require redox-mediated signaling in cardiac and vascular cells.

α1-adrenoceptor; coronary vasomotion; reactive oxygen species

ENDOTHELIN (ET) is an endogenous factor that produces robust and long-lasting coronary vasoconstriction (8, 9, 28). Therefore, it is imperative that the release and/or production of ET by coronary and myocardial cells are carefully regulated. Although the principle driving force for the control of coronary blood flow is the myocardial metabolism, this intimate relationship between cardiac metabolism and coronary blood flow may be modified by a variety of conditions and stimuli (5). This is especially important for vasoconstrictor influences, which can potentially compromise myocardial perfusion and thereby impair cardiac function. In fact, ET is involved in coronary vasoconstriction-induced myocardial ischemia.

The sympathetic nervous system plays an important role in matching myocardial oxygen supply to myocardial oxygen demand through the influence of norepinephrine release from the terminal nerve endings on the coronary vasculature. Norepinephrine can cause α-adrenergic vasoconstriction as well as β-adrenergic vasodilation in various species (4, 6). In a previous study (16), we showed that, unlike in other organs, α1-adrenergic constriction in the coronary circulation is not mediated via the activation of α1-adrenoceptors on vascular myocytes. Rather, α1-adrenoceptor agonists activate the receptor on cardiac myocytes, and the resulting coronary constriction is mediated through ET-1 (4). Moreover, it has been reported that adult cardiac myocytes do not express the gene for proET-1, the precursor peptide for ET (22), and direct measurement of the ET-1 concentration in the supernatant of phenylephrine (PE)-treated myocytes yields concentrations below the vasoactive threshold. α1-Adrenergic activation in vivo causes constriction of coronary arterioles, whereas in the in vitro state, these microvessels do not contract under this stimulation (27).

On the basis of these observations, we hypothesized that cardiac myocytes produce an ET-releasing factor, and the release of this factor occurs during α1-adrenergic stimulation of cardiac myocytes, which induces ET release by vascular endothelial cells and causes vasoconstriction.

However, two unsolved problems remain. First, the ET-releasing factor in α1-adrenergic stimulated cardiac myocytes is unknown. Because Winegrad et al. (29) identified angiotensin as the ET-releasing signal in cardiac myocytes against hypoxia, we hypothesized that α1-adrenergic stimulation releases angiotensin in cardiac myocytes, which stimulates the release of ET-1 in coronary arterioles. Second, the role of ROS in the α1-adrenergic-ET-1 axis is unclear. Recent reports have shown that angiotensin stimulates membrane-bound NADPH oxidase, which generates ROS within vascular endothelial cells (23), and ROS mediates ET-1 gene induction in vascular endothelial cells (2). In addition, it has been reported that the generation of ROS by PE is rapid in cardiac myocytes (1). In most cell types, superoxide (O2•−) is rapidly converted by SOD to H2O2. In cultured vascular smooth muscle cells and cardiac myocytes, O2•− as well as H2O2 mediates the myogenic effects of angiotensin (30). Recently, we reported that an increase of cardiac metabolism upregulates O2•− and H2O2.

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production in cardiac myocytes, which is vasoactive, and that the mitochondrial electron transport chain is the main source in the production of $O_2^{•-}$. Thus, we hypothesized that ROS have an important role in α1-adrenergic-ET-1 axis vasoconstriction. The aim of this study was to test these two above-mentioned hypotheses.

MATERIALS AND METHODS

This investigation conformed to the Guidelines on Animal Experiments of Fukushima Medical University, the Japanese Government Animal Protection and Management Law (no. 105), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

Adult male Wistar rats weighing 148 ± 8 g from Japan SLC (Shizuoka, Japan) were used for experiments on isolated arterioles and isolated cardiac myocytes. Rats were anesthetized with pentobarbital (Shizuoka, Japan) were used for experiments on isolated arterioles and hearts were detached from the perfusion apparatus and placed in a NaOH) containing (in mM) 10 HEPES, 30 taurine, 113 NaCl, 4.7 KCl, 0.6 KH$_2$PO$_4$, 0.6 Na$_2$HPO$_4$, 1.2 MgSO$_4$, 0.032 phenol red, 12 NaHCO$_3$, and 10 KHCO$_3$. After the blood had been rinsed out and contractility had ceased, the perfusion was switched to a buffer containing the above constituents along with 0.5 mg/ml type II collagenase (Worthington, Lakewood, NJ), 0.14 mg/ml trypsin, and 12.5 μM CaCl$_2$. After perfusion of the heart for 10–12 min and identification of isolated myocytes in the perfusate from the heart, hearts were detached from the perfusion apparatus and placed in a "stop" solution containing the perfusion buffer with 10% BSA (Sigma Chemical (St. Louis, MO). TA0201, an ET type A (ET$_A$) receptor antagonist, was kindly donated by Tanabe Chemical (Tokyo, Japan).

Isolation of cardiac myocytes. Cardiac myocytes were enzymatically isolated from rat hearts with a modified Langendorff setup (21). After the excision of the heart, the aorta was cannulated, and the preparation was suspended in a perfusion apparatus. The left ventricle was initially perfused (retrograde from the aorta) at 37°C with oxygenated, Ca$^{2+}$-free HEPES buffer (titrated to pH 7.45 with 5 M NaOH) containing (in mM) 10 HEPES, 30 taurine, 113 NaCl, 4.7 KCl, 0.6 KH$_2$PO$_4$, 0.6 Na$_2$HPO$_4$, 1.2 MgSO$_4$, 0.032 phenol red, 12 NaHCO$_3$, and 10 KHCO$_3$. After the blood had been rinsed out and contractility had ceased, the perfusion was switched to a buffer containing the above constituents along with 0.5 mg/ml type II collagenase (Worthington, Lakewood, NJ), 0.14 mg/ml trypsin, and 12.5 μM CaCl$_2$. After perfusion of the heart for 10–12 min and identification of isolated myocytes in the perfusate from the heart, hearts were detached from the perfusion apparatus and placed in a "stop" solution containing the perfusion buffer with 10% BSA (Sigma Chemical) and 12.5 μM Ca$^{2+}$. The heart was minced into small pieces, which were further triturated in the stop buffer. After microscopic confirmation of the presence of myocytes, cells were filtered and placed in a 50-ml conical tube. CaCl$_2$ was added in a series of four steps to arrive at a final concentration of 1 mM. Cells were pelleted by centrifugation (1,500 rpm) for 5 min, and the supernatant was discarded. Cells were resuspended in stop buffer with Ca$^{2+}$ and small aliquots were then used for cell counts (hemocytometer) to enable dilution or concentration (via centrifugation) to a final concentration of 100,000 cells/ml. Viability of the myocytes was determined by trypan blue exclusion and rod-shaped configuration. On average, >80% of the cells exhibited a rod-like configuration.

Isolation of coronary arterioles. Single arterioles (passive diameter: 42–94 μm) were dissected from the left ventricular free wall or the septum of the rat heart as previously described (17, 27) and placed in ice-cold PSS of the following composition (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl$_2$, 1.17 MgSO$_4$, 1.2 Na$_2$HPO$_4$, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS with 1% BSA. The solution was buffered to pH 7.4 at 4°C. The heart was placed under a dissection microscope in a 4°C chamber, and each arteriole with the surrounding ventricular muscle was carefully excised, transferred to a temperature-controlled dissection dish (4°C) containing PSS, and dissected free of the muscle tissue. The vessel was transferred to a lucite chamber and cannulated at both ends using micropipettes (outer diameter: 20–60 μm, depending on the size of the vessel) that had matched resistances. The arteriole was tied to each pipette using 11-0 suture. The preparation was then transferred to the stage of an inverted microscope.

Leaks were assessed by measuring pressure at zero flow, which should equal pressure in the inflow reservoir pressure, in the absence of leaks. Any preparations with leaks were excluded. The height of these reservoirs was set to obtain the desired intraluminal pressure (60 mmHg). Vessels that failed to maintain pressure were excluded from analysis. The internal diameter of each coronary arteriole was measured under a charge-coupled device camera (Olympus) with a computer caliper system. The vessel was slowly warmed to 37°C and allowed to develop spontaneous tone.

In vitro measurement of myocyte $O_2^{•-}$ and $H_2O_2$ production. In isolated myocytes, ROS production was measured by dihydroethidium (DHE) staining (10 μM) and dichlorodihydrofluorescein (DCF) staining (10 μM). Isolated cardiomyocytes were plated on a slide glass and incubated for 5 min at 37°C with DHE or DCF. To assess the distribution of DHE or DCF, cardiomyocytes were scanned by an Olympus IX71 inverted microscope. We measured DHE or DCF fluorescence intensities in 20–24 isolated myocytes from each heart and then averaged the values.

Isolated cardiac myocyte ROS production was measured in the quiescent state of myocytes and a 20-min PE (50 μM)-stimulated state. In addition, to examine the effect of angiotensin on ROS generation, myocytes were treated with olmesartan (1 μM), an angiotensin II type I receptor antagonist, and enalaprilat (1 μM), an angiotensin-converting enzyme inhibitor. After 20 min of incubation, the supernatant was discarded, and myocytes were then stimulated for 20 min with PE and stained with DHE and DCF. To examine the source of ROS generation, myocytes were stimulated with apocynin (0.3 mM), a NADPH oxidase inhibitor, and rotenone (2 μM), a mitochondrial electron transport chain complex I inhibitor. To separate $O_2^{•-}$ and $H_2O_2$ produced from myocytes, myocytes were stimulated with tempol (10 mM), a SOD mimetic, and catalase (10,000 U/ml), a catalytic dismutase for $H_2O_2$. After 20 min, the supernatant was discarded, and myocytes were then stimulated with PE for 20 min and stained with DHE and DCF.

We measured the level of $H_2O_2$ using an electrochemical detection system (Apollo 4000, WPI) from 0.5-ml aliquots of the supernatant derived from the suspensions of cardiac myocytes. Samples of the supernatant were obtained from the quiescent state of myocytes and the 20-min PE (50 μM)-stimulated state and after pretreatments of rotenone (2 μM), tempol (10 mM), or catalase (10,000 U/ml) in the PE-stimulated state. Each electrode was calibrated using serial dilutions of $H_2O_2$, and the current recorded from the supernatant was then calculated as $H_2O_2$ concentration (24).

Changes in coronary arteriolar diameter with vasoactive factors from cardiac myocytes. The supernatant of cardiac myocytes was added to isolated coronary arterioles to establish the effects on vessel diameter. Aliquots of myocyte supernatant (100, 200, and 500 μl) with quiescent and PE-stimulated myocyte supernatant were added to the vessel bath. Vascular diameter was measured at 5 min after the addition of supernatant and after a 10-min washout. The addition of the supernatant (500 μl) was repeated in the presence of olmesartan (1 μM), enalaprilat (1 μM), the ET$_A$ receptor antagonist TA0201 (1 μM), and apocynin (3 μM). To examine the effect of $H_2O_2$ from PE-stimulated cardiac myocytes, catalase (10,000 U/ml) with olmesartan and catalase with TA0201 were added to the vessel bath, and changes in the vasomotor tone with the supernatant of PE-stimulated cardiac myocytes were then measured. To determine the effect of $O_2^{•-}$ or $H_2O_2$ released from cardiac myocytes to the vessel separately, the vascular response to the supernatant (500 μl) from PE-stimulated myocytes treated with tempol (10 mM) or catalase (10,000 U/ml) was examined. Moreover, to determine the effect of angiotensin or $H_2O_2$ released from cardiac myocytes to the vessel separately, the vascular response to the supernatant (500 μl) from PE-stimulated myocytes treated with rotenone (2 μM) was examined in the presence of olmesartan (1 μM) and catalase (10,000 U/ml) in the vessel bath separately.
Measurements of ET-1 in the vessel bath. Plasma ET-1 levels in the vessel bath were determined by a radioimmunoassay using an I\textsuperscript{125}-labeled ET-1 assay system (Amersham) (15).

Data analysis and statistics. All statistical analyses were performed with StatView software (Abacus Concepts, Berkeley, CA). Data from the experiments were compared using repeated-measures ANOVA with Scheffe’s test as a post hoc multiple-comparison test. Vascular diameters were normalized to the diameter with tone before the administration of the supernatant. Data are expressed as means ± SE; N is the number of myocytes and n is the number of measured samples. Significance was accepted at P < 0.05 in all experiments.

RESULTS

Production of O\textsuperscript{2−} and H\textsubscript{2}O\textsubscript{2} in cardiac myocytes. DHE and DCF signal intensities increased in PE-stimulated myocytes (Figs. 1 and 2, respectively). DHE oxidation in PE-stimulated/quiescent myocytes was 4.2 ± 0.4 (N = 8 myocytes and n = 160 measured samples). DCF oxidation in PE-stimulated/quiescent myocytes was 5.3 ± 0.6 (N = 8 myocytes and n = 182 measured samples). Tempol decreased DHE signal intensities [DHE oxidation in PE-stimulated/quiescent myocytes: 1.2 ± 0.2 (N = 8 myocytes and n = 180 measured samples, P < 0.01 vs. without tempol)] and increased DCF signal intensities [DCF oxidation in PE-stimulated/quiescent myocytes: 24.2 ± 2.2 (N = 8 myocytes and n = 186 measured samples, P < 0.01 vs. without tempol)]. Catalase decreased DCF signal intensities [DCF oxidation in PE-stimulated/quiescent myocytes: 0.92 ± 1.2 (N = 8 myocytes and n = 160 measured samples, P < 0.01 vs. without catalase)] but did not change DHE signal intensities. Apocynin did not change DHE and DCF signal intensities compared with its absence in PE-stimulated myocytes [DHE oxidation in PE-stimulated/quiescent myocytes: 4.6 ± 0.6 and DCF oxidation in PE-stimulated/quiescent myocytes: 4.8 ± 0.8 (N = 8 myocytes and n = 172 measured samples each)]. In contrast, the effects of PE were enhanced by rotenone [DHE oxidation in PE-stimulated/quiescent myocytes: 20.4 ± 1.2 and DCF oxidation in PE-stimulated/quiescent myocytes: 25.2 ± 0.9 (N = 8 myocytes and n = 168 measured samples, P < 0.01, respectively)]. Olmesartan and enalaprilate did not change DHE and DCF intensities in PE-stimulated myocytes.
creased the H$_2$O$_2$ concentration to 4.6 μM in supernatant from phenylephrine-stimulated cardiac myocytes. Data are shown as means ± SE; N = 8 each.

From these results, we speculated that coronary arteriolar vasoconstriction induced by α$_1$-adrenoceptor-stimulated cardiac myocytes is modulated by the generation of ROS in the cardiac myocyte mitochondrial electron transport chain complex I pathway.

**Plasma concentrations of ET-1 in the vessel bath.** Although plasma levels of ET-1 were not detected in coronary arterioles with nonexposure of myocyte supernatant, after exposure of supernatant from PE-stimulated myocytes, plasma levels of ET-1 increased 0.08 ± 0.04 pg/ml after exposure of supernatant from PE-stimulated myocytes (N = 8, P < 0.01).

**DISCUSSION**

Our study reports new findings that bear on the understanding of the mechanisms by which products of myocardial α$_1$-stimulation, mimicking myocardial ischemia, modulate coronary arterial tone.

First, α$_1$-adrenergic cardiac myocyte stimulation releases angiotensin and H$_2$O$_2$, which depend on mitochondrial ROS generation. We have previously reported that ET-1 is produced in coronary microvessels in response to a chemical signal from cardiac myocytes and that this signal is modified by α$_1$-adrenergic stimulation of cardiac myocytes, which implies that these cells produce a substance or substances that stimulate the...
Production of ET by coronary arterioles (17). We identified angiotensin I as a cardiac myocyte-releasing factor that stimulates the vascular production of ET. In our experiments with supernatant from quiescent myocytes, myocytes did not seem to release angiotensin I or II, because exposure of olmesartan or enalaprilate to coronary arterioles did not alter the vasoreactive properties of the supernatant. On the other hand, when myocytes were stimulated with PE, vasoconstriction of coronary arterioles to the supernatant, which was reversed by pretreatment of coronary arterioles with olmesartan or enalaprilate, was observed. This phenomenon indicates that PE-stimulated myocytes indeed release angiotensin I, which was changed to angiotensin II with angiotensin-converting enzyme in vessels. Because the vascular response to α1-adrenergic receptors depends on mitochondrial ROS generation (10), and our previous report indicated that ROS production by cardiac myocyte mitochondria modulates coronary artery tone (24), we examined the relationship of α1-adrenergic stimulation of cardiac myocyte and mitochondria ROS generation. ROS could be produced by many different pathways in addition to the mitochondrial pathway, such as NADPH oxidase and xanthine oxidase as well as TNF-mediated pathways in patients with heart failure (12, 26). In this study, ROS generation, which was determined by histofluorescence in PE-stimulated cardiac myocyte, was not changed by an angiotensin II type 1 receptor antagonist, an angiotensin-converting enzyme inhibitor, or a NADPH oxidase inhibitor; however, it was increased by the addition of the mitochondrial electron transport chain complex I inhibitor rotenone. Surprisingly, vasoconstriction was changed by the supernatant of cardiac myocytes stimulated with PE and ROS modulators such as rotenone or tempol. The SOD mimic tempol decreased O$_{2}^{-}$ and increased H$_{2}$O$_{2}$ in cardiac myocytes; as a result, tempol changed vasoconstriction to vasodilation after PE-stimulated myocyte supernatant exposure, probably due to a weaken angiotensin effect and an augmentation of the H$_{2}$O$_{2}$ vasodilative effect. On the other hand, decrease of the H$_{2}$O$_{2}$ effect by catalase treatment in cardiac myocytes augmented vasoconstriction with PE-stimulated myocytes supernatant. Moreover, vasodilation changed from vasoconstriction after treatment with the angiotensin II type 1 receptor antagonist or angiotensin-converting enzyme inhibitor after PE-stimulated cardiac myocyte supernatant was eliminated with catalase. These results suggest that α1-stimulation of cardiac myocyte generated O$_{2}^{-}$, which mediates angiotensin I release as
well as converts H$_2$O$_2$, which is metabolic vasodilator (24). From our study, rotenone treatment of cardiac myocytes increased the level of H$_2$O$_2$ and presumably increased angiotensin I by augmentation of O$_2^{•−}$ stimulation. We also determined vascular responses to supernatant from myocytes treated with rotenone in the presence of olmesartan or catalase separately. Treatment with rotenone in PE-stimulated cardiac myocytes augmented not only the release of H$_2$O$_2$ as a vasodilative component but also as vasoconstrictive component. As the net effect of the two components, rotenone weakened vasoconstriction but not vasodilation with PE-stimulated cardiac myocyte supernatant because the degree of the augmentation of the vasoconstrictive effect may be slightly more potent. However, further study is needed to clarify this mechanism. Although the level of angiotensin I was not determined, angiotensin I released from cardiac myocyte is converted to the active angiotensin II form at the blood vessel. Therefore, direct confirmation that angiotensin II causes ET release in coronary arterioles is important. However, we were unable to measure the levels of angiotensin production by cardiac myocytes. As the reason, we speculate that the angiotensin concentration might be too low to measure. However, there are several reports that have shown that cardiac myocytes release angiotensin in the rat or cat (3, 29). Moreover, previous studies have indicated that angiotensin II can turn on the synthesis of ET-1 in different vascular cell types, including cultured vascular smooth muscle cells (18) and rat vascular endothelial cells (20). Winegrad et al. (29) reported that angiotensin II can stimulate endothelial cells to secrete ET, and, in some tissues, the vasoconstricting effect from the ET released can be substantially greater than the direct effect of angiotensin on the vascular smooth muscle. Nishida et al. (19) reported that angiotensin II increases prepro-ET mRNA in rat coronary microvascular endothelial cells in culture. In addition, Hong et al. (11) reported that ROS mediate angiotensin II-induced ET-1 gene expression in vascular smooth muscle cells. Therefore, we speculate that even though the angiotensin concentration was too low to measure, its concentration was sufficient to stimulate the release of ET but not sufficient to produce direct vasoconstriction. Thus, angiotensin operates through an amplification system that is spatially sharply focused on the vascular smooth muscle. However, further study is needed.

To our knowledge, the mechanism by which ROS releases angiotensin I may well be a new finding, although several reports have shown that angiotensin II generates ROS through the NADPH oxidase pathway (13, 14). However, further study is needed to clarify this mechanism.

Second, vasoconstriction with PE-stimulated cardiac myocyte supernatant was decreased by treatment of vessels with apocynin as well as the ETA antagonist, with the latter added to vessels. Aiello et al. (3) showed that angiotensin II stimulates NADPH oxidase in vessels. From these results, angiotensin II-induced ROS through NADPH oxidase are mediators to release ET-1 in coronary arterioles (Fig. 7).

Methodological considerations. We used an in vitro system to investigate the regulation of ET-1 production by studying enzymically isolated cardiac myocytes and isolated coronary arterioles (17). A potential problem is that enzymatic isolation damages or even kills cells. We minimized the problem of dead cells by using only preparations with >80% rod-shaped cells. There is the possibility that dead cells may release vasoactive substances, which could complicate our findings. However, supernatant from preparations with mainly dead cells was not vasoactive (data not shown). Therefore, we consider that the responses to the supernatant involve vasoactive substances released by viable cardiac myocytes. The main advantage of our in vitro system is that we can distinguish the production of factors by cardiac myocytes from those by vascular cells. Many substances are both vasoactive and cardioactive because their receptors are present on both vascular cells and cardiac myocytes. With our system, we can block the receptors on the vasculature without affecting myocytes, and vice versa. This is especially important for the ETA receptor, which, when stimulated, causes vasoconstriction as well as an increase of inotropy of myocytes (7), which, in turn, may increase both the production of vasodilators and extravascular compression. Thus, the administration of an ETA receptor antagonist in vivo also has indirect effects on the vasculature and may therefore result in overestimation of the true vascular effects of ET-1 when tested in vivo.

Clinically, our findings are important in patients with heart failure. Heart failure results in increased sympathetic nervous system activity (25), aimed at maintaining blood pressure, through an increase in heart rate and peripheral vasoconstriction to ensure adequate perfusion of the brain. However, the enhanced sympathetic stimulation of cardiac myocytes will also result in enhanced ET-1 production by the coronary vasculature. Moreover, because heart failure is often accompanied by endothelial dysfunction, nitric oxide production is decreased, which may result in inadequate negative feedback on vascular ET-1 production. Thus, coronary blood supply may be limited by an increased ET-1-induced vasoconstriction, whereas myocardial oxygen demand is increased because of the increased workload of the heart.

Conclusions. In summary, we show that α$_1$-adrenergic cardiac myocyte stimulation releases angiotensin, which stimulates release of ET-1 in coronary arterioles, and that the mitochondrial electron transport chain complex I in cardiac
myocytes and NADPH oxidase in coronary arterioles plays important roles in this α₁-adrenergic-ET-1 axis vasoconstric-
tion.

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