Metabolic regulation of coronary vascular tone: role of endothelin-1

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Received 13 March 2002; accepted in final form 14 July 2002

Merkus, Daphne, Dirk J. Duncker, and William M. Chilian. Metabolic regulation of coronary vascular tone: role of endothelin-1. Am J Physiol Heart Circ Physiol 283: H1915–H1921, 2002. First published July 18, 2002; 10.1152/ajpheart.00223.2002.—Coronary tone is determined by a balance between endogenously produced endothelin and metabolic dilators. We hypothesized that coronary vasodilation during augmented metabolism is the net result of decreased endothelin production and increased production of vasodilators. Isolated rat myocytes were stimulated at 0, 200, and 400 beats/min to modify metabolism. Supernatant from these preparations was added to isolated coronary arterioles with and without blocking vasoactive pathways (adenosine, bradykinin, and endothelin). Chronically instrumented swine were studied while resting and running on a treadmill before and after endothelin type A (ETα) receptor blockade. The vasodilatory properties of the supernatant increased with increased stimulation frequencies. Combined blockade of adenosine and bradykinin receptors abolished vasodilation in response to supernatant of stimulated myocytes. ETα blockade increased vasodilation to supernatant of unstimulated myocytes but did not affect dilation to supernatant of myocytes stimulated at 400 beats/min. In vivo, ETα blockade resulted in coronary vasodilation at rest, which waned during exercise. Thus endothelin has a tonic constrictor influence through the ETα receptor at low myocardial metabolic demand but its influence decreased during increased metabolism.

CORONARY BLOOD FLOW is tightly coupled to myocardial oxygen consumption (MV02), a process termed metabolic dilation. Despite the seminal importance of metabolic dilation in titrating flow to changes in metabolism, it is incompletely understood how cardiac myocytes communicate a change in metabolic activity to the coronary vasculature. One consensus is that cardiac myocytes produce vasodilatory substances, such as adenosine, nitric oxide (NO), prostacyclin, bradykinin, and CO2 in direct relation to MV02 (3, 7–9). In addition to the production of dilators, cardiac myocytes also produce constrictors, such as angiotensin II (6).

Moreover, we have evidence to suggest that cardiac myocytes produce substances that induce endothelin-mediated constriction of the coronary vasculature. Specifically, we (1, 17) previously found that α-adrenergic stimulation of cardiac myocytes resulted in endothelin-dependent vasoconstriction both in vivo and in vitro. A recent study showed that cardiac myocytes in vitro respond to changes in PO2 by altering the balance of vasoactive factors that they secrete. In quiescent noncontracting myocytes, an increased PO2 results in decreased production of dilatory substances (including adenosine) and an increased production of a constrictor (22, 23). This constrictor was identified as angiotensin I, which was converted by the vasculature to angiotensin II, which then caused vasoconstriction in an endothelin-dependent manner. We hypothesized that an increase in metabolic activity of cardiac myocytes would decrease the influence of these vasoconstrictive factors and increase the influence of vasodilators. Thus alterations in the production of vasoconstrictors could contribute to metabolic regulation. Therefore, we measured the contribution of endothelin to metabolic regulation both in vitro and in vivo. We first ascertained the contribution of endothelin to regulation of coronary arteriolar diameter in an in vitro preparation, in which isolated cardiac myocytes were electrically stimulated to contract at different rates and aliquots of the fluid bathing the myocytes were administered to isolated coronary arterioles in the presence and absence of an endothelin type A (ETα) receptor antagonist. To understand whether the in vitro results would be applicable to the in vivo setting, coronary hemodynamics were evaluated in chronically instrumented swine running on a treadmill before and after the blockade of endothelin-mediated constriction with an ETα receptor antagonist. Results from our study demonstrate that endothelin contributes to the regulation of vascular tone but the contribution of this constrictor wanes as metabolism increases. Thus it appears that during increases in cardiac metabolism, there is concomitant increased production of vasodilators and decreased production of vasoconstrictors. We propose that regul-
lation of coronary blood flow involves dynamic regulation of productions of constrictors and dilators by cardiac myocytes depending on the myocardial oxygen metabolism.

METHODS

All experimental procedures and protocols used in this study were reviewed and approved by the Animal Care and Use Committees of our institutions and conformed to the "Guiding Principles in the Care and use of Animals" of the American Physiological Society.

In Vitro Experiments

**General procedures.** Male Wistar rats (175–250 g; Harlan Sprague Dawley) were anesthetized with pentobarbital so-
cytes were allowed to settle under gravity between the CaCl2
or the septum of the rat heart, as previously described for

Dissection of coronary arterioles. MV
factors was measured by the withdrawal of the myocyte

Isolation of cardiac myocytes. Myocytes were isolated using
a modified Langendorff setup. Briefly, the heart was perfused with buffer containing (in mM) 123 NaCl, 2.6 KCl, 1.2
H2PO4, 7 MgSO4, 1.2 H2O, 25 HEPES, 11 glucose, 20
taurine, 20 creatine, and 1 CaCl2 (pH 7.4) for 3 min, after
which the buffer was replaced by one with the same compo-
sition but without calcium for 6 min. Collagenase type II (0.6
M, 500
° C and allowed to

In Vivo Experiments

**General preparation.** Seven Landrace × Yorkshire pigs of
either sex were used in the present study. Adaptation of animals to the laboratory conditions started 1 wk before the
day of surgery and continued until 7 days postoperatively.

Other preparations containing at least 70% rod-shaped cells were used.

Myocytes (500,000 cells in 4 ml) were stimulated for 20
min at different rates (0, 200, and 400 beats/min) in a cus-
tom-designed chamber, and their production of vasoactive
factors was measured by the withdrawal of the myocyte
supernatant, which was then added (in a bioassay) to the
isolated arterioles (see below). MVo2 was measured by stim-
ulating myocytes in an airtight chamber and withdrawing
supernatant at fixed time intervals. The supernatant was then injected into an ABL5 automated blood gas analyzer
(Radiometer) and Po2 was measured. Po2 was converted into
MVo2 per myocyte using the following formula

\[
MVo2 = \frac{ao2 \cdot \text{volume} \cdot \Delta\text{Po2}}{760 \cdot \Delta t \cdot \# \text{ cells}}
\]

where aO2 is the oxygen solubility in ml O2 per ml fluid per
atm (equal to 0.0214), 760 is the conversion from atm to
mmHg, volume refers to the volume of the chamber (4 ml),
\(\Delta\text{Po2}\) is the change in Po2 between two measurements, and \(\Delta t\)
is the time between two measurements.

**Dissection of coronary arterioles.** Single arterioles (40–130
\(\mu\)m passive diameter) were dissected from the left ventricle or the septum of the rat heart, as previously described for
other species (11), and placed in ice-cold PSS containing 1%
BSA (USB-Amersham). The PSS was composed of (in mM)
145.0 NaCl, 4.7 KCl, 2.0 CaCl2, 1.17 MgSO4, 1.2 NaH2PO4,
5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 3-(N-morpho-
lino)propanesulfonic acid, buffered to pH 7.4 at 4°C and
filtered (dissection buffer). Unless otherwise mentioned, all
drugs were obtained from Sigma.

The vessels were cannulated at both ends with micropipettes (~20–60 \(\mu\)m outer diameter, depending on the size of the
vessel) connected to pressurized reservoirs filled with
PSS buffered at pH 7.4 at 37°C. The height of these reservoirs was set to obtain the desired intraluminal pressure (60
mmHg). Vessels that failed to maintain pressure were ex-
cluded from analysis. The internal diameter of coronary
microvessels was measured with a videomicroscope (Zeiss
Inverted Scope and Sony CCD-IRIS camera and video cali-
per). The vessel was slowly warmed to 37°C and allowed
to develop spontaneous tone.

**Protocol.** Aliquots of myocyte supernatant (100, 200,
and 500 \(\mu\)l) were added to the vessel bath (4 ml vol). Vascular

diameter was measured 5 min after the addition of the
myocyte supernatant, which was then repeated in the presence of various receptor antagonists in the following concentrations: adenosine receptor
antagonist 8-(parasulfophenyl)theophylline (8PSPT; 50
\(\mu\)M), bradykinin B2 receptor antagonist HOE-140 (1
\(\mu\)M), ETa receptor antagonist JKC-301 (5 \(\mu\)M; American Peptide)
(15, 16, 21), and angiotensin AT1 receptor antagonist losar-
tan (1 \(\mu\)M).

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myocytes; † experiments were compared using ANCOVA with MVware (Abacus Concepts; Berkeley, CA). Data from the in vivo Data Analysis and Statistics the exercise protocol was repeated.

After completing the exercise protocol, the animals were collected when hemodynamics had reached a steady state. Subsequently, the animals ran on a treadmill at 5 km/h until hemodynamic parameters had reached a new level. LV dP/dt, aortic blood pressure, and coronary blood flow were continuously measured and blood samples collected when hemodynamics had reached a steady state. After completing the exercise protocol, the animals were allowed to rest on the treadmill for 90 min, after which the swine received the selective ETA receptor antagonist EMD-122946 [3 mg/kg; a gift from Dr P. Schelling, Merck Darmstadt (12)] infused intravenously over 10 min, and 5 min later swine received the selective ETA receptor antagonist EMD-122946 [3 mg/kg; a gift from Dr P. Schelling, Merck Darmstadt (12)] infused intravenously over 10 min, and 5 min later.

To assess the possible contributions of adenosine and bradykinin in mediating dilation with increased stimulation rate (oxygen metabolism), we performed experiments in the presence and absence of the adenosine receptor antagonist 8PSPT and the bradykinin B2 receptor antagonist HOE-140 (Fig. 2). Administration of 8PSPT and HOE-140 decreased the dilation of the coronary arterioles to the supernatant of myocytes stimulated at 400 beats/min (P < 0.05), whereas a combination of 8PSPT and HOE-140 completely abolished the dilation (Fig. 2) (P < 0.05 vs 400 beats/min).

Administration of JKC-301 to the coronary arterioles increased the vasodilator properties of the supernatant (Fig. 3). This increase was inversely related to the rate of stimulation, i.e., the increased dilation was significantly larger in quiescent myocytes and myocytes stimulated at 200 beats/min than in myocytes stimulated at 400 beats/min (P < 0.05). Thus it appears that the vasoconstrictor effects of endothelin decreased during augmented oxygen metabolism.

Previously, a paradigm for the production of endothelin was found to be related to the production of myocytes were electrically stimulated. The increase in MV\(_2\)O\(_2\) was dependent on contraction rate. Stimulation of myocytes at 100, 200, and 400 beats/min resulted in 20-, 29-, and 41-fold increases in MV\(_2\)O\(_2\), respectively.

Supernatant of cardiac myocytes (either quiescent or stimulated to contract at 200 or 400 beats/min) was added to isolated coronary arterioles, and the vasodilatory response was graded to metabolism (Fig. 1). Supernatant from myocytes stimulated at 200 beats/min caused modest dilation (compared with 0 beats/min) and that from myocytes stimulated at 400 beats/min resulted in a further increase in vasodilator properties of the supernatant (P < 0.05).

During the first week after surgery, the animals received intravenous injections of 25 mg/kg amoxicillin (SmithKline Beecham Pharma) and 5 mg/kg gentamycin (AUV) on a daily basis to prevent infection. The catheters were flushed daily with PSS containing 2,000 IU/ml heparin.

Protocol. Studies were performed 1 to 2 wk after surgery with animals exercising on a motor-driven treadmill. With swine lying quietly on the treadmill, resting hemodynamic measurements consisting of LV dP/dt, aortic blood pressure, left atrial pressure, and coronary blood flow were obtained, and arterial and coronary venous blood samples were collected. Subsequently, the animals ran on a treadmill at 5 km/h until hemodynamic parameters had reached a new stable level. LV dP/dt, aortic blood pressure, and coronary blood flow were continuously measured and blood samples collected when hemodynamics had reached a steady state. After completing the exercise protocol, the animals were allowed to rest on the treadmill for 90 min, after which the swine received the selective ET\(_A\) receptor antagonist EMD-122946 [3 mg/kg; a gift from Dr P. Schelling, Merck Darmstadt (12)] infused intravenously over 10 min, and 5 min later the exercise protocol was repeated.

Data Analysis and Statistics

All statistical analyses were performed on StatView software (Abacus Concepts; Berkeley, CA). Data from the in vivo experiments were compared using ANCOVA with MV\(_2\)O\(_2\) as covariate, whereas data from the in vitro experiments were compared using ANOVA with repeated measures with Scheffe’s test as a post hoc multiple-comparison test. Vascular diameters were normalized to the diameter with tone before administration of the supernatant. Data are presented as means ± SE. Significance was accepted at P < 0.05 in all experiments.

RESULTS

In Vitro Experiments

Quiescent myocytes consumed 23 ± 15 nl O\(_2\)/min per 100,000 cells. MV\(_2\)O\(_2\) increased dramatically when the

![Graph](http://ajpheart.physiology.org/Downloadedfrom)
and stimulated endothelin production, then losartan should have produced a similar effect as JKC-301.

**In Vivo Experiments**

The increase in MV02 during exercise resulted in vasodilation leading to an increase in myocardial oxygen supply (Table 1), whereas myocardial oxygen extraction was unaltered (Fig. 5). Blockade of ETA receptors resulted in an increase in myocardial oxygen supply in excess of MV02 (P < 0.05, Table 1), so that myocardial oxygen extraction was reduced and coronary venous O2 saturation increased (Fig. 5). In support of our hypothesis, the effect of ETA receptor blockade decreased during exercise (P < 0.05).

**DISCUSSION**

The present study demonstrates that (in vitro) endothelin-mediated coronary constriction is initiated by a signal from the cardiac myocytes that induces the release of endothelin from the arteriolar endothelium. Moreover, this vasoconstrictor activity is inversely related to cardiac metabolic activity. These in vitro observations have significance to the intact coronary circulation because in vivo endothelin contributes to the regulation of vascular tone at rest and, to a lesser extent, during exercise. This implies a regulatory scheme for the control of coronary vascular tone that includes a balance between the release of constrictors and dilators, depending on metabolic rate. Before discussing the implications of our findings, we will first compare the in vitro and in vivo results and evaluate the potential pitfalls in both systems.

**Methodological Considerations**

Because both ETA receptor antagonists used in this study are relatively new, we tested in separate experiments if they block the constrictor response to ET-1 (Fig. 6). Both EMD-122946 (20 μg/ml) and JKC-301 (5 μM) completely abolished the endothelin-induced constriction, indicating that the doses we used were sufficient. Also, in experiments from other investigators, JKC-301 has been used as an endothelin antagonist (15, 16), whereas EMD-122946 has been shown to

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**Table 1. Hemodynamics and determinants of myocardial oxygen delivery**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ETA Receptor Antagonist</th>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>127 ± 10</td>
<td>259 ± 7†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>87 ± 3</td>
<td>82 ± 2†</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td>51 ± 5</td>
<td>121 ± 12†</td>
</tr>
<tr>
<td>O2 sat, %</td>
<td>92.3 ± 0.6</td>
<td>91.3 ± 0.8</td>
</tr>
<tr>
<td>MDO2, μmol/min</td>
<td>243 ± 31</td>
<td>616 ± 64†</td>
</tr>
</tbody>
</table>

Values are means ± SE. ETA, endothelin type A; HR, heart rate; MAP, mean arterial pressure; CBF, coronary blood flow; O2 sat, arterial oxygen saturation; MDO2, myocardial oxygen supply. *P < 0.05 vs. corresponding control; †P < 0.05 vs. corresponding rest.
inhibit the pressor response to endothelin both in vivo and in vitro (12, 21).

We found that endothelin-1 contributes to the regulation of coronary vascular tone in vivo. This observation is in accordance with the study by Takamura et al. (14), showing that combined blockade of the ETA and ETB receptors with tezosentan resulted in coronary vasodilation. In our study and that of Takamura et al. (14), the contribution of endothelin-1-mediated constriction decreased during exercise, indicating that metabolic dilation may be the result of increased production of vasodilators and decreased production of endothelin. It is difficult to estimate the contribution of cardiac myocytes to this decreased production of endothelin (via a yet-to-be identified factor that stimulates the release of endothelin from the vascular endothelium) because an increase in MV2 may increase vascular shear stress, thereby augmenting NO production. Increased NO production decreases endothelin release from the vascular endothelium, which could explain our in vivo but not in vitro findings (10). Also, vasodilator substances produced during heightened metabolic demand may directly counteract the vasoconstrictor effect of endothelin, thereby complicating the interpretation of the results. Furthermore, it is difficult to identify the main contributors to metabolic regulation in vivo because metabolic regulation encompasses redundant systems that act in concert to regulate vascular tone; inhibition of one pathway might be compensated by altered output of the others. Unless more pathways are blocked simultaneously, metabolic regulation appears to be maintained (9). Also, changes in production of vasoactive substances are difficult to measure. The endothelium forms a two-way barrier between the bloodstream and the myocardial interstitium, and can actively metabolize and produce vasoactive and cardioactive substances. Therefore, concentrations of vasoactive substances as measured in the coronary sinus do not necessarily reflect concentrations in the interstitium.

In addition, many substances are both vasoactive and cardioactive because their receptors are present both on vascular and cardiac myocytes. This is especially important for the ETA receptor, which, when stimulated, causes vasoconstriction as well as an increase in inotropy of the myocytes (5), which in turn increases the production of vasodilators. The effect of ETA receptor blockade in vivo may therefore underestimate the true vascular effects of endothelin. With our in vitro system, we can block the receptors on the vasculature without affecting the myocytes and vice versa and thus investigate the communication from myocytes to the coronary vasculature (17). In this in vitro system, the majority of the cells are cardiac myocytes; however, other cell types such as fibroblasts and endothelial cells may be present in small amounts, and may therefore contribute to the vasoactive factors secreted into the supernatant. A study by Emerson and Segal (4) showed that in an intact vessel, endothelial cells can respond to electrical activation but the stimulus in that study was of a much higher frequency and magnitude than that used in our study. Moreover, the response of the vessel was local, suggesting a direct effect rather than secretion of vasoactive factors. Electrically stimulated fibroblasts are capable of secreting transforming growth factor (TGF)-β (18). However, the increase in TGF-β is small compared with control conditions. Moreover, we never actually observed fibroblasts in our setup, so if they are present, there are very few of them. The majority of vasoactive factors will therefore be secreted by cardiac myocytes. Compared with the in vivo situation, the in vitro system has both advantages and disadvantages. The main disad-

![Diagram](Image)

**Fig. 6.** The vasoconstrictor response to endothelial-1 (ET-1) \((n = 4)\) was completely abolished by 20 μg/ml EMD-122946 \((n = 6)\) or 5 μM JKC-301 \((n = 4)\). *P < 0.05 compared with ET-1 alone.
vantage is that by removing the myocytes and coronary arterioles from their natural environment, we lose the close proximity between vessels and myocytes. We thereby introduce the possibility that some short-lived vasoactive factors, such as NO and CO, will not be preserved. Furthermore, it is difficult to mimic in vivo concentrations of endogenous substances. The changes in vascular diameter in response to administration of the supernatant are, therefore, qualitative rather than quantitative. However, because we used the same concentration of cardiac myocytes throughout our protocols, it is possible to compare vasoactive substances produced at the different contraction rates.

Another problem is that enzymatic isolation of cardiac myocytes can cause cell death or injury. Although we minimize the number of dead cells in our preparation by using only preparations with 70% or more alive rod-shaped cells, there is the possibility that dead cells may release vasoactive substances that could potentially complicate our findings. However, the supernatant from preparations with mainly dead cells (80% or more) was not vasoactive (data not shown). Therefore, we believe that the responses to the supernatant are the result of vasoactive substances released by viable cells. This conclusion is strengthened by the observation that adenosine and bradykinin are the main factors involved in the vasodilatory response to the supernatant. These factors were shown to be released in vivo and to contribute to regulation of coronary vascular tone (2, 3, 7). The main advantage of the in vitro system is that metabolic activity of the myocytes can be changed independently of coronary blood supply, and alterations in vascular diameter (i.e., in vascular resistance) do not influence the oxygen supply to the myocytes. Thus, in contrast to the in vivo situation, when receptors on the arterioles are blocked in vitro, the oxygen supply to the cardiac myocytes or the metabolism of the myocytes is not influenced. Hence, the effect of the mediators that are released during increased metabolic activity can be blocked without affecting their production. Thus, by combining in vivo and in vitro measurements, a more complete view of the involvement of endothelin in the process of metabolic regulation can be obtained.

**Implications of Our Findings**

Our experiments support the concept that cardiac myocytes, when acting as oxygen sensors in the heart, produce a vasodilator when \( P_{O_2} \) decreases and a vasoconstrictor when \( P_{O_2} \) increases. Although our results suggest the involvement of endothelin, the mechanism for endothelin production is yet unresolved. We do not believe that cardiac myocytes produce endothelin directly because we (17) reported previously that endothelin concentrations in the myocyte supernatant were too low to cause net constriction, despite the observation that the constrictor influence could be unmasked by using an ETA receptor antagonist. Moreover, another recent study (13) maintained that adult cardiac myocytes do not produce endothelin because they do not express preproendothelin. This implies a process involving the production of a factor (or factors) by cardiac myocytes that induces the release of endothelin from the vasculature. Unfortunately, in our experiments we were unable to identify the endothelin-releasing factor secreted by the cardiac myocytes. In contrast to the study mentioned above, blockade of the angiotensin AT1 receptor with losartan failed to enhance the vasodilatory properties of supernatant from quiescent myocytes in our experiments (Fig. 4), whereas subsequent blockade of the ETA receptor did increase the dilation. These differences may be due to different receptor expression on the coronary arterioles in our experiments versus the aorta in the other study. Nevertheless, blockade of the ETA receptor resulted in coronary vasodilation both in vivo and in vitro, indicating that endothelin is involved in determining basal coronary arteriolar tone, and a reduction of its influence does contribute to metabolic regulation.

Conventionally, metabolic regulation of coronary blood flow is thought of in terms of vasodilation, via the production of vasodilators in response to increases in myocardial work. The vasodilator pathways have, therefore, been the main focus of attention. Adenosine, bradykinin, and NO have each been reported to contribute to metabolic dilation under control conditions or when one or more pathways are blocked (3), although the role of especially adenosine remains controversial (7–9, 19, 20). Interestingly, in our in vitro experiments also suggest that release of adenosine and bradykinin contributes to metabolic dilation of the arterioles. For dilation to occur, it is implicit that intrinsic vasomotor tone is present. Also, when myocardial work decreases to basal levels coronary flow needs to return to baseline as well. Thus if coronary flow regulation is considered to be a dynamic control system, the advantages of having both vasodilators and vasoconstrictors involved become obvious. This way, changes in vascular tone can be fine tuned more quickly by involving simultaneous augmented production of vasodilators and decreased production of vasoconstrictors during elevations in metabolism. Conversely, during decreases in metabolism, rather than waiting for vasodilator influences to dwindle, myocytes can actually actively increase vascular tone and thereby actively decrease coronary blood flow as \( \dot{MVO}_2 \) decreases.

In conclusion, because endothelin is a very potent and long-lasting vasoconstrictor, its production and release need to be carefully regulated. Therefore, the myocytes, which are the beneficiaries of coronary blood flow, are given the ability to fine tune the production of endothelin according to their metabolic status.

This study was supported by American Heart Association Grant 9920433Z, National Heart, Lung, and Blood Institute Grants HL-32788 and HL-65203, and by The Netherlands Heart Foundation Grants 2000D038 and 2000D042.

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