Self-Protection by Cardiac Myocytes Against Hypoxia and Hyperoxia

Saul Winegrad, Daniel Henrion, Lydie Rappaport, Jane Lise Samuel

Abstract—Cardiac muscle must maintain a continuous balance between its energy supply and work performed. An important mechanism involved in achievement of this balance is cross talk via chemical signals between cardiac myocytes and the cardiac muscle vascular system. This has been demonstrated by incubating isolated cardiac myocytes in different concentrations of oxygen and then assaying the conditioned media for vasoactive substances on isolated aortic rings and small-resistance arteries. With increasing oxygen concentrations above 6%, cardiac myocytes produce increasing amounts of angiotensin I, which is converted to angiotensin II by the blood vessel. The angiotensin II stimulates vascular endothelial cells to secrete endothelin and increase vascular tone. Below 6% oxygen, cardiac myocytes secrete adenosine, which acts directly on vascular smooth muscle to block the effect of α-adrenergic agonists and reduce vascular tone. In an intact heart, the net effect of these 2 regulatory systems would be the maintenance of oxygen concentration within a narrow range at the cardiac myocytes. By acting as oxygen sensors, cardiac myocytes modulate vascular tone according to the needs of the myocytes and reduce potential problems of hypoxia and extensive formation of reactive oxygen species. (Circ Res. 1999;85:690-698.)

Key Words: adenosine ■ angiotensin ■ cardiac myocyte ■ endothelin ■ regulation of blood flow

Because the work of the heart normally varies over a wide range (maximum work may be as much as 5 times basal work), energy supply to the heart must be capable of responding similarly. The heart normally extracts most of the oxygen in the coronary blood, forcing the changes in energy supply to come primarily from changes in coronary blood flow. A further limitation on the response of the heart to an increase in its power output is its inability to develop an “oxygen debt.” Unlike skeletal muscle, cardiac muscle must maintain an ongoing balance between energy supply and energy utilization. This balance requires the existence of information transfer between the myocardial cells and the coronary blood vessels. As oxygen tension rises, the rate of formation of potentially harmful oxidants increases and may result in damage to the myocytes.

Until the pioneering studies of Furchgott and Zawadzki, which showed the important role of vascular endothelial cells in the regulation of the contractile state of smooth muscle, there were vague ideas about regulation of vascular resistance by “metabolites,” pH, PO2, and PCO2. After the establishment of the critical role for endothelial cells, the importance of such vasoactive substances as NO, endothelin, prostacyclin and others was demonstrated. The sensitivity of their production by endothelial cells to tension on the vascular wall and to oxygen tension was also shown. Vascular endothelial cells also produce substances that can modify cardiac contractility. The secretion of 2 kinds of endothelium-derived cardioactive substances, one that increases and another that decreases cardiac contractility, has been demonstrated in cultures of endothelial cells and in the coronary venous effluent of isolated perfused working hearts. For the vasoactive effect to be seen, endothelial cells must be present in the assay tissue. To explain these observations, cross talk between cardiac myocytes and vascular endothelial cells was proposed. In this putative mechanism, the cardiac myocyte acts as an oxygen sensor and secretes a substance that stimulates endothelial cells to produce regulatory substances. Because oxygen tension at the cardiac myocyte is the single factor that most accurately reflects the balance between the rates of cardiac work and energy supply within the heart, this mechanism can be important in maintaining a balance between energy supply and cardiac power.

Confirmation of the existence of such a mechanism requires the demonstration of a vasoactive substance the production of which is sensitive to the concentration of oxygen.
Here we report the demonstration that 2 different substances, adenosine and angiotensin, produced by isolated cardiac myocytes, respectively dilate and constrict blood vessels. They are part of an elaborate regulatory mechanism by which cardiac myocytes can modulate the tone in blood vessels to maintain oxygen tension at the cardiac myocytes within a relatively narrow range, avoiding both hypoxia and hyperoxia.

Materials and Methods
After calcium-tolerant cardiac myocytes had been isolated from rat hearts by perfusion with collagenase and the concentration of rod-shaped and round cells measured,12 they were incubated at 37°C with the concentration of oxygen between 20% and 5.0%. Four hours later, the concentrations of rod-shaped and round cells were measured and the solutions gently centrifuged to separate the myocytes. The supernatant was frozen at –80°C and stored for assay for vasoactive substances.

The purity of the cardiac myocyte preparation was determined by fixing the cells, permeabilizing them, staining their nuclei with 4',6-diamidino-2-phenylindole, and visualizing them with fluorescence microscopy. Nuclei of cardiac myocytes have a characteristic elongated shape that allows them to be easily distinguished from those of fibroblasts and endothelial cells (Figure 1). Of a total of 87 cells examined, 81 (93%) were cardiac myocytes, and the remaining 6 cells, all of which lay on the surface of the myocytes, were of another cell type.

Aortic rings were used for assaying the vasoactivity of the myocyte-conditioned medium.13,14 The solution bathing each ring had the same composition as that used in the myocyte incubation. Endothelial cells lining some of the rings were disrupted to determine whether vascular endothelial cells were necessary for an effect of the medium on the tone of the aortic rings. Nω-Nitro-L-arginine methyl ester was added to all solutions to inhibit NO synthase and block the production of NO. The amount of force produced by K+ depolarization was determined by replacing the appropriate amount of NaCl with KCl. The K contraction was repeated after 30 minutes of recovery in normal HEPES buffer. Only rings in which the second K contraction produced at least 95% of the tension of the first K contraction were used for assaying the myocyte-conditioned media. After recovery from the second K contraction, response to 10−5 to 3×10−6 mol/L phenylephrine was measured for each ring, and the drug was washed out. When force had returned to the baseline, the HEPES buffer was replaced by medium conditioned by myocytes at different oxygen concentrations. The conditioned medium was then continuously oxygenated with 100% O2. After a stable level of force had been achieved in the conditioned media, phenylephrine in increasing concentrations was added to determine the effect of the myocyte-conditioned media on the response of the rings to phenylephrine. All changes in force were normalized to the maximum force produced by phenylephrine in the first phenylephrine dose-response curve.

The isolated perfused mesenteric resistance blood vessel was also used to assay myocyte-conditioned medium.13,15 A small vessel 100 to 200 μm in diameter was dissected from the mesentery and cannulated at both ends so that the luminal perfusion could be maintained separate from adventitial superfusion, and the diameter of the blood vessel was continuously measured.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results
Response of Isolated Cardiac Myocytes to Hyperoxia: Production of a Substance That Increases Vascular Tone

Change in Tone of Aortic Rings
Cardiac myocytes in situ in the intact organism are exposed to oxygen concentrations that are <10%, likely in the range of 6% to 7%. Twenty percent or the concentration of oxygen in air constitutes hyperoxia. Replacement of the standard HEPES buffer with HEPES buffer that had been conditioned by isolated cardiac myocytes equilibrated in 20% O2 for 4 hours produced a rise in tension of the aortic rings (Figure 2). In 8 measurements made with 6 different media (duplicate measurements were made with 2 media), each conditioned by cardiac myocytes at 20% O2, the tension generated by the aortic ring continued to rise for 10 to 12 minutes by an equivalent of 27±2% of the maximum increase produced by phenylephrine (P<0.01). The amplitude of the rise in tension was as great as 35% of maximum increment in force produced by the optimal concentration of phenylephrine. During the assay with the aortic rings, all solutions were continuously equilibrated with 100% O2.
The importance of the vascular endothelial cells in the aortic ring to the changes in the tone produced by the myocyte-conditioned medium was determined by comparing the effect of the same medium on pairs of rings with intact and disrupted endothelium in 5 experiments. In the absence of normal endothelial cells, the change in force produced by the conditioned medium was $1 \pm 2\%$ compared with $29 \pm 3\%$ for the same myocyte-conditioned medium on rings with intact endothelium ($P<0.01$) (Figure 2). This indicates that vascular endothelial cells are required for the rise in tension. The role of endothelin in the changes in force was determined by adding 1 mol/L BQ 123, an endothelin receptor A blocker, to both the standard HEPES medium used before the conditioned medium and the myocyte-conditioned medium (Figure 2). In 5 experiments, the change in tension in the presence of endothelin receptor blocker was $1 \pm 3\%$ compared with $29 \pm 3\%$ in the absence of the blocker ($P<0.01$), indicating that cardiac myocytes were producing a substance that stimulated the release of endothelin from the vascular endothelial cells.

To determine whether the amount of the substance that stimulated the endothelial cells to secrete endothelin was sensitive to oxygen tension, we examined the response of aortic rings to media conditioned by cardiac myocytes during equilibration with 5.0% to 95% O2 (Figure 3). A maximum increase in force of $30 \pm 4\%$ occurred at 12% oxygen, a value that is likely to be experienced by cardiac myocytes in the intact heart under physiological conditions. The magnitude of the increase in force in the aortic ring progressively decreased with lower oxygen tension, and it disappeared when the oxygen had been lowered to 6%. The rise was always completely inhibited by either disruption of the vascular endothelial cells or by inclusion of an endothelin receptor A blocker. In the total of 11 experiments over the range of oxygen concentrations from 6% to 95% in which endothelial cells were disrupted, the change in tension was $1 \pm 1\%$ ($P<0.005$ when the results with and without endothelial cells were compared). In 8 experiments with intact endothelium in the presence of endothelin receptor blocker, the change in tension was $2 \pm 3\%$ ($P<0.01$).

The concentration of normally appearing rod cells in the incubation medium was varied over a $\approx 2$-fold range to determine the relation between the concentration of the cardiac myocytes and the amplitude of the effect on the tone.

Figure 2. Top, Original force tracings produced by 4 rings from the same aorta. The medium from an incubation of cardiac myocytes in 20% oxygen was used to replace the standard buffer bathing 3 of the rings (at MCM in a, b, and c from top down). The solution change with the 4th ring (d) was with the standard buffer, and this was used as a control. In a, the endothelial cells were intact, and there was no endothelin receptor blockade. In b, the endothelial cells in the ring had been disrupted. In c, the endothelin receptor blocker was present, and d is a control. Response of tension to increasing concentrations of phenylephrine from 0.01 to 30 $\mu$mol/L in the myocyte incubation medium was done in a, b, and c (at PEDR). All solutions contained $N^G$-nitro-L-arginine methyl ester to prevent synthesis of NO. Increase in force produced by cardiac myocyte incubation solution only occurred when endothelial cells were intact and endothelin receptors were not blocked. Bottom, Bar graph shows the effect of disruption of endothelium or endothelin receptor blockade on the increase in tension produced by medium conditioned by myocytes at oxygen concentration of 20%.
of the aortic rings. There was a very good correlation between the concentration of normal cardiac myocytes and the concentration of the vasoconstricting factor (Figure 4). The data are well fit by either a linear or a power function with a correlation coefficient of 0.86. The extent of vasoconstriction produced by media equilibrated in 10%, 12%, or 20% was equally well related to the concentration of normal cardiac myocytes present during the conditioning of the media (data for 20% only is shown).

**Angiotensin as the Vasoconstricting Substance**

The vasoconstricting effect was not due to endothelin secreted by the isolated cardiac myocytes, because direct measurement of endothelin in the conditioned medium failed to detect any endothelin (the sensitivity of the measurement was 1 pmol/L or better). Although no endothelin in the myocyte-conditioned medium was measured after the exposure to the aortic ring, this is not surprising. Endothelin secretion by vascular endothelial cells is unipolar and preferentially directed toward the vascular smooth muscle.16–18 Both the affinity and the concentration of endothelin binding sites on the endothelial and smooth muscle cells are high, and the volume of the bathing solution is 300 times that of the aortic ring. Because of both dilution and binding of endothelin released by the endothelial cells, one would not expect to detect a significant increase in the concentration of endothelin in the bathing solution.

Angiotensin II can stimulate endothelial cells to secrete endothelin, and in some tissues the vasoconstricting effect

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**Figure 3.** Relation between the concentration of oxygen (in % saturation) and amplitude of the effects of endothelial cell-dependent modulation of tension in aortic rings. Force is given in terms of percentage of maximum phenylephrine force. •, Intact endothelium; ○, Disrupted endothelium. Data are mean ± SEM of at least 4 different preparations at a given oxygen concentration for cells with intact endothelium and 3 preparations for cells with disrupted endothelium, except for single preparations at 12% and 95%.

**Figure 4.** Relation between concentration of rod-shaped myocytes in incubation solution and amplitude of the increase in tension produced by the rings when they are exposed to incubation solutions equilibrated with 20% oxygen. Each point represents a different preparation. Correlation coefficient = 0.86.

**Figure 5.** Force records from 2 rings from the same aorta. In the upper tracing, normal medium was replaced by medium conditioned in 20% oxygen, producing an increase in force. In the lower tracing, normal medium with 1 μmol/L losartan was replaced by medium conditioned in 20% oxygen, with losartan added after the conditioning. Note the absence of change in force in the latter. Ordinate indicates force; abscissa, time.

**Figure 6.** Continuous tracings of 3 rings from the same aorta. In the upper tracing, the incubation solution of cardiac myocytes exposed in 5% oxygen replaced the standard buffer at the point indicated (S.C.). In the middle tracing, the same solution change occurred, but the endothelium in the aortic ring was disrupted. In the lower tracing, there was only a change of solution of the same composition (S.C.), KCl contractions and response of tension to increasing concentrations of phenylephrine from 0.01 to 30 μmol/L (PEDR) in the myocyte incubation medium before and after the solution changes are shown. Endothelial cells were intact in upper and lower rings, and there was no endothelin receptor blockade. Results were the same when endothelin receptors were blocked. Response to phenylephrine but not to KCl was reduced by media conditioned in 5% oxygen.
from the endothelin released can be substantially greater than the direct effect of angiotensin on the vascular smooth muscle.18–20 To test whether angiotensin was the cardiac myocyte–derived vasoconstricting factor, 1 μmol/L losartan, an angiotensin receptor blocker, was added before and along with media conditioned by cardiac myocytes in 7% and 20% oxygen. In all cases, losartan completely eliminated the vasoconstriction (Figure 5). In the presence of losartan, the rise in tension produced by the myocyte-conditioned media was reduced to 3±2% of maximum phenylephrine-induced tension (n=4; P<0.05).

To confirm that angiotensin was the substance responsible for the vasoconstriction, its concentration was measured in media conditioned in 20% oxygen, in which vasoconstriction occurred and in 5% oxygen, which did not produce vasoconstriction. Angiotensin was present in the former but was not detected in the latter (n=4).

Response of Cardiac Myocytes to Hypoxia

Secretion of an Endothelium-Insensitive Vasodilating Substance

Myocyte medium conditioned in 5% oxygen caused no significant change in force in the aortic ring under basal conditions (1±1%; n=9; P>0.05) or in the amplitude of the contraction in 80 mmol/L KCl (−3±4%; n=9; P>0.05). The response to α-adrenergic stimulation by phenylephrine, however, was decreased by ≈75% at every concentration of the drug whether or not the endothelium was intact (Figures 6 and 7). In 5 of the 9 experiments with myocyte medium conditioned in 5% oxygen, the maximum response to phenylephrine was compared with the KCl contraction in paired aortic rings with intact or disrupted endothelial cells. No significant difference was found in the level to which the response to phenylephrine was reduced (29±5% with intact endothelium and 35±4% with disrupted endothelium; P=0.37). These results indicate that in 5% oxygen, cardiac myocytes release a substance that acts directly on vascular smooth muscle to partially block the α-adrenergic pathway without interfering directly with the contractile system per se. The magnitude of the response is sensitive to the concentration of oxygen in the incubation medium, appearing at only 7% oxygen, which is at the lower range of oxygen concentration normally experienced by cardiac myocytes in the intact heart (Figure 8).

Adenosine as the Endothelium-Insensitive Factor Released at Low Oxygen Concentration

The α-adrenergic inhibiting factor released by isolated cardiac myocytes resembles adenosine in the following 3 important ways: (1) it is released at low concentrations of oxygen, (2) it acts directly on vascular smooth muscle, and (3) it can produce vasodilation by blocking the effect of adrenergic agonists. The vasodilator effect of myocyte-conditioned medium from incubation in hypoxic conditions was inhibited by addition of 10 μmol/L theophylline, an adenosine receptor blocker, to the medium before it was applied to the aortic ring in an assay. It is very unlikely that the effect of theophylline on the force of the aortic ring was caused by an inhibition of phosphodiesterase, because the latter action would have
changed force in the opposite direction to what was observed. To eliminate this concern, 1 \text{\mu mol/L} of the more specific adenosine receptor blocker 8-(3-chlorostyryl) caffeine was used. With this adenosine receptor blockade, there was no significant difference from control phenylephrine dose response (Figure 7).

To confirm that the factor is adenosine, the concentration of adenosine was measured in myocyte-conditioned medium and compared with both the concentration of oxygen in the medium during the incubation and the degree to which maximum force produced by phenylephrine was reduced (Figure 9). The concentration of adenosine varied from 29 to 214 nmol/L depending on the oxygen concentration. Among the 7 media in which adenosine was measured, 6 showed an exponential relation to oxygen concentration. A seventh, incubated in 5% oxygen, had less adenosine than would have been expected from the relation defined by the other 6 media. There was an excellent correlation between the concentration of adenosine and the relative inhibition of maximum phenylephrine-activated force, including the outlier in the relation between adenosine and oxygen concentrations. The data show that adenosine is probably responsible for the effect of myocyte-conditioned media on adrenergic stimulation of vasoconstriction. It is not totally surprising to find a single outlier in the relation between oxygen and adenosine concentrations, because there are several factors besides oxygen that can influence the adenosine concentration produced by incubating myocytes, and it is difficult to completely control them.

**Effect on Resistance Blood Vessels**

For adenosine and angiotensin secreted by cardiac myocytes in the intact heart to regulate coronary blood flow according to local oxygen tension, it is necessary for both substances to diffuse from the cardiac myocytes through the extracellular space to the blood vessels, because if they had to enter the capillaries first, their concentration would have been much reduced when they reached the arterial vascular cells. Adenosine is known to act directly on vascular smooth muscle, and it does not require endothelial cells for its vasodilating effect. On the other hand, the vasoconstriction produced by angiotensin in this system does require the presence of endothelial cells and the secretion of endothelin.

To address this question, media conditioned by cardiac myocytes incubated in 20% oxygen were assayed on the adventitial side of small-resistance arteries isolated from the mesenteric circulation of the rat. The lumen of the vessels was perfused separately with normal HEPES buffer, and no mixing between the 2 solutions occurred. Flow and pressure were controlled, allowing a continuous measurement of vessel diameter to be used as an indication of vessel tone and resistance. In 3 mesenteric vessels, the myocyte-conditioned media produced a 22% decrease in vessel diameter, equal to more than a doubling of vessel resistance, because resistance is a fourth-power function of radius (Figures 10 and 11). This decrease in diameter was completely reversed when the conditioned medium was replaced by normal buffer solution. It was completely inhibited by adding 1 \text{\mu mol/L} losartan to the conditioned medium to block angiotensin receptors.

Cardiac myocytes have been shown to produce angiotensin I, but there is no unequivocal evidence for their production of angiotensin II, although angiotensin II is produced in the intact heart. We examined the effect of \text{\mu mol/L} capto-
pril, an inhibitor of the angiotensin-converting enzyme (ACE) that converts angiotensin I to angiotensin II, on the vasoconstriction produced by media conditioned by cardiac myocytes at 20% oxygen. Addition of the ACE inhibitor to the conditioned medium completely inhibited the increase in vascular tone (Figure 11), indicating that the cardiac myocytes had released angiotensin I, which had to be converted to angiotensin II for the vasoconstriction to occur. Captopril was equally effective in inhibiting the increase in tension that myocyte media conditioned in 20% oxygen produced in aortic rings. In 2 experiments with paired aortic rings, there was no detectable change in tension in the presence of 1 μmol/L captopril. Endothelin receptor A blockade with 1 μmol/L BQ 123 also inhibited the increase in small vessel tone. The pathway for vasoconstriction produced by the cardiac myocyte–conditioned medium, therefore, includes release of angiotensin I; conversion to angiotensin II by ACE, presumably on the surface of the endothelial cells19,20,24; and the subsequent secretion of endothelin by the endothelial cells in response to angiotensin II.

**Damaged Cells Do Not Produce the Vasoactive Substances**

It was essential to rule out the possibility that substances released by cells damaged during the isolation procedure or the incubation were responsible for the alteration in the tone of the aortic rings. Because the amplitude of the change in vascular tone at any given oxygen tension was linearly related to the concentration of normal, rod-shaped cells with normal striation patterns, we examined the relation between the concentration of damaged cells and the amplitude of the change in vascular tone. Incubation media formed by myocyte preparations with different percentages and concentrations of round cells were assayed on aortic rings with and without intact endothelial cells. There was no correlation between the increase in force and the concentration of damaged cells with or without intact endothelial cells. In fact, when the aortic rings did not have intact endothelial cells, there was no observable effect on the tension of an aortic ring.
from damaged cells formed during the incubation as long as the concentration of round cells did not exceed 60,000/mL. Above this concentration, tension increased sigmoidally with increasing concentration of damaged cells (Figure 12). The same lack of correlation between number of damaged cells and response of the aortic rings to the incubation solution existed whether the number of cells that deteriorated during the incubation or the number that deteriorated during both the isolation and the incubation was considered.

The characteristics of the increase in vascular tone at high concentrations of damaged cells differed from those found with concentration of damaged cells $<60,000/mL$. Because increase in tone related to the concentration of damaged endothelial cells occurred in the absence of normal endothelial cells, it could not have been a result of the same substance as that released by normal myocytes. Damaged cells at any concentration did not produce a vasodilatory substance. There was no relation between the concentration of damaged cells even at the highest concentrations and decrease tone of the aortic rings.

Incubation of aortic rings with even higher concentrations of damaged cells had no effect on the tension of the rings if the damaged cells had been suspended in fresh buffer immediately before exposure of the aortic rings. Apparently, a vasoconstricting substance different from that released by normal cardiac myocytes is released during the deterioration and rounding up of the cells, but it must achieve a minimum concentration for a change in vascular tone to occur.

For these reasons, data were used in the study only from preparations of isolated cardiac myocytes, with at least 70% of rod cells at the beginning of the incubation and fewer than 50,000 damaged cells per mL.

**Discussion**

Preparations of isolated cardiac myocytes can act as oxygen sensors and release angiotensin I and adenosine according to the oxygen concentration in their immediate vicinity. The preparations consist almost exclusively of cardiac myocytes, with a small degree of contamination from other cell types. Although it is possible that the oxygen sensors are other cell types present in the preparation, this is unlikely in view of their small number and the fact that the active substances that are secreted are adenosine and angiotensin I, both known to be released by cardiac myocytes. The oxygen sensor is most likely the cardiac myocyte, but even if another cell type in the preparation is the oxygen sensor, it is sensing the same extracellular concentration of oxygen as the cardiac myocytes.

Within the range of oxygen tensions that are near physiological ($\sim$5% to 12%), myocytes are quite sensitive to the level of oxygen in the medium. When the concentration of oxygen is $>6\%$, angiotensin I is produced by the cardiac myocytes and converted to angiotensin II by converting enzyme in the blood vessel, most likely on the surface of the endothelial cells. The angiotensin II stimulates vascular endothelium to secrete endothelin, which produces an increase in vasomotor tone (Figure 13). At oxygen concentration $<6\%$, adenosine is released and acts directly on the vascular smooth muscle primarily to diminish the rise in tension normally produced by $\alpha$-adrenergic stimulation. From the nature of the change in the dose-response curves to phenylephrine, it appears that adenosine acts by altering the $\alpha$-adrenergic receptor or something just downstream from the receptor. The ability of the contractile system per se in vascular smooth muscle to generate force is unaffected by either substance. When the net effects of the combination of angiotensin and adenosine are considered, it becomes clear that there is only a narrow window of oxygen concentration, $\approx6\%$, at which isolated myocytes do not release vasoactive substances. It appears that cardiac myocytes try to maintain oxygen concentration in their immediate environment within a very narrow range, avoiding hyperoxia as well as hypoxia.

Adenosine and angiotensin provide the cardiac myocytes with a mechanism for regulating their own blood supply according to their needs. They can do this by changing the resistance of coronary blood vessels, thereby maintaining the concentration of oxygen in the immediate environment of the myocytes within a relatively narrow range (Figure 13). Increased tone in the blood vessels occurs when oxygen concentration rises above a level of $\approx50$ torr, and decreased tone occurs when the oxygen tension falls. Because the oxygen tension at the cardiac myocyte is probably the best single parameter for monitoring the balance between energy supply and energy use, this mechanism effectively provides a way for cardiac myocytes to regulate their own blood flow. The 2 chemical signals secreted by the cardiac myocytes are well suited to perform their function of modulating vascular tone, because each can diffuse through the extracellular space to its target and avoid the considerable dilution that would occur if they had to enter the capillaries first. Angiotensin I diffuses through the extracellular space and is converted to the active angiotensin II form at the blood vessel, where its concentration is sufficient to stimulate the release of endothelin but not sufficient to produce direct vasoconstriction. Thus, angiotensin operates through an amplification system that is spatially sharply focused on the vascular smooth muscle.
In the intact heart, the endothelium-sensitive regulatory system should act to prevent the rise in oxygen concentration around the cardiac myocytes above \( \approx 50 \) tor. The combination of the 2 regulatory mechanisms should prevent major changes in the concentration of oxygen provided to the myocyte in either direction and help stabilize the intracellular oxygen tension. This stability would be highly desirable, because the alternation of lower and higher oxygen tensions appears to increase the likelihood of damage to the cardiac myocytes by oxidants.\(^{23}\) By limiting or preventing rise in oxygen concentration, the regulatory system should limit the production of reactive oxygen species in the vicinity of the cardiac myocytes and help prevent the damage that such compounds can produce in the cardiac myocytes.

There is evidence in the intact animal of systems that are quantitatively very similar to those observed with the isolated cardiac myocytes.\(^{26}\) When the oxygen partial pressure in the arterial blood in open-chested dogs was varied from 30 to 400 torr and intracellular oxygen tension was estimated from the extent of carbon monoxide binding to hemoglobin, the production of reactive oxygen species in the vicinity of the cardiac myocytes and help prevent the damage that such compounds can produce in the cardiac myocytes.

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References