Does coronary vasodilation after adenosine override endothelin-1-induced coronary vasoconstriction?

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Loghin C, Sdringola S, Gould KL. Does coronary vasodilation after adenosine override endothelin-1-induced coronary vasoconstriction? Am J Physiol Heart Circ Physiol 292: H496–H502, 2007. First published September 1, 2006; doi:10.1152/ajpheart.00818.2006.—Endothelin-1 is a powerful coronary vasoconstrictor that is overexpressed in coronary artery disease. Adenosine is a powerful coronary vasodilator used for myocardial perfusion imaging to identify flow-limiting coronary artery stenosis. Therefore, in an animal model we tested the hypothesis that intracoronary endothelin-1 may cause myocardial perfusion abnormalities by positron emission tomography (PET) at resting conditions that may persist or only partially improve after intravenous adenosine stress in the absence of myocardial scar and flow-limiting stenosis. Fourteen dogs underwent serial PET perfusion imaging with rubidium-82 before and after subselective intracoronary infusion of endothelin-1, followed by intravenous and then intracoronary adenosine. Small physiological doses of endothelin-1 infused into the mid-left circumflex coronary artery caused quantitatively significant resting perfusion abnormalities that normalized after intracoronary adenosine but not consistently after intravenous adenosine used for diagnostic imaging. After effects of adenosine abated, resting perfusion defects returned, lasting up to 5 h in some animals. Cumulative doses of endothelin-1 caused perfusion defects that did not normalize after intravenous adenosine. In an animal model without myocardial scar or flow-limiting stenosis, intracoronary endothelin-1 causes visually apparent, quantitatively significant, long-lasting myocardial perfusion defects at resting conditions that may persist or only partially improve after intravenous adenosine used for diagnostic imaging. These results may potentially explain resting perfusion abnormalities or heterogeneity by clinical PET that may persist or only partially improve after adenosine stress perfusion imaging in the absence of myocardial scar and flow-limiting stenosis.

IN DIAGNOSTIC MYOCARDIAL PERFUSION imaging, the resting perfusion image serves as a baseline for comparison to the exercise or pharmacological stress image where a new or worsening stress-induced perfusion abnormality indicates flow-limiting stenosis. This paradigm of rest-stress perfusion imaging is based on the concept of coronary flow reserve and perfusion imaging during pharmacological stress for assessing coronary artery stenosis as first reported from this laboratory (7–10). In the absence of attenuation artifacts as with positron emission tomography (PET), a persisting fixed perfusion defect is clinically interpreted as myocardial scar or hibernating myocardium due to flow-limiting stenosis.

However, we have also described resting myocardial perfusion defects that improve or disappear during dipyridamole stress (10) in the absence of myocardial scar or flow-limiting stenosis. In a large clinical study, we demonstrated (15) that myocardial perfusion heterogeneity at rest and/or after dipyridamole stress quantified by Markovian homogeneity analysis is closely associated with early nonobstructive coronary artery disease (CAD). However, the mechanisms underlying these resting perfusion abnormalities in the absence of myocardial scar or flow-limiting stenosis have not been identified.

Endothelin-1 (ET-1) is the strongest known arteriolar vasoconstrictor peptide and contributes to resting coronary vasoconstrictor tone (19) and coronary spasm due to localized paracrine effects more than blood concentrations. Vasoconstriction due to ET-1 and associated reduction in coronary flow is at least twice as potent as the flow reduction caused by inhibiting nitric oxide synthesis with Nω-nitro-L-arginine (L-NMMA) that does not change coronary phasic flow or maximum flow capacity despite reduction in coronary artery diameter (3). There is corresponding uneven heterogeneous distribution of myocardial perfusion (16) among different segments of the same coronary artery or different coronary arteries after acetylcholine-induced coronary vasoconstriction (29). ET-1 is mitogenic for smooth muscle cells and is associated with atherosclerosis progression (14). Elevated plasma levels of ET-1 are found in patients with chest pain and normal coronary arteries (13), in diabetes, obesity (20), hypertension (25), CAD, acute coronary syndromes, congestive heart failure (22), and slow coronary flow transit time at arteriography (1) and after coronary stenting (30), all associated with coronary endothelial dysfunction.

Adenosine is a powerful coronary vasodilator used for myocardial perfusion imaging to identify flow-limiting coronary artery stenosis. It is predominantly a direct smooth muscle vasodilator (4, 18, 23, 24, 28). Therefore, we tested in an animal model the hypothesis that intracoronary ET-1 may cause myocardial perfusion abnormalities by PET at resting conditions that may persist or only partially improve after intravenous adenosine stress in the absence of myocardial scar and flow-limiting stenosis.

METHODS

Experimental Preparation

The experimental protocol was approved by the Animal Welfare Committee of the University of Texas Health Sciences Center at Houston. After an overnight fast, healthy adult hound dogs (n = 14) of both sexes, 21–35 kg, were anesthetized with 30 mg/kg pentobarbital sodium (Nembutal) and underwent endotracheal intubation and positive pressure ventilation. Anesthesia was maintained with nitrous oxide, oxygen, and isoflurane. A catheter (n = 12) was inserted into the left femoral artery to measure blood pressure and infuse adenosine (adenosine triphosphate); the femoral vein was used for intravenous injections of adenosine and intracoronary infusions of ET-1 (endothelin-1). Prior to adenosine stress imaging, each dog underwent subselective intracoronary angiography with a four-unit coronary catheter. In 3 dogs, the mid-right coronary was catheterized and angiography was performed to assess the right coronary flow reserve.

After recovery, the dogs were fasted overnight before the study. Each dog underwent PET perfusion imaging with rubidium-82 at resting conditions that may persist or only partially improve after intravenous adenosine. This was followed by serial PET perfusion imaging with rubidium-82 after subselective intracoronary ET-1 infusion to confirm the hypothesis that intracoronary ET-1 may cause resting myocardial perfusion abnormalities. After effects of ET-1 abated, serial PET perfusion images were then acquired in this dog to confirm the hypothesis that intracoronary ET-1 may cause resting myocardial perfusion abnormalities.
mechanical ventilation with adequate anesthesia maintained by small supplemental doses during the experiment. Arterial blood gases were maintained within physiological range by adjusting the mechanical ventilator with supplemental oxygen, core body temperature was maintained at 37°C with a homeothermic blanket, and stable body position was maintained throughout the duration of the imaging protocol by using a specially designed portable cradle for both coronary arteriography and PET imaging without moving the dog strapped to the portable cradle.

Arterial access was obtained via the right femoral artery by standard Seldinger technique with an arterial micropuncture kit (Cook, Bloomington, IN) with a 6-F arterial sheath. All animals received 100 IU/kg of initial heparin bolus, with 50 IU/kg every hour for the duration of the experiment. A 6-F standard JL3 VistaBrite coronary guide catheter (Cordis-Cardiology, Miami Lakes, FL) was positioned under fluoroscopic guidance into the left main coronary artery ostium. After a stable position for the guide catheter was obtained, a 0.014-in.-diameter Hi-Torque Whisper guide wire (Guidant, Indianapolis, IN) was placed in the left circumflex coronary artery (LCx). Over the coronary wire, a 2.3-F, 150-cm-long, 0.042-in.-diameter Rapidtransit infusion catheter (Cordis-Cardiology) was positioned in the LCx, with the tip in the midsegment of the LCx artery proximal to the takeoff of the first large obtuse marginal branch. The guide wire was removed, the guide catheter was withdrawn into the aortic root, and the small catheter was left in place in the mid-LCx.

**Experimental Protocol**

Contrast angiograms were obtained to document the LCx as the dominant coronary artery and the small catheter position in the mid-LCx. With contrast solution diluted 50% with normal saline, different injection rates were tested to determine the rate at which back flow occurred into the proximal segment of the coronary artery. No back flow was observed at infusion rates of <5 ml/min. Dogs were then moved into the PET scanner without changing position on the special cradle, and a repeat subselective angiogram was performed under fluoroscopy on the PET imaging table to ensure that the small intracoronary infusion catheter positioned in the mid-LCx had not changed during transportation.

PET imaging was carried out with the University of Texas-designed Posicam BGO multislice tomograph HZL/mPower (Positron, Houston) as previously described (5, 11, 21, 26, 27) with a reconstructed resolution of 10-mm full-width-half-maximum. Images were acquired in two-dimensional mode with extended septa to minimize scattered counts with random coincidences corrected from the singles count rate. Images were reconstructed with filtered back projection, with a Butterworth filter order of 5 and 0.04 cycles/mm corresponding to a cutoff of 0.16 for the input pixel dimensions of 2 × 2 × 2.6 mm, displayed with image dimensions of 256 × 256.

On the basis of a 5-min positioning transmission scan, dogs were precisely positioned in the PET scanner and laser guides aligned to external body markers were used to check correct position for every image acquisition. With a rotating rod source containing 4–5 mCi of 68Ge, transmission images to correct for photon attenuation contained ~70–90 million counts. Emission images obtained after intravenous injection of 935–1,110 MBq (25–30 mCi) of generator-produced rubidium-82 (82Rb) contained ~20–30 million counts.

Our protocol used the following imaging sequence as illustrated in Fig. 1.

**Step 1.** Immediately after completion of a resting perfusion image with intravenous 82Rb, 3 mg/ml adenosine (Fujisawa Healthcare, Deerfield, IL) was infused subselectively into the LCx for 4 min at 2 ml/min. Two minutes before the end of infusion, a second dose of 82Rb was injected intravenously and images were obtained to confirm position of the coronary infusion catheter in the mid-LCx, to document the distribution and size of the myocardial LCx territory perfused distal to the catheter, and to quantify the relative increase in activity over baseline induced by intracoronary adenosine.

**Step 2.** ET-1 (Sigma-Aldrich, St. Louis, MO) was then infused into the LCx via the subselective intracoronary infusion catheter at an initial dose of 1.5–3.5 ng·kg⁻¹·min⁻¹ for 10 min in nine dogs. Myocardial perfusion imaging was repeated with intravenous 82Rb. In six dogs, it was necessary to administer repeated smaller doses of ET-1 in order to obtain a perfusion defect on PET images. The solution of ET-1 was prepared to provide an infusion rate of 2–2.5 ml/min for any given dose rate in order to avoid backfilling demonstrated only at above two times this infusion rate at coronary arteriography. No tachyarrhythmia developed, and no animal died because of ET-1 administration.

**Step 3.** After the ET-1 image was acquired, adenosine was then given intravenously at a dose of 0.142 mg·kg⁻¹·min⁻¹ for 6 min. 82Rb was administered intravenously 3 min before the end of adenosine infusion and imaging was repeated.

**Step 4.** Adenosine was then given as an intracoronary injection via the coronary infusion catheter at the same dose and rate as the initial intracoronary adenosine injection. 82Rb was administered 2 min before the end of the intracoronary adenosine infusion, and imaging was repeated.

**Step 5.** After effects of the last adenosine injection had abated, another emission scan was again performed. If a perfusion defect persisted in the LCx territory, the sequence of intravenous and intracoronary adenosine was repeated. The images showing a persistent defect were used as a reference for analysis of subsequent scans after additional adenosine injections. This follow-up imaging provided up to three serial protocol sequences of ET-1-induced perfusion defects, intravenous adenosine, and intracoronary adenosine, resulting in a total of 23 protocol sequences for the 9 dogs in this protocol.

**Step 6.** In five additional dogs, instead of infusion of ET-1 as in step 2 above, the nitric oxide synthesis inhibitor L-NMMA (Paragon Biochemical) was infused into the LCx via the coronary infusion catheter at doses ranging between 100 and 400 μg·kg⁻¹·min⁻¹ for 10 min at the same flow rate to avoid backfilling. Perfusion images were again obtained after administration of intravenous 82Rb.

**Step 7.** A final emission scan was acquired at the end of each experiment to document persistence or resolution of the perfusion defect.
Quantifying Relative Changes in PET Images

Activity in each cardiac image data set was normalized to the maximum 2% of pixels in the whole heart data set in order to obtain a relative normalized scale as well as the original scale of absolute counts. The purpose of this first normalization of all activity in the heart to its maximum counts was to enable combination of data from all studies in all animals with the least measurement variability so that small relative regional changes below resting could be reliably measured. We used relative changes on PET perfusion images for four reasons. 1) Relative perfusion defects, i.e., relative coronary flow reserve, are independent of heart rate and perfusion pressure, whereas absolute flow and absolute coronary flow reserve are highly dependent on heart rate and blood pressure changes. 2) The relative perfusion defects after intravenous injection of radiotracer are comparable to relative defects of clinical imaging and need to be studied as relative defects if they are to be relevant to clinical PET perfusion imaging as now most commonly performed. 3) The reproducibility of quantifying relative defects is very good, with one SD of repeated measurements of relative defects being 0.5% in this study. For comparison, in our comprehensive review of 23 publications, the SD of absolute perfusion expressed as a percentage of mean flow is 24% (SD 12) for absolute resting perfusion and 29% (SD 12) for stress absolute perfusion, reflecting much greater variability than the SD of 0.5% for relative defects in this study. 4) Since our scientific question addresses only small relative changes below resting perfusion, we designed this approach to provide precise measurements of small relative regional perfusion defects in resting perfusion images without needing to measure relative changes above resting levels, coronary flow reserve, or absolute perfusion having substantially greater methodological variability than the relative changes expected here.

Small relative regional changes in the LCx distribution were determined on whole heart-normalized images as follows. A pixel size of 2 × 2 mm in the tomographic plane best showing the perfusion defect in the distribution of the LCx after intracoronary ET-1 was selected that also had the highest counts on the resting baseline image, and x, y, and z coordinates were recorded. For each location with the highest baseline counts in the LCx distribution on the resting baseline image, the pixel value normalized to the whole heart was recorded for all images—at baseline and for each intervention.

To obtain the primary end point data, the normalized LCx pixel value on the image after an intervention was divided by the normalized pixel value on the resting baseline image before the intervention at the same LCx location; this ratio is multiplied by 100 to express the LCx pixel value after the intervention as a percentage of the resting baseline pixel value before the intervention. The baseline pixel coordinates were used to locate and record the pixel values on all subsequent images for determining the changes after subsequent interventions with ET-1 and adenosine or l-NMMA.

Our method of quantifying these relative changes in radionuclide uptake in the LCx distribution has some important specific characteristics. Small changes in relative normalized radionuclide uptake in the LCx distribution can be precisely determined independently of activity in the left anterior descending coronary artery LAD region and without the biological and methodological variability of absolute perfusion measurements. Furthermore, relative perfusion defects are relatively independent of heart rate and blood pressure changes compared with absolute flow measurements. Moreover, the marked increased perfusion in the LCx distribution after intracoronary adenosine is normalized out for the following reason. At baseline, perfusion is uniform and pixel values normalized to the maximum activity are uniformly 100% throughout the heart, including the LCx area. After administration of intracoronary adenosine into the LCx and intravenous 82Rb, the activity in the LCx area is the maximum activity in the heart where the pixel values are therefore also 100%. Thus the LCx pixel value normalized to maximum activity at baseline is 100%, and the same pixel value normalized to maximum activity after intracoronary adenosine is also 100%. Consequently, the ratio of the normalized pixel values after adenosine to baseline is either 1.0 or 100% and does not reflect an increase due to intracoronary adenosine.

Intracoronary adenosine with intravenous 82Rb after the baseline image was used solely for confirming the position of the small subselective catheter in the LCx, not for obtaining end point data. For testing our hypothesis on resting perfusion defects, assessing coronary flow reserve or the increases in perfusion over baseline after adenosine was not important since testing our hypothesis required precisely measuring relative small decreases in perfusion below resting levels.

If our measurement technique incorporated a wide range from zero to four times baseline, then the measurements of small decreases below resting values would have less precision and greater variability, like the high- versus low-range options on a voltmeter. Since our scientific question addresses only the small relative changes below resting perfusion, we designed this approach to provide precise measurements of small relative regional decreases in resting perfusion images without need to measure relative changes above resting levels, coronary flow reserve, or absolute perfusion having substantially greater methodological variability than the relative changes expected.

We intentionally did not normalize activity in the LCx distribution to the LAD distribution because 1) intravenous adenosine would increase the perfusion in the LAD distribution that would then change the LCx-to-LAD ratio regardless of small positive or negative changes in the LCx distribution that were of specific interest in this protocol and 2) referencing the LCAx activity to the LAD activity after intravenous adenosine would in effect measure the relative coronary flow reserve of the LCx compared with the LAD. However, as explained above, assessing relative coronary flow reserve would not address our hypothesis about relative resting perfusion defects.

Based on alignment of external markers, superimposition of the cardiac images, and the x, y, and z coordinates, there was no misregistration among baseline resting images and subsequent scans obtained after ET-1 and adenosine injections. Repeated readings of the entire data set showed a measurement variability of <0.5% relative uptake compared with the reported 24–29% variability in absolute flow measurements. In this study, there was no misregistration of attenuation and emission scans as we have previously reported for clinical studies (17).

Changes for each intervention study were quantified as percentage of the resting baseline pixel value before the intervention in the same LCx pixel as defined above. Therefore, for reporting the changes from resting baseline, the pixels selected on the baseline image had a value of 100%, with the value of the same LCx pixel after ET-1 expressed as some percentage below 100%.
Statistical Analysis

All statistical analyses were carried out with SPSS version 11.5 (SPSS, Chicago, IL). Data are reported as means (SD). Differences among the means of continuous variables were analyzed with an independent or paired two-tailed t-test. Levene’s test for equality of variances was used to validate the t-test results. Analysis of variance was carried out for significance of variance, with Games-Howell post hoc test for unequal variances. Linear regression analysis was used to evaluate whether ET-1 dose predicted the quantitative response to adenosine. Spearman’s nonparametric correlation test was used for correlating ET-1 dose and the subsequent response to intravenous adenosine. A two-tailed P value of <0.05 was considered statistically significant.

RESULTS

High-quality images were obtained. For each rest perfusion image, the dose of $^{82}$Rb infused intravenously averaged 24.7 mCi (SD 0.8), the total number of counts for the baseline rest image data set averaged 23.1 million counts (SD 2.7 million), and the heart-to-lung ratio averaged 13.6 to 1 with SD of 4.6. The adenosine images were comparable with 24.7 mCi (SD 1.2) $^{82}$Rb injected, 23.8 million counts (SD 2.8 million), and heart-to-lung ratio of 13.6 to 1 with SD of 4.6. The total counts for each image data set for the small chest and heart size of the dogs compared with humans produced very good images with high heart-to-lung ratios.

Figure 2 illustrates the series of PET perfusion images on this protocol: at resting control baseline, after intracoronary adenosine to confirm the location of the infusion catheter in the LCx, after intracoronary ET-1 infusion, after intravenous adenosine, after intracoronary adenosine used for quantifying the improvement in the ET-1-induced resting perfusion defects, and after intravenous adenosine. Depending on the dose of intracoronary ET-1, the perfusion defect induced by ET-1 did not always improve after intravenous adenosine, as illustrated in Fig. 3, showing PET perfusion images in the same sequence. However, in all experiments, the ET-1-induced resting perfusion defects normalized after intracoronary adenosine.

Figure 4 shows the value of the LCx pixel as percentage of the baseline pixel values for all 23 complete protocol sequences obtained with PET perfusion imaging at resting control baseline, after initial intracoronary adenosine, after intracoronary ET-1, after intravenous adenosine, and after intracoronary adenosine and the final image with a persisting ET-1-induced defect after the adenosine effects had worn off. Intravenous adenosine at doses comparable to those used in clinical practice only partially counteracted the vasoconstrictor effect of ET-1 as opposed to intracoronary adenosine that induced vasodilation to a similar extent before and after intracoronary ET-1.

To analyze the differing effects of ET-1 on resting perfusion, the 23 complete protocol sequences were categorized into two groups based on the response to intravenous adenosine (Table 1). In group 1 ($n=8$), the ET-1-induced perfusion defect improved after intravenous adenosine as in Fig. 2. In group 2 ($n=15$), the ET-1-induced perfusion defect was visually not improved or relatively worse after intravenous adenosine, as shown in Fig. 3. Table 1 shows the differences in the severity of the perfusion defects related to the cumulative dose of ET-1 and the response to intravenous adenosine.

There was no significant difference between the mean body weights in the two groups. The weight-based ET-1 infusion rate was not significantly different between the two groups [1.9...
(SD 0.7) vs. 2.3 (SD 0.9) ng·kg⁻¹·min⁻¹; P = 0.275), but this dose rate does not indicate the total cumulative dose of ET-1 given, which was significantly different between the two groups [44.3 (SD 13.9) vs. 67.5 (SD 28.9) ng; P = 0.017].

There was no significant difference between resting control baseline percent uptake relative to maximum whole heart activity of the two groups [91.2% (SD 3.9) vs. 92.0% (SD 5.2); P = 0.675]. The response to intracoronary adenosine expressed as percentage of baseline was similar between groups 1 and 2 [102% (SD 5.5) vs. 101.3% (SD 5.2); P = 0.98]. After initial ET-1 infusion(s) sufficient to produce a visible resting perfusion defect, there was no significant quantitative difference between the normalized percent uptake in the LCx territory in both groups [89.3% (SD 7.3) vs. 90.2% (SD 8.1); P = 0.789], reflecting a similar severity of the ET-induced defect in both groups [−12.7% (SD 6.7) vs. −11.1% (SD 11.9); P = 0.72].

Table 1. Response to intravenous adenosine:
group characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 8)</th>
<th>Group 2 (n = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>27.0 (4.4)</td>
<td>29.2 (3.3)</td>
<td>NS</td>
</tr>
<tr>
<td>ET dose, ng·kg⁻¹·min⁻¹</td>
<td>1.9 (0.7)</td>
<td>2.3 (0.9)</td>
<td>NS</td>
</tr>
<tr>
<td>ET total dose, ng</td>
<td>44.3 (13.9)</td>
<td>67.5 (28.9)</td>
<td>0.017</td>
</tr>
<tr>
<td>PET parameters</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>91.2 (3.9)</td>
<td>92.0 (5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>AD ic pre-ET</td>
<td>102.0 (5.5)</td>
<td>101.3 (5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>ET</td>
<td>89.3 (7.3)</td>
<td>90.2 (8.1)</td>
<td>NS</td>
</tr>
<tr>
<td>∆ET - baseline</td>
<td>−12.7 (6.7)</td>
<td>−11.1 (11.9)</td>
<td>NS</td>
</tr>
<tr>
<td>AD iv</td>
<td>93.4 (6.0)</td>
<td>77.7 (14.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>∆AD iv - ET</td>
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<td>−12.5 (9.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AD ic post-ET</td>
<td>98.7 (5.5)</td>
<td>100.5 (4.3)</td>
<td>NS</td>
</tr>
<tr>
<td>∆AD ic - ET</td>
<td>8.7 (4.3)</td>
<td>10.5 (8.5)</td>
<td>NS</td>
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</table>

Data are means (SD). Baseline uptake data are expressed as % of maximum ⁸²Rb uptake of the whole heart data set; all other positron emission tomography data are expressed as % of peak activity in the left circumflex coronary artery area normalized to activity of the same area on the baseline scan. Endothelin-1 (ET) total dose represents total cumulative dose of intracoronary ET calculated based on subject weight and total duration of administration. AD, adenosine; pre-ET, injection before ET administration; post-ET, injection after ET administration; iv, intravenous; ic, intracoronary; ∆, difference; NS, nonsignificant.

After intravenous adenosine administration, group 1 showed visual improvement of the resting perfusion defect, quantified by a significantly different percentage of resting baseline pixel values compared with group 2 [93.4% (SD 6.0) vs. 77.7% (14.5); P = 0.008]. The magnitude of the change, e.g., improvement for group 1 and worsening for group 2, was also significantly different between the two groups [+4.1% (SD 2.0) vs. −12.5% (SD 9.2); P < 0.001]. The worsening of relative perfusion defects in the LCx distribution after intravenous adenosine in animals receiving high cumulative doses of ET-1 indicates that the normal surrounding or remote myocardium vasodilated after intravenous adenosine more than the LCx region that was vasoconstricted by high cumulative doses of ET-1 subselectively into the LCx coronary artery. In other dogs with less severe LCx vasoconstriction due to a lower cumulative ET-1 dose, intravenous adenosine vasodilates the LCx bed as much as the surrounding remote myocardium such that the relative defect is abolished. All ET-1-induced defects normalized after intracoronary adenosine, thereby proving functional vasoconstriction as the cause of the resting perfusion defects, not myocardial necrosis.

In contrast to the intravenous administration of adenosine, a subsequent intracoronary adenosine dose overcame the vasoconstrictor effect of ET-1 and induced similar complete defect resolution in both groups, with normalized percent uptake of 98.7% (SD 5.5) in group 1 vs. 100.5% (SD 4.3) in group 2 (P = 0.473) after intracoronary adenosine. Defect resolution was also indicated by the difference between intracoronary adenosine and ET-1 images, expressed as percent change in LCx uptake of 8.7% (SD 4.3) for group 1 vs. 10.5% (SD 8.5) for group 2 (P = 0.668).

Figure 5 shows the relative change in LCx uptake as percentage of the resting control baseline pixel values, over the protocol time line, for group 1 and group 2. The only significant difference is in the response to intravenous adenosine, whereas ET-1-induced resting defects and responses to intracoronary adenosine before and after ET-1 administration were similar between the two groups.

The total cumulative dose of ET-1 correlated well with the quantitative change in the ET-1-induced defect after intrave-
nous adenosine, expressed as the difference between adenosine and ET-1 percent change in LCx uptake (P = 0.009; correlation coefficient = −0.534). In a linear regression test, the total dose of ET-1 predicted the intravenous adenosine response intensity (P = 0.055; confidence interval: −0.336 to −0.004).

In the five dogs receiving intracoronary l-NMMA, the perfusion defects were significantly less severe than after ET-1 [-2.48% (SD -4.11) for l-NMMA vs. -10.13% (SD 7.66) for ET-1; P = 0.009], as expected since ET-1 is well recognized as the most powerful of endothelial vasoconstrictors. Consequently, ET-1 without l-NMMA was used for the remainder of the experiments.

**DISCUSSION**

Intracoronary ET-1 causes visually apparent, quantitatively significant, localized, long-lasting resting myocardial perfusion defects that may persist or only partially improve after intravenous adenosine in doses comparable to those used in clinical diagnostic imaging in the absence of myocardial scar or flow-limiting stenosis. The degree of improvement after intravenous adenosine is inversely related to the total cumulative dose of ET-1. Since our canine model has normal coronary arteries without endothelial dysfunction, flow-limiting stenosis, or myocardial scar and the ET-1-induced perfusion defects normalized after intracoronary adenosine, our study demonstrates experimentally the concept that myocardial perfusion defects at rest and/or after intravenous adenosine stress may be due to a vasoactive mediator and not due to myocardial scar and flow-limiting stenosis.

Our data support the possibility that excess production of vasoconstrictors associated with severe endothelial dysfunction may partially explain the clinical finding of resting perfusion abnormalities (10) or perfusion heterogeneity (15) that partially improves or normalizes after adenosine or dipyridamole stress in the absence of myocardial scar or flow-limiting stenosis. The results of this study do not conflict with our initial demonstration of reduced coronary flow reserve and stress-induced perfusion defects after pharmacological stress due to flow-limiting stenosis (7–10). Rather, our study extends our understanding of myocardial perfusion imaging at rest and after adenosine stress.

**Study Limitations**

We used exogenous intracoronary ET-1 in an experimental model with normal coronary arteries in order to control precisely the experimental conditions for causing myocardial perfusion abnormalities. In addition, ET-1 is a powerful vasoconstrictor that is overexpressed in endothelial dysfunction associated with coronary atherosclerosis.

Measuring plasma levels of ET-1 was not essential for our study for several reasons. 1) Given the known abluminal path of endothelin secretion toward the myocardial interstitium rather than into the bloodstream, plasma endothelin concentrations do not reflect interstitial concentration of the peptide at arteriolar smooth muscle level (6). 3) There is substantial myocardial extraction of endothelin with coronary sinus levels lower than plasma levels, leaving unknown interstitial concentrations at which endothelin exerts its effects. 4) At the doses used, endothelin has no significant effects on systemic arterial pressure, cardiac output, or heart rate, despite significant decreases in coronary blood flow (2). 5) It was important in our protocol to demonstrate a relation between total cumulative endothelin dose and the changes in resting perfusion defects after intravenous adenosine without making assumptions about interstitial concentrations extrapolated from plasma concentrations. 6) Our model was designed to demonstrate an imaging concept, not to study the biology of endothelin and/or nitric oxide that is well known. Endothelin overexpression or imbalance with nitric oxide production may be only one of many potential vasomotor abnormalities causing resting vasoconstriction and perfusion abnormalities associated with endothelial dysfunction.

Determining absolute myocardial perfusion in milliliters per gram per minute was also not essential to our study for several reasons. 1) We previously demonstrated (12) that relative coronary flow reserve, i.e., relative perfusion defects, is not dependent on heart rate and blood pressure changes whereas absolute coronary flow reserve using absolute flow measurements is highly dependent on changes in heart rate and blood pressure. 2) Our review of 23 publications reporting absolute perfusion measurements by PET showed a great variability of 24% (SD 13) for rest and 29% (SD 12) for stress perfusion, expressed as the SD and mean value in milliliters per minute per gram. 3) Mathematical models for calculating absolute perfusion corrected for varying radionuclide extraction magnify differences in radionuclide uptake into greater differences in absolute perfusion (31) that could obscure or exaggerate the differences we observed. 4) The assumptions inherent in these mathematical models for calculating absolute myocardial perfusion are open to question for the relatively small but significant relative resting perfusion defects after intracoronary endothelin. 5) We wished to demonstrate results using relative uptake values since accurate determination of the arterial input function required for models of absolute perfusion is technically difficult and makes PET data acquisition so complex that it introduces substantial variability and is rarely used in clinical practice. 6) Absolute quantification of myocardial perfusion also requires assumed corrections for partial volume errors that are equally questionable. 7) In our study, quantification of relative myocardial uptake is optimal for the specific hypothesis tested independent of varying heart rate and blood pressure and without the assumptions or model calculations on the primary data inherent in determining absolute perfusion.

**Conclusions**

Our experimental study demonstrates that intracoronary ET-1 causes visually apparent, quantitatively significant, long-lasting resting myocardial perfusion defects by PET imaging that may persist or only partially improve after intravenous adenosine used for diagnostic imaging in the absence of myocardial scar and flow-limiting stenosis. These results may potentially explain in part the resting perfusion abnormalities on clinical PET images that may persist or only partially improve after adenosine stress (10) and the resting perfusion heterogeneity by PET perfusion imaging associated with early nonobstructive coronary artery disease and endothelial dysfunction (15).
REFERENCES


