Temporal Heterogeneity of Myocardial Blood Flow in Anesthetized Dogs

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SUMMARY
Temporal variation in perfusion to small segments of the myocardium was studied in 19 open-chest dogs. In six control dogs, three or four differently labeled 7-10 μ microspheres were injected simultaneously into the left atrium to assess the variability in measured myocardial perfusion due to the microsphere technique. In 13 other dogs, microspheres were injected four times at 5 minute intervals while various hemodynamic parameters (mean aortic pressure, peak systolic pressure, heart rate, left ventricular end-diastolic pressure, and Vmax) were stable (less than 10% variation in any one parameter). The left ventricle was divided into 96 segments, the mean weight ± SD of each segment was 0.95 ± 0.17 grams. The flow to each segment was expressed as a percent of the mean flow of the three or four measured flows to that segment, and the difference between the largest and the smallest percent of each segment was taken as a measure of the variability of flow to that segment. The average variability of segmental flow (mean ± SD) when the three to four differently labeled microspheres were injected was 14.0 ± 4.7%; and the variability when differently labeled microspheres were injected sequentially was 31.0 ± 10.8% (P < 0.001). Furthermore, in the sequentially injected animals the magnitude of temporal variation was similar in various subdivisions of the ventricle (layers, walls, apex to base). The mean and standard deviation of the variability of flow to the endo, mid, and epicardial layers were 28.7 ± 10.3, 30.0 ± 11.3 and 34.5 ± 12.4%, respectively. These changes may reflect either spontaneous or local autoregulatory changes in precapillary sphincters or arterioles.

SPONTANEOUS VARIATION in flow to skeletal muscles has been demonstrated and recent studies suggest that this phenomenon may also occur in the left ventricular myocardium. Several investigators using the labeled microsphere technique, have shown a considerable variation of flow to small segments of the left ventricle in dogs with unobstructed coronary arteries. Technical errors in microsphere technique have been studied in this laboratory and probably account for less than one-half of the measured variability. Also, heterogeneity of myocardial blood flow was small in regard to spatial position in the left ventricle. Therefore, a major factor contributing to variation in regional flow could be a temporal variation, that is, spontaneous or local autoregulatory changes of precapillary sphincters or arterioles. The magnitude of these factors (technical artifact, spatial heterogeneity, and temporal heterogeneity) needs to be established when trying to assess changes in myocardial blood flow. The present study was designed to measure the temporal heterogeneity of flow, as well as reconfirm the magnitude of technical artifact and spatial heterogeneity.

Methods

Studies were made on 19 dogs weighing 22.8 ± 2.6 kg, SD. They were anesthetized with sodium pentobarbital, 25 mg/kg i.v. After tracheal intubation, the dogs were ventilated with room air using a Harvard respiratory pump. The right femoral artery and vein as well as the right brachial artery were isolated and catheterized. A midsternal thoracotomy was performed and the heart was suspended by a pericardial cradle. A cannula was placed in the left atrial appendage and a solid-state transducer was placed into the left ventricle via the left atrial incision. Left ventricular pressure, dp/dt, and Vmax were determined from the high-fidelity left ventricular pressure tracings. Aortic pressure was measured with a Statham P23Db strain gauge. Electrocardiogram and pressure signals were recorded on a Brush/Gould Multi-Channel Recorder, as well as a Hewlett-Packard 3960 FM Tape System for later playback and analysis. Pressure analysis was done by hand as well as on a PDP-11/35 computer.

Myocardial perfusion was measured with 7-10 μ microspheres labeled with, 85Sr, 140Ce, 52Cr, and 48Sc. For each flow measurement, between 1.15 × 106 to 21.6 × 106 microspheres were suspended in between 0.1 and 3.1 ml of saline and injected into the left atrium. Prior to injection, the vial containing the microspheres and one drop of Tween-90 was vigorously agitated mechanically for at least...
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four minutes. Microscopic examination of microspheres dispersed in the manner described above showed that in excess of 98% of the spheres were completely dispersed. Occasionally small groups of three to five spheres were observed. Starting 30 sec before injection and continuing until 3 min after injection, blood was withdrawn simultaneously from the right brachial and right femoral arteries at 2.06 or 3.09 ml per minute using a Harvard pump. The variance in segmental flow secondary to the microsphere technique was studied by injecting 3 or 4 differently labeled microspheres simultaneously into the left atrium of six dogs. In these studies, differently labeled spheres were mixed and agitated together for 4 min prior to injection. The spheres were injected over a 20 sec period and flushed in with 5 ml of saline warmed to 37°C over a 30 sec period. In 13 additional dogs, temporal variation in segmental flow was studied by injecting 3 or 4 different isotopes at 5 min intervals into the left atrium. In this case spheres were injected over a 10 sec period and flushed with 5 ml of warmed saline over 20 sec. During this period of time the electrocardiogram, aortic pressure and left ventricular pressure were monitored.

Following the study, the animals were killed with an injection of potassium chloride. The heart was excised and the free walls of the right ventricle, the left atrium, great vessels, valves, surface vessels, and epicardial fat were removed. Utilizing the posterior descending coronary as a starting point, the left ventricle was divided into four equal levels of eight segments each, and each segment was divided into three layers — endocardium, mid-wall and epicardium — of approximately equal thickness. Thus the left ventricle was divided into 96 segments, and the relative geometric position of each segment was constant from animal to animal (fig. 1). Subsequently, the myocardial segments were weighed (to the nearest mg), placed in glass tubes, and counted for five minutes each in a three-inch, well-type sodium iodide gamma counter. The average weight of the segments was 0.95 g ± 0.17, sn. The reference blood samples were divided into aliquots so that their counting geometry was similar to that of the myocardial samples. Energy windows utilized were 46Sc 700-1500 keV, 89Sr 400-600 keV, 51Cr 270-370 keV, and 131Ie 126-175 keV. Isotope separation was performed utilizing standard techniques.

The myocardial blood flow was calculated using the following formula: MBF = Cm × 100 × RBF ÷ CR, where MBF = myocardial blood flow in cc/100 g per min, Cm = counts per gram of myocardium, RBF = reference blood flow (rate of withdrawal from reference arteries), CR = total counts in the reference blood. The counts in the femoral and brachial blood samples were averaged. The number of spheres present in the brachial and femoral reference samples was rarely identical. The average difference between simultaneous reference samples was 5.57 ± 3.73% (mean ± sn). If there was a greater than 25% difference between any pair of reference samples, that flow was eliminated. This required deleting six flows. Thus of the 19 animals studied and 70 flows done, only six flows were eliminated because of reference flow measurements.

The blood flow, sample weight, and geometric reference number (see fig. 1) for each segment were punched on computer paper tape. Subsequent analysis was performed with a PDP-11/35 computer. Results were analyzed using, where appropriate, either a one- or two-way analysis of variance. Tukey’s test was applied to test intergroup differences. The results are expressed as the mean ± 1 standard deviation.

Results

In all 19 animals studied (table 1), total left ventricular perfusion was determined. The average total flow for the simultaneously injected dogs was not significantly different from the average total flow from the sequentially injected dogs (97.8 ± 28.1 and 85.7 ± 24.3 for the two groups respectively. Averages are from the first flow determination in each animal.). There were, however, some differences in sequential flows to the animals 2, 3, 6, 10, 17. This probably represents a difference in reference arterial flow sampling.

In the 19 animals studied the distribution of segmental flow was analyzed. Figure 2 demonstrates heterogeneity of a single flow to 96 segments. Normal

![Diagrammatic representation of the 96 segments used to divide the left ventricle. The left ventricle was divided into four levels (A-D), base to apex. Each level was divided into eight subsections (2 each for the anterior, lateral, septal and posterior walls). Each sub-section was divided into three layers (epicardium, mid-wall and endocardium). Each individual segment was assigned a geometric reference number (1, . . . 96) so that similar segments could be compared from study to study.](http://circ.ahajournals.org/)

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In the simultaneous mean flows it appears that the flow estimates made with Cr labelled microspheres are less than those made with the other labelled spheres. In 3 additional awake dogs, 4 groups of labelled microspheres were injected. The mean flow obtained with Cr labelled spheres compared with the other three labels was the highest value in one, second highest in two, and third and fourth in the two other animals respectively.

The overall mean flow for all dogs obtained with the three isotopes was 92.7; the overall mean flow obtained with Cr labelled spheres was 90.6. Thus we believe the tendency for Cr flow in the simultaneous dog studies to appear low is a chance observation.

Abbreviation: Sc = scandium; Sr = strontium; Cr = chromium; Ce = cerium.

Table 2 demonstrates the perfusion to various large segments of the left ventricle in the animals studied by simultaneous and sequential flow measurements. For the first flow measured in each animal, the flow to each major subgroup was normalized by dividing by the mean flow to all 96 segments. The distribution of perfusion, when studied by simultaneous or sequential injections, appears to be the same in each group of dogs studied. The mean ± 1 sd endo/epi ratio for the sequential and simultaneous groups was 1.15 ± 0.12 and 1.15 ± 0.09, respectively.

It was considered important that the sequentially-injected group have a stable hemodynamic condition. Average hemodynamic parameters were also calculated for each animal. In only one animal was there a greater than 10% change in one of the parameters and that flow was deleted from the study. Most changes were much less than 10% and averaged 3.25 ± 1.62%. Table 3 lists the average hemodynamic parameters for all dogs during the sequential injection of the four radioactive isotopes.

Figure 3 shows the distribution of perfusion in the left ventricle for the radioisotopes injected simultaneously in one dog. As can be seen, the mean calculated flow did not vary appreciably between the radioisotopes injected. The solid symbols indicate the values for the four isotopes injected. The range varied from approximately 85 to 115% of the normalized mean.

Figure 4 shows the perfusion of the left ventricle for four sequential radioisotope injections in one dog. As can be seen, the mean calculated flow did not vary appreciably between the first and fourth injection. The solid symbols indicate the values for the first flow determination and the band width indicates values of subsequent injections. Notice the large variability in flow for a particular segment.

Using the data of the type available from figure 3 or figure 4, mean flows were determined for each segment by averaging the three or four simultaneous or
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Table 2

Perfusion of Major Subgroups

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>Simultaneous</td>
<td>105.2</td>
<td>97.9</td>
<td>97.6</td>
<td>99.1</td>
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<tr>
<td>Sequential</td>
<td>105.4</td>
<td>96.8*</td>
<td>97.9</td>
<td>102.0</td>
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<td>Layers</td>
<td>Epi</td>
<td>Mid</td>
<td>Endo</td>
<td></td>
</tr>
<tr>
<td>Simultaneous</td>
<td>95.5</td>
<td>100.0</td>
<td>104.4</td>
<td>103.0</td>
</tr>
<tr>
<td>Sequential</td>
<td>95.2</td>
<td>100.0</td>
<td>104.9</td>
<td>103.0</td>
</tr>
<tr>
<td>Walls</td>
<td>Post</td>
<td>Sept</td>
<td>Ant</td>
<td>Lat</td>
</tr>
<tr>
<td>Simultaneous</td>
<td>96.1</td>
<td>98.0</td>
<td>103.0</td>
<td>103.0</td>
</tr>
<tr>
<td>Sequential</td>
<td>100.8</td>
<td>99.0</td>
<td>97.7</td>
<td>102.6</td>
</tr>
</tbody>
</table>

Normalized flows in percent are obtained by dividing the absolute flow to that subgroup by the mean flow of all 96 segments.

Abbreviations and description of major subgroups as noted in figure 1.

*Indicates: Sequential A different from B; all epi, mid, and endo layers are different from one another; the sequential anterior wall is different from lateral wall when tested by Tukey test (P < 0.05).

the three or four sequential flows to that segment. Thus segmental flow was available in three different forms: 1) absolute flow in cc/100 grams, 2) segmental flow normalized by dividing by the mean flow of all the flows to that segment, or 3) segmental flow normalized by dividing by the mean of the four flows to that segment as well as dividing by the mean flow of the 96 segments. The third flow was determined to eliminate the effect of reference flow difference.

Table 4 indicates an analysis of flow variability between animals with simultaneous and sequential radioisotope injections. The range in percent (highest minus lowest flow in normalized percent) for each of the 96 segments for the four flows was averaged in each animal. In this table, data presented are normalized in two ways. The mean was calculated from the range of values for each of the 96 segments. For example, the mean range in Level A for the simultaneously injected dogs was 13.4 ± 4.8% as opposed to 28.6 ± 11.8% for the dogs with the sequential injections. As noted in table 4, the flow variability appeared to be much greater in the animals with the sequential injections. Temporal heterogeneity is demonstrated when the data is analyzed by normalization method number 2 or number 3. Thus, even when mean left ventricular perfusion of the sequentially injected animals was set at unity (method 3), there was still a significant increase in variability of flow to each segment. However, the range of variability is smaller when method 3, which minimizes the effect of reference flow sampling, is used.

Figure 5 indicates the distribution of the range of perfusion of the 96 segments for all dogs studied. In this plot, the middle band width indicates the range of perfusion variability for the dogs with simultaneous injections. The larger outside bands indicate the range

Figure 3

Distribution of perfusion of four simultaneous flows. In this figure the solid symbols indicate the values for flow 1. The data have been normalized by dividing by the mean flow of that segment. The shaded area indicates the range of values for the four isotopes injected.

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### Table 3

Hemodynamic Parameters

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>Ao P (mm Hg)</th>
<th>LV (mm Hg)</th>
<th>Peak dp/dt (mm Hg/sec)</th>
<th>Vmax (sec-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>140</td>
<td>106.7/89.9</td>
<td>104/1.7</td>
<td>2140</td>
<td>76.2</td>
</tr>
<tr>
<td>± SD</td>
<td>26.2</td>
<td>20.6/17.0</td>
<td>13.5/6.6</td>
<td>856</td>
<td>10.8</td>
</tr>
</tbody>
</table>

The mean and one standard deviation (SD) are listed for parameters measured during the sequential radioisotope injections among animals. Intra-animal differences in these parameters were much less (average 3.25 ± 1.6%) Abbreviations: HR = heart rate; Ao P = aortic pressure; LV = left ventricular systole and end-diastolic pressure; dp/dt = maximal rate of left ventricular pressure rise; Vmax = maximal velocity of contractile element velocity at zero load.
of perfusion for the four sequential injections. There appears to be fairly consistent variability in flow.

**Discussion**

The primary purpose of this investigation was to determine if temporal flow heterogeneity to small adjacent segments of the myocardium was present and to assess its magnitude. Before accomplishing this, the range of measurement variability associated with the labeled microsphere technique needed to be established. The simultaneous injection of four differently labeled radioisotopes demonstrates that the average range of variability associated with the microsphere technique is approximately 14% of mean flow. The variability is primarily dependent upon the number of spheres injected, radioisotope counting errors, and differences in reference arterial samples.

In a sequential radioisotope injection, variability due to nonhomogeneity of tissue in the ventricular segment and weighing errors are held constant by using each segment as its own control. The variability measured in these studies represents temporal changes in segmental flow as well as variability due to the microsphere technique. These variations in sequential flow were not thought to be due to change in total coronary blood flow or the hemodynamic parameters. The sequential studies were done with the animal in a stable state, as evidenced by measurements of cardiac hemodynamics and total coronary blood flow. In the sequential flow investigation, the range variability of individual segmental flow averaged 31%. This is much higher than the variation.

**Table 4**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Simultaneous</strong></td>
<td>12.3 ± 4.3</td>
<td>10.2 ± 4.7</td>
<td>12.2 ± 3.2</td>
<td>10.1 ± 4.8</td>
</tr>
<tr>
<td><strong>Sequential</strong></td>
<td>28.7 ± 10.2</td>
<td>15.2 ± 4.7</td>
<td>30.0 ± 11.3</td>
<td>15.6 ± 4.6</td>
</tr>
</tbody>
</table>

The first column under each subgroup denotes average range of variability normalized by mean of four flows. Second column denotes data normalized by mean of 4 flows divided again by 96 segment mean to lose the effect of reference flows.

A t-test performed on average flow variations from all segments in the simultaneous group versus all segments from the sequential group demonstrated the flow variations to be significantly different.
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(14%) seen in the simultaneous studies. This variation is thought to represent the average range of variation in flow to an individual segment over a 15 minute period.

Another part of this investigation concerned the spatial, and in particular, the transmural distribution of blood flow. Although many investigators have studied this problem, some investigators\textsuperscript{10, 11} have concluded the endocardium received less blood than the epicardium. Other investigators, using different techniques,\textsuperscript{12, 13} reported that endocardial and epicardial flow measurements are not significantly different. In the present study, there appears to be a small transmural gradient of myocardial blood flow through the wall, which confirms the results of other investigators.\textsuperscript{5, 14, 16}

The physiologic significance of regional flow changes in both space and time is not clear. In regard to the spatial distribution of flow, one may speculate that this may result from a demand due to higher stress or tension development in a particular region of the heart. Since discrete finite analysis of stress variation within the myocardial wall is not yet available, this speculation cannot be confirmed.

In regard to the temporal distribution of regional blood flow, this could be due either to spontaneous or autoregulatory changes in precapillary sphincters or arterioles. The spontaneous opening and closing of vascular beds has been seen in other muscle groups.\textsuperscript{1} Once again the reason for this is not clear, although it has been speculated that it is secondary to stress distribution. In the myocardium, opening and closing of capillary beds has been seen in response to hypoxia.\textsuperscript{18} Preliminary results from another laboratory,\textsuperscript{17} have also demonstrated temporal heterogeneity of myocardial perfusion in the canine heart. With injection of microspheres at 10–20 second intervals, a flow periodicity of between 45 and 90 seconds was detected.

This study documents that regional blood flow to the heart is a complex system that is related to both spatial and temporal factors. It notes that if the small segment of the heart is used for measurements at different times, changes may partially reflect either measurement errors or temporal heterogeneity. If a different segment is used, there is also a small change in flow due to position (spatial heterogeneity). The magnitude of these factors is established and it is incumbent upon the investigator, when assessing changes in myocardial blood flow, to analyze either large areas of the left ventricle or the total left ventricle if meaningful data are to be obtained.

Acknowledgment

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