Myocardial Micronecrosis Produced by Microsphere Embolization

Role of an α-Adrenergic Tonic Influence on the Coronary Microcirculation

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SUMMARY. Microspheres approximately 25 or 50 μm in diameter were systemically embolized from the left ventricular cavity. The number of microspheres given was empirically chosen to minimize the possibility of more than one microsphere lodging in an arteriole (3 mg/kg), yet was sufficient to allow for adequate histological assessment. The dogs were sacrificed after 24 hours, and focal areas of myocytolytic necrosis were noted in the myocardium. Groups of dogs were given pretreatment with drugs 10 minutes before embolization. Dogs pretreated with phentolamine (n = 8) and prazosin (n = 2) did not reveal any areas of myocardial necrosis after embolization with 25-μm microspheres. Cardiac lesions were also prevented in four of five dogs pretreated with verapamil. In contrast, cardiac lesions were not prevented by pretreatment with yohimbine (n = 2), dipyridamole (n = 3), propranolol (n = 2), or atropine (n = 2). Drug pretreatment with phentolamine or verapamil was not able to prevent cardiac lesions after embolization with 50-μm microspheres. Furthermore, despite a greater number of microspheres physically present in the subendocardial layer, the necrotic lesions were more frequent in the mid-wall and epicardial layers. Lesions produced by 25- or 50-μm emboli were also significantly smaller in the endocardium. Systemic embolization with microspheres excluding the coronary circulation did not produce cardiac lesions. We conclude that mechanical interruption of the coronary circulation with a 25-μm microsphere may be a necessary but not sufficient condition to produce cardiac necrosis. An α-adrenergic mechanism is also involved in the production of these lesions. The distinct transmural differences in distribution and size of the embolic lesions suggest the possibilities of a nonuniform transmural α-adrenergic tonic influence on the coronary microcirculation and/or a nonuniform transmural arteriolar hierarchy within the left ventricular myocardium. (Circ Res 54: 74–82, 1984)
droperidol 20.0 mg/ml, methylparaben 1.8 mg/ml (Pitman-Moore) intravenously (iv), pentobarbital (15 mg/kg, iv), or local anesthesia (subcutaneous 2% lidocaine in the femoral area). The group of dogs receiving Innovar and the two dogs receiving local anesthesia were conscious throughout the study and were trained to lie quietly during the procedure. The inner aspect of the left thigh was shaved and scrubbed with a Betadine solution. A cut down was performed at the mid-thigh level, and the left femoral artery was isolated. A 110-cm 8F pigtail catheter was inserted into the femoral artery and passed retrograde up into the ascending aorta. Heparin (5000 units) was given intra-arterially (ia). The aortic pressure was recorded. Some dogs received no drug pretreatment; the pretreated dogs received either: (1) phentolamine 1.0 mg/kg, ia; (2) prazosin, 1.0 mg/kg, ia; (3) yohimbine, 1.0 mg/kg, ia; (4) verapamil, 0.2 mg/kg bolus, followed by an iv infusion of 0.01 mg/kg per min, for 10 minutes; (5) dipyridamole, 0.5 mg/kg, ia; (6) propranolol, 2.0 mg/kg, ia; or (7) atropine, 0.5 mg/kg, ia. After 10 minutes, the pigtail catheter then was advanced into the left ventricular cavity, with the continuous pressure recording as a guide.

Microsphere Embolization

Unless noted otherwise, nonradioactive carbonized microspheres (3M Company) were used. Microspheres of approximately 25 μm (26.2 ± 3.4; mean ± SD) and 50 μm (52.5 ± 4.0) in diameter were suspended in a 63% sucrose solution with a few drops of Tween 80 added (1.0 g of microspheres in 100 ml of suspension). The concentration of microspheres was approximately 1,000,000 beads/ml and 120,000 beads/ml for the 25- and 50-μm microsphere suspensions, respectively. Before embolization, the microsphere suspensions were agitated for at least 15 minutes in an ultrasonicator (Bransonic). Either 25-μm (n = 31 dogs) or 50-μm (n = 11 dogs) microspheres were injected into the left ventricular cavity over a 10- to 15-second period at a dose of 3 mg/kg. This nonselective systemic embolization was performed 10 minutes after drug pretreatment. The catheter was then withdrawn into the aorta and the aortic pressure was recorded for a 10-minute period. The catheter was removed, the femoral artery ligated, and the wound was sutured.

Four dogs that received no pretreatment with a drug were embolized in the manner described above with radioactive 25-μm microspheres (85Sr). Arterial reference blood was withdrawn at a rate of 19.4 ml/min from a catheter in the other femoral artery starting just before the embolization and continuing for 1 minute. After sacrifice at 24 hours, several sections of the left ventricular free wall tissue were divided into endocardial, mid-wall, and epicardial portions and counted by γ-spectrometry (Searle Analytic model 1085 with Nuclear Data ND-60 pulse height analyzer). The transmural myocardial distribution and concentration of the 25-μm emboli was determined.

Three dogs premedicated with Innovar underwent the embolization procedure with the tip of the catheter above the coronary ostia. Using the pressure tracing as a guide, the catheter was passed into the left ventricular cavity. The catheter was pulled back to about 2 cm above the aortic valve. Radioactive 25-μm microspheres were injected so as to produce systemic embolization (including the central nervous system) without embolizing the myocardium. After sacrifice at 24 hours, several regions of the brain in these animals were counted to confirm embolization to the central nervous system, and portions of the left ventricular myocardium were counted to confirm that no microspheres entered the coronary vessels. The myocardial tissue was examined histologically for evidence of microvascular necrosis. This portion of the study was performed to evaluate if myocardial necrosis was due to primary extra-cardiac influences, in particular, centrally mediated reflexes.

Histological Studies

Except for three animals, all dogs were sacrificed, 24 hours postembolization. Pentobarbital (30 mg/kg, iv) and heparin (5000 units, iv) were given and the dogs were sacrificed with an iv injection of a concentrated potassium chloride solution. The hearts were removed and the proximal left anterior descending artery was dissected free postmortem. The LAD artery was cannulated and hand injected with a silicone rubber solution (Microfil). The hearts then were fixed in formalin. In the first 22 studies performed, a kidney was also removed postmortem for histological analysis.

Five to seven 0.5-cm thick sectors of an entire left ventricular ring (including septum) taken at the midventricular level were embedded in paraffin. Four- to 5-μm thick sections were stained with hematoxylin and eosin and were evaluated microscopically for evidence of necrosis. Tissue from 21 hearts were subsequently cleared with solvents, as described by Schaper (1971). Thick (approximately 1-mm) sections were sliced free-hand from these cleared specimens and microscopic examination of the microvasculature (defined by the Microfil) in relation to the microspheres was made using epifluorescence (Okun et al., 1979). The kidney samples were sectioned and stained with hematoxylin and eosin, and adequate dispersion of the microsphere emboli was assessed by determining the number of microspheres in an individual afferent glomerular arteriole. Three dogs pre-treated with phentolamine, 1.0 mg/kg, prior to embolization with 25-μm microspheres were sacrificed at 48 hours, 12 days, and 13 days, respectively. Histological examination of the hearts was performed as described above. These more chronic studies assessed whether the short-term protective effect of drugs would be circumvented with time.

Transmural Pattern of Microspheres and Necrosis

The transmural distribution of necrotic foci was quantitatively assessed in the seven non-pretreated dogs embolized with 25-μm microspheres. Four of these animals received radioactive 25-μm microspheres. Six transmural myocardial samples from these animals were divided into approximately equal endocardial, mid-wall, and epicardial pieces in each dog and submitted for γ-spectrometry to obtain the absolute number of microspheres in each layer per gram of tissue. Quantification of necrotic foci was performed on an entire left ventricular ring in each of the seven dogs. Each ring was further divided into an average of 6.0 ± 0.8 sectors. These sectors were photographed and enlarged seven to eight times. Each sector was divided into approximately equal endocardial, mid-wall, and epicardial areas on the photograph. The actual area of each transmural region was calculated by correcting the planimetered area on the photographs with the magnification factor. The number of lesions in each transmural area was counted microscopically under 40X power with occasional confirmation using higher powers. The normalized frequency of lesions was calculated as the number of lesions counted in each layer divided by the area of the layer surveyed.
Two methods were used to assess the size of the necrotic foci produced in the seven non-pretreated dogs embolized with 25-μm microspheres. The criterion for boundaries defining normal and necrotic tissue was the presence of hypereosinophilia and/or contraction bands. The generally ellipsoidal-shaped lesions were sized using an eyepiece micrometer under 100X magnification. The eyepiece micrometer superimposed a linear grid with 1-μm divisions and was able to be rotated to measure the maximum length and width of the lesions. Measurements were made to the nearest micron. As a gross estimate of the two-dimensional sections of the lesions, individual length and width measurements were multiplied to arrive at an area or size index. Measurements were performed on a total of 67 lesions with an average number quantified in each layer per dog of 2.7 ± 2.0, 3.3 ± 1.8, and 3.6 ± 2.6 for the endocardium, mid-wall, and epicardium, respectively. In each dog, the measurements were averaged for each transmural layer. The size or area of the lesion was also determined by point counting methodology on 62 lesions with an average number quantified in each layer per dog of 2.7 ± 2.0, 3.1 ± 3.3, and 3.0 ± 2.9 for the endocardium, mid-wall, and epicardium, respectively. Lesions were identified under 50.4X power and color photographed at 31.5X. A square grid with 50-μm divisions was also photographed under similar magnification. The two photographic slides were then superimposed and projected onto a screen. Point counting quantification of the size of the lesions was performed using the same criteria for lesion boundaries. The lesions produced by 50-μm emboli were assessed only in terms of the maximum length, width, and area index using the eyepiece micrometer. A total of 48 lesions were sized in the two non-pretreated dogs with an average number of quantified in each layer per dog of 7.0 ± 7.1, 7.5 ± 9.2, and 9.5 ± 7.8 for the endocardium, mid-wall, and epicardium, respectively. A total of 126 lesions were sized in eight dogs pretreated with phentolamine with an average number quantified in each layer per dog of 4.3 ± 5.5, 4.4 ± 5.3, and 7.1 ± 5.9 for the endocardium, mid-wall, and epicardium, respectively. The mean measurement value for each transmural layer in each dog was calculated and used for statistical analysis.

Summary data is presented as the mean ± SD. Hemodynamic data were analyzed using analysis of variance (ANOVA) and Student’s paired t-test, where appropriate. In order to satisfy the basic assumption of homogeneity of variances when using ANOVA methods, a variety of transformations were tested on the raw data summarized in the conventional manner in Tables 3 and 4. These transformations probably do not conform to Gaussian distributions, and appropriate transformations are necessary to interpret the results of ANOVA with confidence. The data on frequency of lesions were analyzed statistically after a square root transformation. Point count lesion size, lesion dimensions, and size index were analyzed after logarithmic transformations. The transmural distribution data of 25-μm microspheres per gram of tissue, frequency of lesions, and point count size of lesions produced by 25-μm emboli (Table 3) was analyzed using a one-way ANOVA with repeated measures followed by a Scheffé multiple comparison test for a significant F value. The morphometric data (Table 4) were analyzed in terms of lesion length, width, and size index using a 3 × 3 two-way ANOVA with repeated measures (transmural location). Significant differences between individual groups were compared using the Scheffé test. P < 0.05 was considered to be a significant difference.

Results

Hemodynamics

Only one death occurred as a result of embolization in this study. This occurred in a dog that was pretreated with verapamil and subsequently embolized with 50-μm microspheres. The dogs that received pentobarbital anesthesia (n = 15) had a higher pretreatment heart rate, 165 ± 34 beats/min, compared with dogs receiving Innovar (n = 28), 81 ± 21 beats/min, or local anesthesia (n = 2), 97 ± 5 beats/min, P < 0.01. The dogs that received pentobarbital anesthesia also had higher pretreatment mean arterial pressures: 124 ± 17 mm Hg vs. 100 ± 16 mm Hg and 102 ± 15 in Innovar and local anesthesia, respectively, P < 0.01. Pretreatment with phentolamine (n = 16) significantly increased the heart rate from 123 ± 53 to 147 ± 54 beats/min, P < 0.01, but did not significantly decrease the mean aortic pressure as measured 10 minutes after acute drug administration (just prior to embolization with microspheres), 104 ± 17 mm Hg decreasing to 99 ± 13 mm Hg, P = 0.19. Prazosin (n = 2) slightly increased the heart rate from 61 ± 7 to 63 ± 4 beats/min, and decreased the mean arterial pressure from 105 ± 14 to 100 ± 14 mm Hg. Yohimbine (n = 2) increased the heart rate from 63 ± 13 to 107 ± 35 beats/min, and the aortic pressure from 100 ± 7 to 108 ± 11 mm Hg. Verapamil (n = 6) increased the heart rate from 138 ± 43 to 156 ± 40 beats/min, P < 0.05, and decreased the mean aortic pressure from 125 ± 22 to 99 ± 20 mm Hg, P < 0.01. Dipyridamole (n = 3) caused a minimal effect on the heart rate from 151 ± 45 to 152 ± 36 beats/min, but decreased the mean aortic pressure from 117 ± 30 to 88 ± 16 mm Hg. Propranolol (n = 2) decreased the heart rate from 77 ± 11 to 75 ± 1 beats/min, and the mean aortic pressure from 106 ± 6 to 80 ± 6 mm Hg. Atropine (n = 2) increased the heart rate from 100 ± 34 to 234 ± 8 beats/min, and increased the mean aortic pressure from 105 ± 14 to 109 ± 16 when measured 10 minutes after drug administration.

The heart rate did not significantly change after embolization with microspheres, 129 ± 55 to 124 ± 54 beats/min. However, the mean aortic pressure rose from 100 ± 16 to 109 ± 19 mm Hg after embolization, P < 0.01. The left ventricular diastolic pressure also rose after embolization with microspheres, 3.2 ± 3.5 to 4.2 ± 5.1 mm Hg. The dogs without evidence of cardiac lesions (n = 17) responded hemodynamically to the embolization in a similar manner, compared with the group that had evidence of cardiac necrosis (n = 28), except for the mean aortic pressure. The group with no cardiac lesions had a slight but not significant rise in the aortic pressure after embolization: 96 ± 13 to 98 ± 15 mm Hg. However, the group with cardiac lesions had a significant rise in the aortic pressure after embolization, 103 ± 18 to 116 ± 19 mm Hg, P = 0.03, a significant interaction effect in the analysis of variance.
General Microscopic Findings

The cardiac lesions, when present, were focal, sharply delimited zones of acute necrosis appearing hyperesinophilic with contraction bands (Fig. 1). A mild to moderate acute inflammatory infiltrate was associated with the necrotic myocardial cells. Areas of micronecrosis were generally associated with a surrounding zone of absent Microfil perfusion. However, the necrotic foci were usually smaller than the Microfil deficient area, surrounded by a zone of apparently normal cells (Figs. 1 and 2). The lesions and Microfil-deficient foci were invariably asymmetrical and ellipsoidal in shape, with the long axis generally parallel to the endocardial or epicardial surface and parallel to the adjacent orientation of the muscle fibers.

The adequacy of microsphere dispersion was assessed by examination of kidney tissue and cleared myocardial specimens. Only one microsphere, when present, was associated with a given glomerulus. The cleared myocardial specimens, in general, revealed singular microspheres. Groups of two, and rarely more than two, microspheres were noted in close proximity to each other, occasionally. It is not clear whether this grouping represents inadequate dispersion, or was a consequence of the vascular anatomy. For instance, a vessel that gives off several branches in close proximity may receive microsphere emboli lodging at the ostia of the branch points. This may appear as a cluster of microspheres on a cleared specimen. Thus, the findings in the kidney tissue are probably a more reliable index of adequate dispersion. Figure 3 demonstrates that Microfil appears to be able to pass around the microsphere, suggesting that microspheres by themselves may not be entirely occlusive of the vascular lumen. Thus, if complete occlusion of a vessel is required to produce necrosis, a factor in addition to the presumed partial mechanical obstruction by a microsphere must be postulated.

Drug Pretreatment

Table 1 summarizes the histological findings in the animals embolized with 25-μm microspheres. Foci of myocardial micronecrosis were noted in all animals pretreated with dipyridamole (n = 3), propranolol (n = 2), atropine (n = 2), yohimbine (n = 2), and no drug (n = 7). In contrast, none of the animals pretreated with phentolamine (n = 8) and prazosin (n = 2) showed evidence of cardiac lesions. Only one of five animals pretreated with verapamil showed evidence of myocardial micronecrosis. This animal received only a bolus of verapamil as pretreatment, whereas the four animals without cardiac lesions received both a bolus and a 10-minute infusion of verapamil. The type of anesthesia and the state of consciousness of the animal did not have a bearing on the results. Animals pretreated with phentolamine, prazosin, and verapamil had diffuse Microfil perfusion, even around histologically identified 25-μm microspheres, with no region of absent vascular filling. Embolization with 50-μm microspheres produced lesions in all 11 animals, regardless of pretreatment: no pretreatment (n = 2), pretreatment with phentolamine (n = 8), and verapamil (n = 1) (Table 2). The three dogs acutely pretreated with phentolamine, embolized with 25-μm microspheres, and sacrificed at 48 hours, 12 days, and 13 days did not reveal any evidence of cardiac necrosis.

![Figure 1. An area of myocytolytic necrosis presumably caused by the eccentrically located 25-μm microsphere. The Microfil perfusion of the vasculature outlines an ellipsoidal region devoid of perfusion. Within this Microfil-absent region is a margin of normal-appearing tissue surrounding the necrotic focus. It is possible that this rim of normal-appearing cells was preserved by diffusional processes. Contraction bands (arrows) are present within the lesion H & E, 200X.](http://circres.ahajournals.org/figure/1)
The three dogs that received embolization above the coronaries did not have any cardiac lesions. In these dogs, the presence of radioactive microspheres was documented in the brain by radioactive counting.

**Transmural Pattern of Micronecrosis**

In the seven untreated dogs embolized with 25-μm microspheres, a disproportionate number of lesions were noted in the mid-wall myocardium. In order to quantify this observation, the frequency of lesions in each layer was normalized to the area surveyed. The average area surveyed in each region per dog was, 4.87 ± 0.85 cm² (endocardium), 4.88 ± 0.74 cm² (mid-wall), and 5.04 ± 0.84 cm² (epicardium), with a mean total area of 14.8 ± 2.3 cm². The average number of lesions counted in each layer...
The size of the lesions in each layer was not significantly different using the point-counting method performed on lesions in Table 3. The large variability of the lesion size, even within a given transmural layer, may be a factor in the nonsignificant comparison. This variability appears unavoidable when quantifying two-dimensional sections of three-dimensional phenomena, and is not due to lack of precision of measurements. Nevertheless, the index does give a useful estimation of the relative lesion size differences transmurally. The data in Table 4 were analyzed using a 3 X 3 two-way ANOVA with repeated measures. The repeated measures were the transmural locations and the three groups of data considered were the lesions produced by: (1) 25-μm emboli, no pretreatment (n = 7); (2) 50-μm emboli, no pretreatment (n = 2); and (3) 50-μm emboli with phentolamine pretreatment (n = 8). The lesions produced by 25-μm emboli were smaller than those produced by 50-μm emboli, P < 0.025. Lesion measurements were smaller in the endocardium, compared with the mid-wall or epicardium. Phentolamine pretreatment did not significantly alter lesion dimensions or size in any layer of the dogs embolized with 50-μm microspheres. However, larger

### TABLE 2

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Innovar</th>
<th>Pentobarbital</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3 (3)</td>
<td>2 (2)</td>
<td>7 (7)*</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>0 (6)</td>
<td>0 (2)</td>
<td>0 (8)</td>
</tr>
<tr>
<td>Prazosin</td>
<td>0 (2)</td>
<td>0 (2)</td>
<td></td>
</tr>
<tr>
<td>Yohimbine</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>0 (2)</td>
<td>1 (3)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Diprydamol</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Propranalol</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Above coronaries</td>
<td>0 (3)</td>
<td>0 (3)</td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>10 (23)</td>
<td>5 (9)</td>
<td>17 (34)</td>
</tr>
</tbody>
</table>

Results are expressed as number of animals with myocardial lesions. Total number of animals in each subgroup is denoted within parentheses.

*Includes data from two dogs given local anesthesia and no pretreatment.

### TABLE 3

<table>
<thead>
<tr>
<th>Anesthesia</th>
<th>Beads/g tissue</th>
<th>Frequency of lesions/cm²</th>
<th>Point count of lesion size (mm²)</th>
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</thead>
<tbody>
<tr>
<td>Endocardium</td>
<td>259±120*</td>
<td>0.92±0.86</td>
<td>0.052±0.029†</td>
</tr>
<tr>
<td>Mid-wall</td>
<td>193±87</td>
<td>2.70±1.08‡</td>
<td>0.110±0.072‡</td>
</tr>
<tr>
<td>Epicardium</td>
<td>167±68</td>
<td>1.26±0.84</td>
<td>0.112±0.081</td>
</tr>
</tbody>
</table>

* P < 0.025 compared to epicardium.
† P < 0.025 compared to mid-wall and epicardium.
‡ P < 0.001 compared to endocardium and epicardium.

### TABLE 4

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Length (μm)</th>
<th>Width (μm)</th>
<th>Size Index (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-μm emboli(none pretreatment, n = 7)</td>
<td>246±62*</td>
<td>136±48*</td>
<td>0.037±0.020*</td>
</tr>
<tr>
<td>Mid-wall</td>
<td>543±255†</td>
<td>208±107</td>
<td>0.136±0.156</td>
</tr>
<tr>
<td>Epicardium</td>
<td>473±246</td>
<td>230±52</td>
<td>0.121±0.096</td>
</tr>
<tr>
<td>50-μm emboli(none pretreatment, n = 2)</td>
<td>363±117</td>
<td>204±16</td>
<td>0.076±0.031</td>
</tr>
<tr>
<td>Mid-wall</td>
<td>709±98</td>
<td>325±84</td>
<td>0.231±0.090</td>
</tr>
<tr>
<td>Epicardium</td>
<td>668±134</td>
<td>328±112</td>
<td>0.232±0.125</td>
</tr>
<tr>
<td>50-μm emboli(phentolamine, n = 8)</td>
<td>412±142*</td>
<td>211±100</td>
<td>0.101±0.091*</td>
</tr>
<tr>
<td>Mid-wall</td>
<td>835±127</td>
<td>229±83</td>
<td>0.198±0.085‡</td>
</tr>
<tr>
<td>Epicardium</td>
<td>827±180</td>
<td>360±142†</td>
<td>0.301±0.134</td>
</tr>
</tbody>
</table>

* P < 0.01 compared to mid-wall and epicardium.
† P < 0.01 compared to mid-wall and endocardium.
‡ P < 0.05 compared to epicardium.
numbers of dogs are necessary to adequately reduce the probability of a type II error.

**Discussion**

The micronecrotic lesions produced by the microspheres were of the contraction band or myocytolytic type of necrosis. This type of necrosis is often seen in catecholamine-induced myocardial injury (Reichenbach and Benditt, 1969; Baroldi, 1975), as well as in the "reperfusion" injury seen after temporary coronary occlusion (Sommers and Jennings, 1964; Herdson et al., 1965; Bulklely and Hutchins, 1977). These findings, along with the unusual transmural distribution pattern of necrosis, motivated us to pretreat a group of dogs with an α-receptor-blocking drug, phentolamine. None of the eight animals pretreated with phentolamine and embolized with 25-μm microspheres showed evidence of myocardial necrosis. Necrosis was also prevented in the two dogs pretreated with prazosin. However, pretreatment with the relatively selective α1-blocking agent, yohimbine, did not prevent necrosis, suggesting that the mechanism involved is mediated via α1-adrenergic receptors. Necrosis was prevented in four of five dogs pretreated with verapamil. The efficacy of this drug during embolization may also be related to its effects on the adrenergic receptors. Competitive inhibition of α1-adrenergic receptors by verapamil has recently been demonstrated in the rat myocardium, as well as other tissues (Karliner et al., 1982; Barnathan et al., 1982; Motulsky et al., 1981).

The prevention of necrosis by α-blockade suggests that the microsphere embolus may be a necessary but not sufficient condition to produce necrosis, and, by itself, may not be entirely occlusive within the vascular lumen. Indeed, there is in vivo evidence that the microspheres are not totally occlusive (Harell et al., 1977; Hales and Cliff, 1977). Figure 3 demonstrates that Microfil is able to pass around the microsphere to fill the distal portion of the arteriole. Since the microspheres may not be entirely occlusive of the vessel lumen, an additional factor presumably must be involved to occlude the vascular lumen totally, to produce necrosis. Our findings suggest that this factor appears to involve an α1-adrenergic mechanism. Morhman and Feigl (1978) presented evidence for a competition between α-mediated coronary vasoconstriction and metabolic vasodilation. It was later demonstrated that a significant portion of the α-mediated vasoconstriction occurred distal to the large epicardial coronary vessels (Kelly and Feigl, 1978). Schwartz and Stone (1977) demonstrated an α-adrenergically mediated tonic influence on the coronary vessels that continued to be present, despite ischemia produced by a temporary coronary occlusion. Finally, a study in man (Mudge et al., 1979) suggested an inappropriate vasoconstrictor response resulting in ischemia mediated by the α-adrenergic stimulation of the cold pressor test. Our results indicate that not only can the α-mediated tonic influence compete successfully against local metabolic vasodilation, but this mechanism can persist long enough to produce overt cellular damage and necrosis.

**Possible Mechanisms**

Without direct visualization of the event caused by a microsphere embolus, it is possible, only, to speculate on the mechanism involved. There are several possibilities: (1) local (myogenic) or extracardiac reflex-mediated microvascular vasoconstriction (spasm), (2) a tonic microvascular influence that is released by α-blockade, permitting microspheres to lodge more distally in the vasculature, and (3) local release of catecholamines with direct cellular injury. Catheter-induced large vessel coronary spasm has been routinely noted in the clinical setting. Local irritation of the microvasculature by a microsphere may elicit a similar response. Two studies indicate that microsphere embolization can produce what appears to be coronary spasm (Guzman et al., 1962; West et al., 1962). In these nearly identical studies, lycopodium spores or glass microspheres of 30 to 40 μm were embolized selectively into the left anterior descending artery of anesthetized closed-chest dogs. Selective coronary arteriography of the embolized vessel demonstrated a "cut-off" pattern indicating minimal or no coronary flow. This pattern persisted for at least 50 seconds, and, possibly, for several minutes. Arteriography 3-5 minutes later revealed a fully patent vessel. Additionally, one of the studies measured the coronary sinus blood flow during the embolization procedure and demonstrated, in Figure 1 (West et al., 1962), what can be interpreted as an occlusive event lasting up to 1 minute followed by a reactive hyperemic response. This sequence of events is compatible with the coronary flow response that would occur after a transient spastic episode. Since these were both angiographic studies, it is not clear where the locus of the presumed coronary spasm occurred (large vessel vs. microvascular level). However, if a spastic mechanism were involved in our study, it must have occurred at the microvascular level, since the necrotic lesions are too small to be produced by a large vessel occlusion, nor is the transmural distribution of necrosis consistent with a transient large vessel occlusion. Furthermore, although the two angiographic studies demonstrated apparent complete delineation of the embolized artery after several minutes (presumably, too short a duration to produce necrosis), it is possible that—at the microvascular level—reperfusion may not occur homogeneously. The phenomenon may persist at certain foci, resulting in necrosis, but would not be macroscopically (angiographically) apparent. Finally, Weideman and Tuma (1979) performed a study which directly visualized the effects of 9- or 15-μm microspheres in the hamster cheek pouch and bat wing microcirculation. They observed that microspheres often initi-
ated contractile activity in the arteriolar vessels, sometimes rhythmic in nature. These direct observations along with the indirect findings of Guzman et al. (1982), West et al. (1962), and our present results strongly suggest that a microvascular spastic phenomenon may be involved in the pathogenesis of lesions produced by 25-μm emboli.

A microvascular spastic mechanism does not have to be invoked to explain our findings. α-Adrenergic blockade could have abolished a tonic influence on the microvasculature that permitted the 25-μm microspheres to lodge more distally in the vasculature at a point where diffusional processes can preserve the myocardial cells. However, this possibility would not negate the fact that the α-mediated tonic influence could not be overridden by the local metabolic vasodilation that presumably occurred prior to cell necrosis in the untreated animals. Furthermore, the morphometric measurements performed on the lesions produced by 50-μm emboli (Table 4) indicates that phenolamine pretreatment did not significantly alter the size of the lesions. It would be expected that if α-blockade permitted the microspheres to lodge more distally, the resultant lesions would be smaller than lesions in the untreated group. Finally, it is possible that the embolization may release local catecholamines which exert direct toxic effects on the cells. This mechanism to explain the necrosis appears unlikely, since phenolamine was unable to prevent necrosis after embolization with 50-μm microspheres. In addition, β-blockade was ineffective in preventing lesions produced by 25-μm emboli. The inability of α-blockade to prevent necrosis after embolization with 50-μm microspheres suggests that mechanical blockade (even partial) at this level of the vasculature represents the upper limit of substrate delivery via direct or diffusional processes. Alternatively, vasomotion of these larger arterioles may not be affected by α-blockade. This would be a rather precise localization of α-adrenergic influences on the coronary microvasculature. However, it seems reasonable to assume that there must be a critical level of the vasculature where mechanical blockade, even partial, should inevitably result in cellular damage. It may be quite fortuitous that the correct size of microspheres (25 μm) was initially chosen, which led to our findings.

Nonuniform Transmural Pattern of Micronecrosis

Necrosis was more frequent in the mid-wall and epicardial layers than in the endocardial layer, despite the fact that more microspheres were present in the endocardium (Table 3). This unusual and unexpected finding may suggest a transmural variability in sympathetic innervation and/or α1-receptor density in the left ventricular myocardium. Guidicelli et al. (1980) demonstrated a greater decrease in epicardial compared to endocardial flow after left stellate ganglion stimulation and β-blockade. Johannesen et al. (1982) confirmed these findings and suggested a nonuniform transmural influence of sympathetic nerve stimulation even in the presence of "maximal" coronary dilation with adenosine. Our results are consistent with these findings. The transmural variation in lesion size and frequency also raises the possibility of a nonuniform transmural vascular hierarchy. This notion has been alluded to by Bassingthwaighte et al. (1974) but not quantified. A 25-μm arteriole in the epicardium or mid-wall may give rise to a greater number of post-arteriolar branches than the endocardial arterioles. Thus, occlusion of an epicardial or mid-wall 25-μm arteriole may have more significant consequences. No necrosis was noted in the hearts of the three animals that were embolized just above the coronary ostia. This suggests that the myocardial necrosis was not due to a primary central, or a secondary humoral mechanism. However, a cardiocardiac reflex mediated via spinal pathways cannot be excluded (Malliani et al., 1972). No evidence of cardiac necrosis was noted in those dogs pretreated with phentolamine at the time of embolization and followed over several days. This suggests that the mechanism resulting in necrosis is relatively an "all or none" type of phenomenon occurring probably within the first several hours or less.

The findings in this study may have some relevance to other studies. Jørgensen et al. (1967) demonstrated that platelet aggregation induced by intracoronary infusion of adenosine diphosphate can produce myocardial necrosis. Haft et al. (1972a) presented evidence that catecholamine-induced myocardial lesions may be caused by platelet-aggregated plugs. The catecholamine-induced myocardial injury was prevented by inhibition of platelet aggregation (Haft et al., 1972b). Platelet aggregates may represent the in vivo analog to microsphere emboli. The progressive genetic cardiomyopathy of the Syrian hamster has been shown to be prevented by verapamil treatment (Jasmin and Bajusz, 1975). Factor et al. (1982b) believe that the etiology of the cardiac lesions may be due to microvascular spasm that can be prevented with verapamil. The initial myocardial lesions found in the Syrian hamster are similar to those produced in our embolization studies (focal myocardolysis) and the transmural distribution pattern of lesions are similar (preference to the mid-wall). The results of this study also raise the possibility that the use of labeled macroaggregated albumin for myocardial perfusion studies in humans (Endo et al., 1970; Ashburn et al., 1971) may not be entirely benign. These particles range from 20 to 70 μm in diameter. The half life of the particles range from 5 hours (macroaggregated albumin) to 9 hours (human albumin microspheres). Finally, our results should raise issues regarding certain analyses of infarct size in relation to the region at risk after large coronary artery occlusion. Jugdutt et al. (1979) found a linear relationship between the mass of the infarct
and the area at risk with an intercept (no necrosis) when the mass at risk was approximately 20 g. A similar analysis was performed by Koyanagi et al. (1982) for each transmural layer. Our results at the microvascular level would suggest that such analyses would not be generally applicable for the coronary microcirculation.

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References


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