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EXPERIMENTAL STUDIES

Enhancement of Salvage of Reperfused Ischemic Myocardium by Diltiazem

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Concomitant use of pharmacologic agents may be required for maximal salvage of ischemic myocardium by reperfusion. Accordingly, in dogs with induced thrombotic coronary occlusion, the effects of intravenous diltiazem given 30 minutes before administration of streptokinase on myocardial blood flow and myocardial salvage were evaluated. Two independent types of end points were employed. Positron emission tomography was utilized for noninvasive assessment of myocardial perfusion and infarct extent. Direct measurements included quantification of myocardial infarction by assay of creatine kinase activity in myocardial homogenates. Infarct extent averaged 27.9 ± 11.4% of left ventricular weight in 10 control dogs in which coronary occlusion was maintained for 24 hours. In eight dogs given streptokinase alone, the infarct extent averaged 16.7 ± 10.0% of left ventricular mass ($p < 0.05$ versus control). In nine other dogs given diltiazem (15 μg/kg per min continuously until death was induced) beginning 30 minutes before streptokinase, infarct extent averaged 9.4 ± 6.7% of left ventricular mass ($p < 0.05$ compared with reperfusion alone). At the dose administered, diltiazem did not alter blood flow, heart rate or mean arterial pressure after coronary occlusion or thrombolysis.

The region at risk, determined in 16 dogs from perfusion images obtained with positron tomography and oxygen-15–labeled water after coronary occlusion, was similar in the three groups (30.6 ± 7.3% of the left ventricle in six control dogs, 31.8 ± 4.5% in five dogs with reperfusion alone and 30.5 ± 11.6% in five dogs with reperfusion plus diltiazem). Infarct size quantified in terms of the extent of myocardium exhibiting less than 50% of peak carbon-11–labeled palmitate uptake 24 hours after occlusion and expressed as the percent of the region at risk averaged 89.6 ± 11.4% in control dogs, was significantly reduced to 45.1 ± 29.8% in dogs with reperfusion alone and was reduced further to 22.3 ± 16.4% in dogs given diltiazem and reperfusion. Thus, concomitant treatment with diltiazem markedly enhances salvage of reperfused myocardium after coronary thrombolysis.

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Coronary thrombolysis is a promising means for salvaging ischemic myocardium. Results from experimental animals have documented salvage and suggested that it may be facilitated by concomitant administration of pharmacologic agents (1). Calcium channel blockers offer particular promise because of the potentially deleterious effects of intracellular influx of calcium that accompanies reperfusion. Furthermore, they appear to exert beneficial effects on the mechanical function of myocardium subjected to ischemia followed by reperfusion (2,3).

One of the difficulties encountered in determining whether adjunctive pharmacologic approaches are beneficial is that of quantifying the extent of infarction in the setting of reperfusion. Despite the availability of methods for assessing infarct size at necropsy, determination of the infarct extent with conventional techniques in vivo is subject to error. Positron emission tomography permits noninvasive estimation of myocardial perfusion and metabolism and sequential delineation of myocardium at risk. It has been utilized for evaluation of interventions designed to limit myocardial damage (4). However, to avoid ambiguous interpretation, results should be referenced to direct measurements under comparable conditions.

The present study was undertaken to determine whether calcium channel blockade with diltiazem enhances salvage of myocardium by reperfusion in dogs with thrombotic coro-

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nary occlusion and in which coronary thrombolysis is induced by streptokinase. Direct end points included assessment of myocardial creatine kinase depletion and delineation of the distribution of radiolabeled microspheres in myocardium. Positron emission tomography was employed to define the extent of myocardium undergoing infarction that could be characterized noninvasively and to evaluate sequential changes in risk region and the extent of apparent injury. Correlative studies were undertaken to determine whether the effects of the pharmacologic intervention coupled with reperfusion would distort relation between tomographically measured perfusion and perfusion measured directly and between tomographically estimated injury and infarct size measured directly. The results obtained indicate that diltiazem enhances salvage of myocardium subjected to reperfusion.

Methods

Animal preparations. Dogs of either gender were fasted overnight, premedicated with subcutaneous morphine sulfate (1 mg/kg body weight) and anesthetized with intravenous sodium pentothal (12.5 mg/kg) and alpha-chloralose (60 mg/kg). After endotracheal intubation, ventilation was maintained with room air using a Harvard respirator. An intravenous catheter was placed in a peripheral vein for infusion of drugs. Catheters were placed in the abdominal aorta and vena cava through the femoral artery and vein. A left atrial catheter was introduced through the other femoral artery and advanced across the aortic and mitral valves under fluoroscopic guidance. A modified Amplatz catheter was inserted into the left common carotid artery.

Arterial pressure and the electrocardiogram were monitored and recorded with a Gould two-channel recorder. Before induction of coronary occlusion, a loading dose of lidocaine, 1 mg/kg, was given intravenously, followed by a continuous infusion of 50 μg/kg per min.

Coronary thrombosis was induced as described previously (4). After initial coronary arteriography had been performed, a 0.021 inch (0.053 cm) guide wire was advanced into the left anterior descending coronary artery and the coronary catheter was withdrawn. A copper coil (diameter 3 mm, length 5 mm) was advanced over the guide wire with a small (diameter 1.2 mm) flexible catheter until the coil lodged in the left anterior descending coronary artery. The guide wire was removed and the small intracoronary catheter was positioned approximately 1 cm proximal to the coil. This catheter was used for injections of contrast medium and for intracoronary administration of streptokinase.

To verify complete occlusion of the left anterior descending coronary artery by thrombus at the site of the coil, angiography was repeated after ST segment elevation had developed. In animals given streptokinase, angiography was repeated to document restoration of patency of the artery. The coil was then removed by pulling a fine suture to which it had been attached. Thus, reperfusion was maintained without the need for heparinization.

In animals to be given diltiazem, a polyvinyl catheter was inserted in the left external jugular vein, tunneled beneath the skin and exteriorized between the scapulae. Sustained infusion of diltiazem was accomplished with the use of a portable infusion pump (Auto-Syringe, Inc.). The other catheters were removed, incisions were closed and the animals were allowed to recover from anesthesia.

Ventricular myocardial samples. On the next day, animals were anesthetized again and coronary angiography was repeated to confirm the persistence of occlusion in control dogs and the persistence of patency of the left anterior descending coronary artery in dogs given streptokinase. Twenty-four hours after coronary occlusion, cardiac arrest was induced with an overdose of saturated potassium chloride given intravenously. The heart was removed rapidly and rinsed promptly in saline solution at 0 to 4°C. The left anterior descending artery and its branches were examined to determine whether thrombus was present and to detect any signs of trauma from manipulation of catheters. Samples of left ventricular myocardium were obtained for assay of radioactivity associated with microspheres. Tissue samples were obtained for assay of myocardial creatine kinase and protein in normal myocardium from the posterior left ventricle and from homogenates of the remainder of the left ventricle.

Experimental protocols. Three groups of dogs were studied. In 15 control dogs, occlusion of the left anterior descending coronary artery was maintained for 24 hours. Thrombolytic agents were not given and the copper coil was left in place until the heart was excised. In the other two groups, comprising 26 dogs, intracoronary streptokinase (4,000 IU/min for 90 minutes) was administered beginning 2 hours after coronary occlusion. Thirteen of the dogs were not given diltiazem. They constituted the "reperfusion alone" group. The other 13 dogs were given diltiazem (generously supplied by Marion Laboratories, Inc.) intravenously at a dose of 15 μg/kg per min beginning 90 minutes after coronary occlusion, that is, 30 minutes before the onset of infusion of streptokinase. The infusion of diltiazem was continued until the heart was excised. The dose of diltiazem was selected to minimize alterations of heart rate or blood pressure and to be sufficient for effective calcium channel blockade.

For assessment of myocardial blood flow and myocardial viability, two types of experiments were performed. Effects of thrombolysis with or without diltiazem on myocardial blood flow and infarct extent were determined with the use of radiolabeled microspheres injected into the left atrium, and by direct assay of radioactivity in myocardial samples and direct analysis of myocardial creatine kinase depletion at necropsy. In other experiments, effects of thrombolysis

with or without diltiazem on myocardial blood flow and metabolism were assessed sequentially by positron emission tomography with oxygen-15–labeled water and carbon-11–labeled palmitate, respectively. In these experiments, regional myocardial blood flow was quantified with radiolabeled microspheres as well. Some dogs were evaluated according to both protocols.

Dogs that developed ventricular fibrillation after coronary occlusion or reperfusion and those that did not survive for 24 hours were excluded. The number of animals excluded from the two groups subjected to reperfusion (with and without diltiazem) was the same, that is, 11 from each group. These dogs did not have perfusion data different from dogs that survived. Animals with no region of myocardium after coronary occlusion exhibiting a flow of less than 25% of that in normal regions (measured with microspheres) were excluded (n = 8) because of the lack of a substantial region at risk.

Myocardial blood flow. Regional myocardial blood flow was measured with 15 μ radiolabeled microspheres according to the standard reference withdrawal method (5). For each determination of flow, 3 to 4 million microspheres labeled with cesium-14, chromium-51, tin-111, strontium-85 or scandium-46 were injected into the left atrium. Flow was determined throughout three 1 to 1.5 cm thick sections of myocardium. Each was divided into central, lateral and septal ischemic zones and one posterior normal region. Samples were trimmed of epicardial fat and connective tissue and subdivided into endocardial and epicardial layers.

Flow was determined in some animals before induction of ischemia and in all animals 60 to 90 minutes after occlusion of the left anterior descending coronary artery and again 24 hours after occlusion. Microspheres were injected 1 hour after reperfusion in dogs given streptokinase.

Determination of infarct size. The extent of myocardial infarction was determined at necropsy by assay of creatine kinase activity in left ventricular homogenates as described previously (6). Tissue samples used for analysis of the distribution of microspheres were kept frozen throughout the procedure for assay of radioactivity and added to the remainder of the heart before homogenization for assay of total creatine kinase activity. Total creatine kinase per gram of left ventricle was compared with creatine kinase per gram in normal myocardium from the posterobasal left ventricular wall for calculation of infarct size expressed as a percent of left ventricular weight.

Positron emission tomography. Sixteen dogs were evaluated by positron emission tomography. They were placed within a plexiglass shell positioned within a tomograph (PETT VI) such that the entire left ventricle was within the field of view. Data acquisition was performed in high resolution mode (full width at half maximum = 0.7 cm) with simultaneous acquisition of data from seven transverse sections with a separation between slices of 1.4 cm (7). Emission scans were corrected for attenuation with transmission data acquired with an external ring source of germanium-68. Myocardial perfusion and metabolism were assessed with oxygen-15–labeled water ($H_2{}^{15}O$) and carbon-11–labeled palmitate (^{11}C-palmitate) respectively.

For each determination of the distribution of myocardial blood flow, 45 to 55 mCi of $H_2{}^{15}O$ in saline solution was injected intravenously as a bolus. Six consecutive 10 second images were collected beginning at the time of injection. Data collection in the interval from 10 to 50 seconds after injection was used for determination of relative tissue myocardial blood flow. Approximately 10 minutes later, when activity from $H_2{}^{15}O$ (half-life = 2.1 minutes) had decayed to near background levels, 30 mCi of oxygen-15–labeled carbon monoxide ($C^{15}O$) was administered by inhalation to label the blood pool, permitting correction for intravascular tracer consistent with a method previously developed and validated in our laboratory (8). Flow in ischemic zones was expressed in terms of flow as a fraction of flow in normal regions.

Approximately 10 minutes later, when activity from $C^{15}O$ had decayed to near background levels, a bolus of 25 to 35 mCi of ^{11}C-palmitate was injected intravenously. Ten consecutive images over intervals of 2 minutes each were col-

Table 1. Heart Rate and Mean Arterial Pressure after Coronary Occlusion and After Reperfusion

	Control (n = 15)	Reperfusion Alone (n = 13)	Reperfusion Plus Diltiazem (n = 13)
Ischemia for 1 hour			
Heart rate (beats/min)	102 ± 25	104 ± 13	105 ± 24
Mean arterial pressure (mm Hg)	137 ± 5	120 ± 19	116 ± 21
Ischemia for 2 hours			
Heart rate (beats/min)	98 ± 40	95 ± 18	106 ± 15
Mean arterial pressure (mm Hg)	144 ± 5	119 ± 14	115 ± 21
Reperfusion for 1 hour			
Heart rate (beats/min)	—	138 ± 46	123 ± 51
Mean arterial pressure (mm Hg)	—	118 ± 10	110 ± 15

Values are means ± SD. n = number of dogs.

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lected beginning with the time of injection. In animals given streptokinase, tomography was repeated 1 hour after reperfusion.

For analysis of blood flow or distribution of palmitate, 1 cm^3 transmural regions of interest were selected from normal septal, posterior and posterolateral regions of the left ventricle and from anterolateral, anterior and anteroseptal ischemic zones. Identical regions of interest were analyzed in both $H_2{}^{15}O$ and ^{11}C-palmitate tomograms.

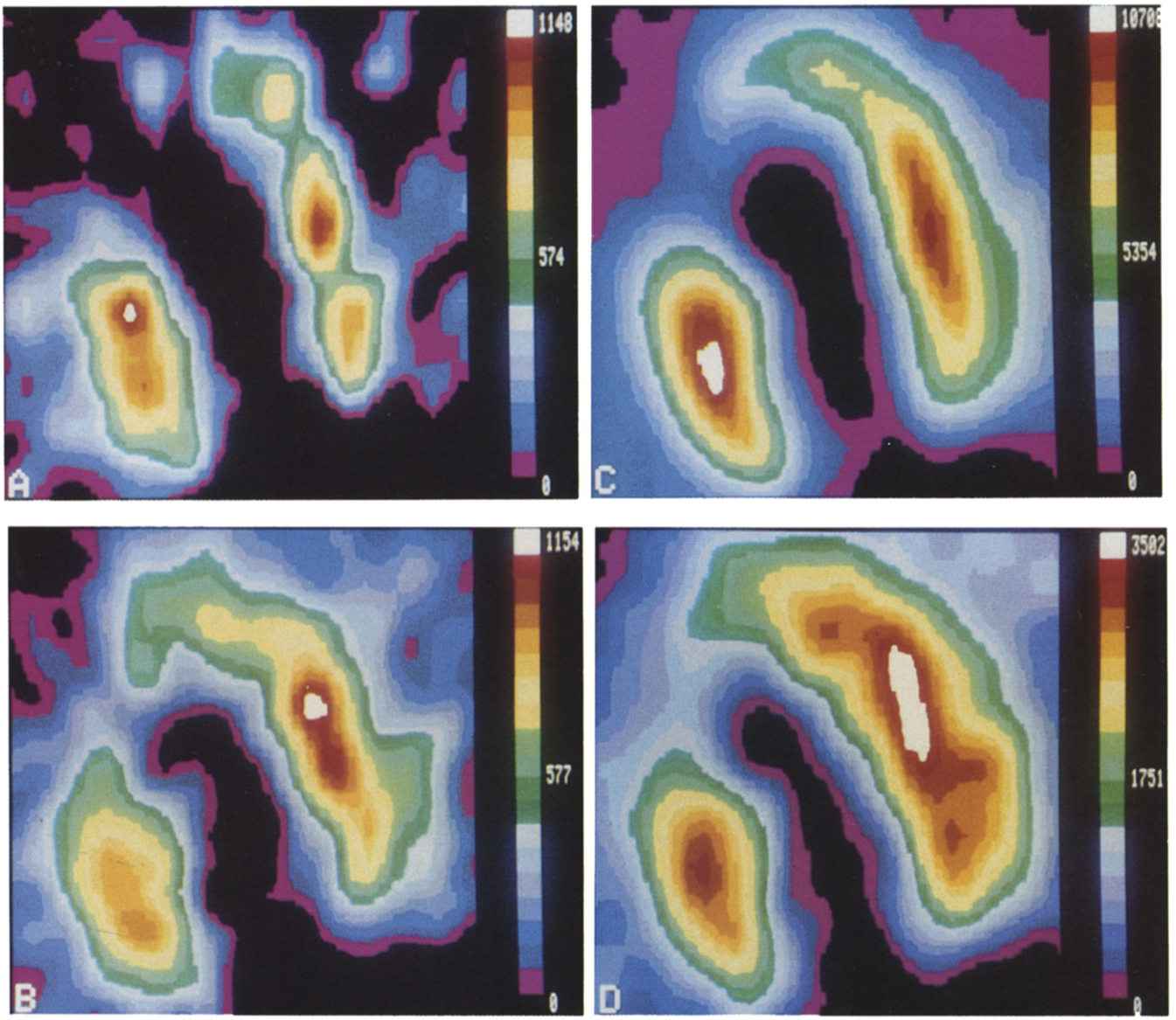
Isocount contours representing 50% of peak myocardial counts were constructed. The percent of myocardium subjected to reduced perfusion was defined as the region at risk, and the percent exhibiting reduced metabolism was defined as the region of injury. Regions exhibiting reduced uptake of palmitate under these conditions correlate closely with zones of infarction that are identifiable histologically and enzymatically (9).

Statistical analysis. Dogs exhibit considerable variation of infarct size in response to occlusion of the left anterior descending coronary artery and in response to interventions designed to limit infarct size. Accordingly, we employed nonparametric tests for analysis of differences (10). Comparisons between groups were made with the Mann-Whitney U test. Sequential measurements within a group were com-

Table 2. Myocardial Blood Flow (ml/min per g) Determined With Radiolabeled Microspheres in 41 Dogs

	Control (n = 15)	Reperfusion Alone (n = 13)	Reperfusion Plus Diltiazem (n = 13)
Ischemia (for 2 hours)			
Lateral ischemic			
Endocardium	0.15 ± 0.19	0.17 ± 0.23	0.09 ± 0.08
Epicardium	0.14 ± 0.14	0.18 ± 0.21	0.15 ± 0.08
Central ischemic			
Endocardium	0.08 ± 0.09	0.06 ± 0.05	0.07 ± 0.07
Epicardium	0.13 ± 0.08	0.20 ± 0.14	0.12 ± 0.06
Septal ischemic			
Endocardium	0.16 ± 0.18	0.10 ± 0.11	0.13 ± 0.09
Epicardium	0.21 ± 0.19	0.23 ± 0.15	0.17 ± 0.11
Posterior normal			
Endocardium	1.04 ± 0.62	0.83 ± 0.07	0.93 ± 0.53
Epicardium	0.97 ± 0.61	0.78 ± 0.18	0.84 ± 0.55
Reperfusion (for 1 hour)			
Lateral ischemic			
Endocardium	—	0.65 ± 0.29	0.74 ± 0.40
Epicardium	—	1.03 ± 0.31	0.83 ± 0.43
Central ischemic			
Endocardium	—	1.02 ± 0.59	0.66 ± 0.33
Epicardium	—	1.16 ± 0.42	0.81 ± 0.38
Septal ischemic			
Endocardium	—	0.90 ± 0.51	0.81 ± 0.43
Epicardium	—	0.97 ± 0.39	0.89 ± 0.47
Posterior normal			
Endocardium	—	0.91 ± 0.29	1.13 ± 0.88
Epicardium	—	0.86 ± 0.31	1.16 ± 0.97
Reperfusion (for 22 hours)			
Lateral ischemic			
Endocardium	0.03 ± 0.05	0.33 ± 0.19*	0.54 ± 0.40*
Epicardium	0.08 ± 0.05	0.53 ± 0.20*	0.70 ± 0.29*
Central ischemic			
Endocardium	0.03 ± 0.03	0.44 ± 0.28*	0.45 ± 0.23*
Epicardium	0.13 ± 0.14	0.61 ± 0.31*	0.62 ± 0.27*
Septal ischemic			
Endocardium	0.04 ± 0.06	0.53 ± 0.36*	0.53 ± 0.14*
Epicardium	0.06 ± 0.05	0.57 ± 0.26*	0.65 ± 0.25*
Posterior normal			
Endocardium	0.73 ± 0.48	0.90 ± 0.12	1.15 ± 0.21
Epicardium	0.67 ± 0.45	0.90 ± 0.11	1.08 ± 0.37

Values are means ± SD for endocardial and epicardial halves of transmural sections. *Values significantly different from those in the control group ($p < 0.05$).



pared with the Wilcoxon signed rank test. Differences at the 5% level were considered to be significant.

Results

Persistent occlusion of the left anterior descending coronary artery after 24 hours was documented in all 15 control dogs. In all 26 dogs subjected to reperfusion, intracoronary streptokinase restored patency of the left anterior descending coronary artery and its branches. Repeat angiography immediately before termination of the study documented the persistence of patency in all but one animal (excluded from subsequent analysis).

Mean arterial pressures and heart rates were similar in the three groups throughout the interval of ischemia as shown in Table 1. Infusion of diltiazem did not significantly alter heart rate or blood pressure before reperfusion as shown

Figure 1. Transverse reconstructed tomograms from a single plane through the middle of the left ventricle of a control dog after coronary occlusion. All images have been corrected for vascular activity with the use of blood pool images obtained at each interval after inhalation of oxygen-15–labeled carbon monoxide. In this and all subsequent tomograms, anterior myocardium is depicted at the **top**, posterior at the **bottom**, lateral wall on the **left** and interventricular septum on the **right**. **A** and **B** illustrate perfusion images obtained after injection of oxygen-15–labeled water. **C** and **D** depict the distribution of carbon-11–labeled palmitate. Tomograms shown in **A** and **C** were obtained 90 minutes after occlusion of the left anterior descending coronary artery, and tomograms in panels **B** and **D** were obtained 24 hours after occlusion. A large flow defect is observed in the anterior region supplied by the occluded artery, with a corresponding diminution of uptake of ^{11}C-palmitate. In this example from a dog with persistent occlusion, perfusion and palmitate uptake remained consistent over 24 hours. In this transverse slice, the discontinuity of tracer uptake in the posterior myocardium is due to the mitral valve apparatus. The 20 grade linear color scale indicates counts per pixel with the peak counts per pixel indicated at the white grade.

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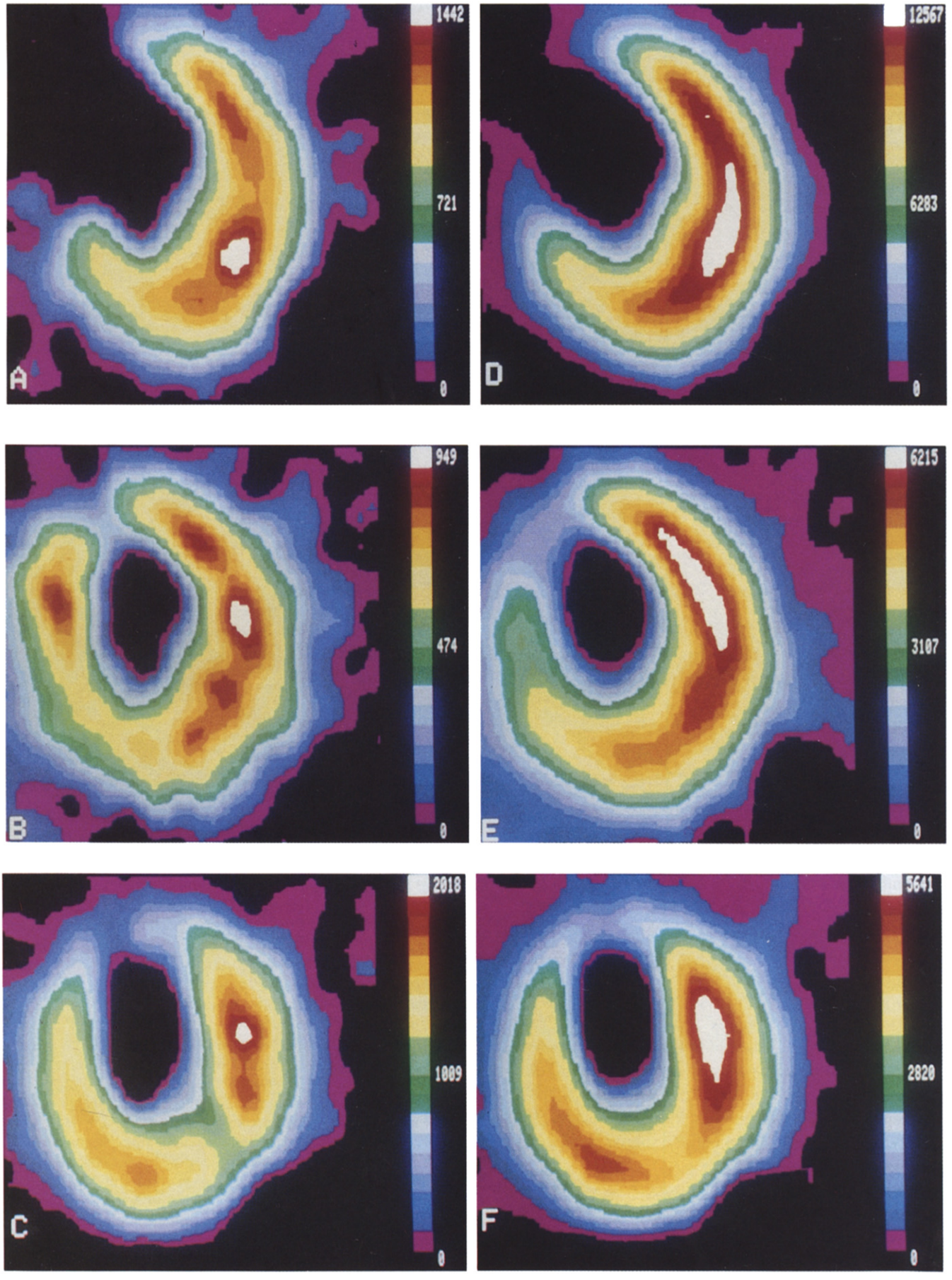

Figure 2. Transverse reconstructed tomograms from a dog given streptokinase alone after coronary occlusion, depicting perfusion (**A, B, C**) and uptake of carbon-11–labeled palmitate (**D, E, F**). Tomograms in **A** and **D** were obtained 90 minutes after coronary occlusion; those in **B** and **E** were obtained 1 hour after reperfusion and those in **C** and **F** were obtained 22 hours after reperfusion (24 hours after occlusion). Perfusion was not different from normal 1 hour after thrombolysis, but was markedly diminished at 24 hours despite maintained macrovascular patency documented angiographically. Uptake of palmitate was restored only partially 1 hour after reperfusion. Restoration was similar at 24 hours.

by the similarity of values 1 and 2 hours after occlusion. Administration of streptokinase was associated with significantly higher heart rates as a result of arrhythmias accompanying reperfusion. There was, however, no difference with respect to heart rate or mean arterial pressure in the two groups subjected to reperfusion, that is, dogs that had and dogs that had not been given diltiazem.

Effects on regional myocardial blood flow (Table 2). The extent of ischemia was similar in each of the three groups, with transmural flows in the anterior ischemic zone averaging 0.14 ± 0.14, 0.16 ± 0.15 and 0.12 ± 0.09 ml/min per g for dogs with occlusion alone, those with reperfusion alone and those with reperfusion plus diltiazem, respectively.

Thrombolysis restored flow in ischemic zones to near normal (1.02 ml/min per g) levels. Transmural blood flow in the previously ischemic anterior region 1 hour after reperfusion was not statistically different in dogs subjected to reperfusion whether or not they were also treated with diltiazem (0.94 ± 0.42 ml/g per min in dogs with reperfusion alone compared with 0.83 ± 0.43 ml/min per g for those with diltiazem).

Despite restoration of perfusion early after thrombolysis and maintenance of macrovascular patency 24 hours after occlusion, flow in reperfused regions at 24 hours was significantly lower than flow in normal regions, averaging 0.50 ± 0.26 ml/g per min in dogs with reperfusion alone and 0.58 ± 0.26 ml/min per g in dogs with reperfusion plus diltiazem (normal flow = 1.01). Nevertheless, perfusion in both groups was significantly greater than that in dogs with persistent occlusion (0.07 ± 0.08 ml/min per g).

Effects on myocardial creatine kinase depletion. Infarct extent was determined at necropsy by analysis of myocardial creatine kinase depletion in 10 control dogs with occlusion alone, 8 dogs with reperfusion alone and 9 dogs with reperfusion and diltiazem. Infarct extent averaged 29.9 g or 27.9 ± 11.4% of left ventricular weight judging from creatine kinase depletion from hearts in dogs with persistent occlusion. Thrombolysis alone reduced the extent to 18.1 g or 16.7 ± 10.1% of left ventricular weight ($p < 0.05$). Thrombolysis combined with diltiazem further reduced the infarct extent to 8.4 g or 9.4 ± 6.7% of left ventricular weight ($p < 0.05$, compared with results after reperfusion alone), reflecting an additional 44% decrease of infarct size compared with the reduction induced by reperfusion alone.

Results with positron tomography. Sixteen dogs were evaluated by positron emission tomography including six control dogs with persistent occlusion, five given streptokinase alone and five given streptokinase and diltiazem. Because the tomograph used does not permit gating needed to correct for cardiac motion, absolute quantification of the myocardial distribution of radioactivity was not possible. Accordingly, results were expressed as relative activity, that is, the ratio of activity in the anterior myocardium subtended by the left anterior descending coronary artery compared with that in normal myocardium.

Relative myocardial blood flow was assessed by analysis of $H_2{}^{15}O$ content in a region of myocardium corrected for activity in the blood pool. In control dogs with occlusion alone, no increase in relative blood flow to the ischemic zone was evident 24 hours after occlusion as illustrated by the tomograms in Figure 1. Coronary thrombolysis induced with streptokinase resulted in substantial restoration of flow in the previously ischemic anterior region (Fig. 2). Similar increases were evident when treatment with diltiazem was implemented 90 minutes after occlusion followed by thrombolysis with streptokinase (Fig. 3). Twenty-four hours after occlusion, flow was diminished compared with its initial augmentation in zones subjected to reperfusion although flow still exceeded that evident before reperfusion (Fig. 2). The same phenomenon was seen despite administration of diltiazem (Fig. 3). Flows in the center of ischemic zones are summarized in Figure 4. Relative flow measurements obtained by positron emission tomography correlated with determinations of flow based on analysis of the distribution of radiolabeled microspheres measured directly (Fig. 5).

Uptake of ^{11}C-palmitate in ischemic myocardium soon after reperfusion and in myocardium subjected to reperfusion for 22 hours, that is, 24 hours after occlusion, is summarized in Figure 4. Sequential tomograms obtained during the interval from 6 to 12 minutes after intravenous administration of ^{11}C-palmitate and corrected for ^{11}C-palmitate activity in the blood pool are shown in Figures 1 through 3. Uptake of ^{11}C-palmitate in control dogs with occlusion alone was diminished after the onset of ischemia and similarly diminished 24 hours after occlusion. Reperfusion partially restored uptake of ^{11}C-palmitate in the previously ischemic region, but a residual region of decreased uptake persisted. Twenty-four hours after occlusion, uptake of ^{11}C-palmitate in dogs subjected to reperfusion was significantly greater than that in control dogs with persistent occlusion although uptake had diminished modestly in comparison with that 1 hour after reperfusion. In dogs given diltiazem as well as streptokinase, uptake of ^{11}C-palmitate was 33% greater than that in previously ischemic zones in comparison with values in dogs subjected to reperfusion alone.

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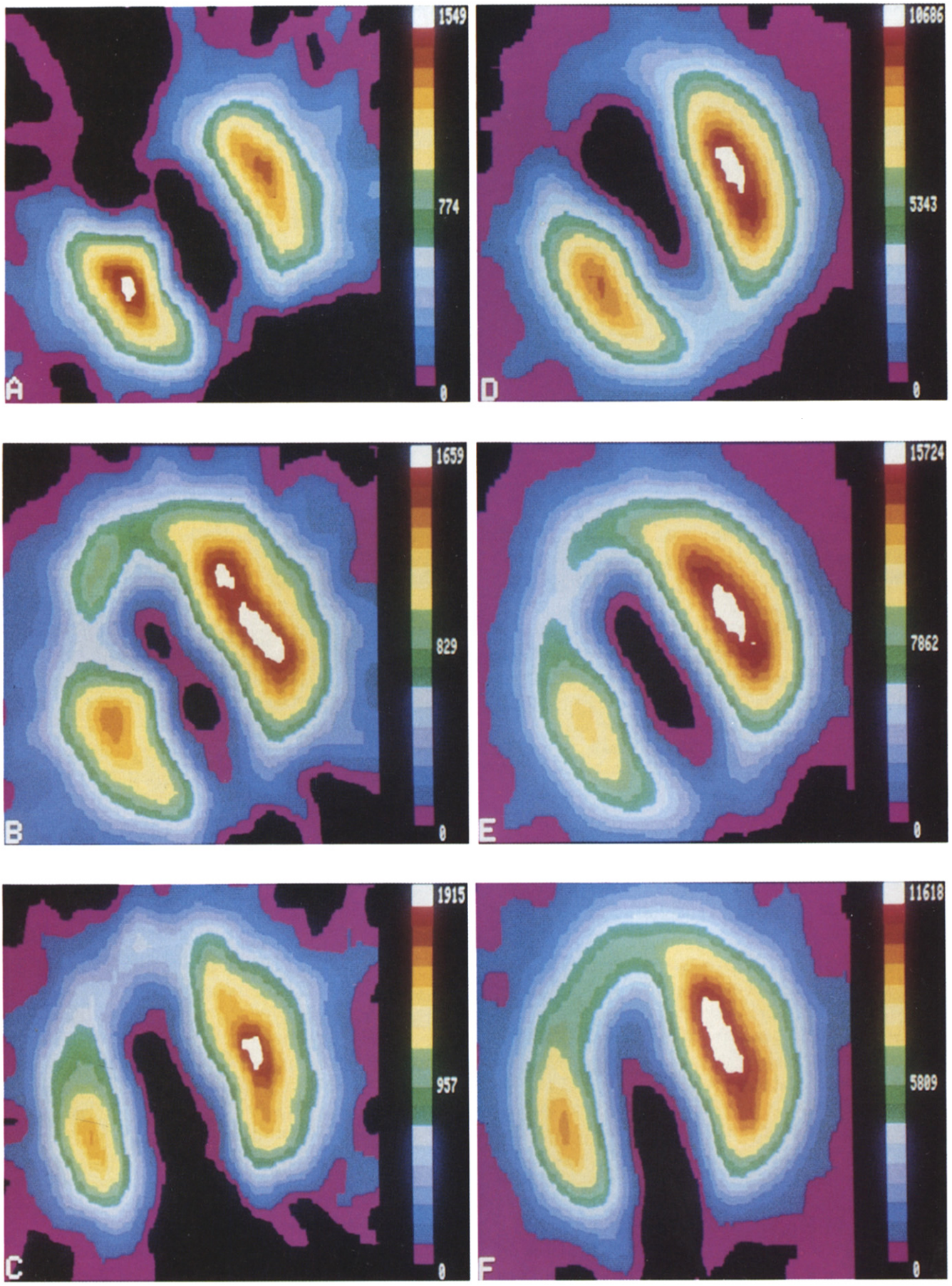


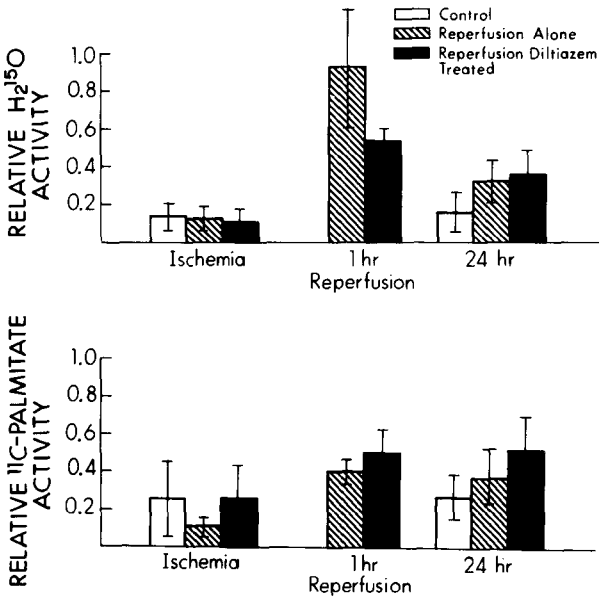
Figure 3. Transverse reconstructed tomograms from a dog given intravenous diltiazem before induction of thrombolysis with streptokinase. Perfusion is depicted in **A**, **B** and **C**. The distribution of carbon-11–labeled palmitate is shown in **D**, **E** and **F**. Time points are the same as those in Figure 3; conditions were: **A** and **D**, occlusion; **B** and **E**, 1 hour after thrombolysis; **C** and **F**, 22 hours after reperfusion. Perfusion was diminished at 24 hours compared with its early augmentation just as in dogs given streptokinase alone. However, uptake of palmitate is improved compared with the restoration in dogs given streptokinase alone, with substantial improvement evident at 24 hours.

Regions at risk assessed from perfusion images obtained after occlusion and before reperfusion, and zones of infarction estimated from the palmitate images obtained 24 hours after occlusion are summarized in Table 3. Regions at risk constituted 20.6 to 40.0% of the left ventricle. Values were similar among the three groups of dogs studied.

Tomographically estimated infarct size was reduced by an average of 43% by reperfusion alone. It was reduced slightly more in animals given diltiazem as well as streptokinase. Expressed as a percent of the region at risk, reperfusion alone decreased tomographically estimated infarct size by 50%. Administration of diltiazem followed by reperfusion decreased tomographically estimated infarct size by 51% more than the reduction elicited by reperfusion alone. Because of the small sample size, however, this additional 50% reduction was not statistically significant.

Combined results. When results obtained by direct measurement in myocardium and those obtained from tomographic assessments before death were combined, the consistency of changes observed with both was evident (Fig. 6). Thrombolysis reduced infarct size from 27.3 ± 9.2 to 17.1 ± 8.7% of left ventricular weight ($p < 0.05$). Thrombolysis coupled with administration of diltiazem further reduced the infarct extent to 9.3 ± 6.2% ($p < 0.05$, compared with results with reperfusion alone).

Figure 4. Tomographically delineated $H_2{}^{15}O$ (**top**) and ^{11}C-palmitate (**bottom**) expressed as the ratio between values in anterior, previously ischemic myocardium and normal tissue obtained in six control dogs, five dogs subjected to reperfusion alone and five dogs subjected to reperfusion plus diltiazem. Values represent means ± SD.



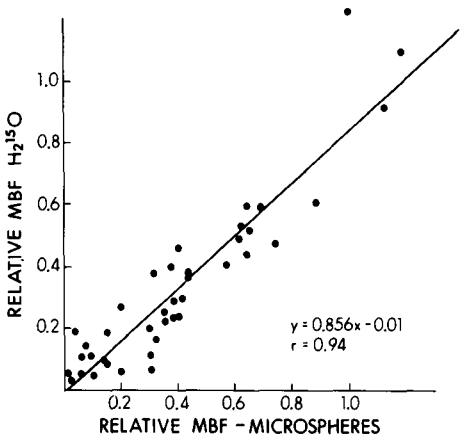


Figure 5. Correspondence between measurements of relative (anterior/normal) myocardial blood flow (MBF) obtained tomographically ($H_2{}^{15}O$) and those acquired with radiolabeled microspheres. Although flow is underestimated slightly by positron emission tomography, the correspondence between results obtained with the two types of determinations is close.

Discussion

The results of this study indicate that administration of diltiazem before thrombolysis increases salvage of myocardium compared with salvage achieved by thrombolysis alone.

Methodologic considerations. This study was undertaken to evaluate preservation of myocardium by pharmacologic agents under conditions avoiding thoracotomy for several reasons. Even with chronically instrumented prep-

Table 3. Extent of Region at Risk and Infarct Size Determined Tomographically in 16 Dogs

	Region at Risk (% left ventricular mass)	Infarct Size (% region at risk)
Control (n = 6)	30.6 ± 7.3 (20.6 to 38.6)	89.6 ± 11.4 (75.8 to 102.3)
Reperfusion alone (n = 5)	31.8 ± 4.5 (26.0 to 35.8)	45.1 ± 29.8* (11.9 to 78.7)
Reperfusion plus diltiazem (n = 5)	30.5 ± 11.6 (29.4 to 40.0)	22.3 ± 16.4* (3.6 to 58.3)

Values are means ± SD. Values in parentheses represent range of values for each group. Region at risk was determined as the percent of left ventricle exhibiting less than 50% of maximal $H_2{}^{15}O$ content after coronary occlusion. Infarct extent was measured as the percent of left ventricle with less than 50% of maximal ^{11}C-palmitate uptake 24 hours after occlusion and expressed as the percent of the region at risk. *Values significantly different from control values, $p < 0.05$.

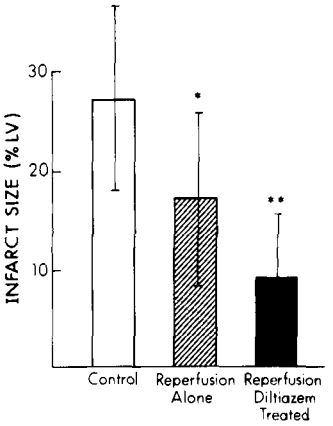


Figure 6. Pooled results of the extent of infarction, expressed as the percentage of left ventricular mass based on tomographic determinations of infarct size and direct measurement of myocardial creatine kinase depletion (n = 15 control dogs, 13 with reperfusion alone and 13 with reperfusion plus diltiazem). Values represent means ± SD. *Values significantly different from controls; **Values significantly different from those with reperfusion alone, $p < 0.05$ for both.

arations, effects of isolation and instrumentation of the coronary artery may lead to induction of collateral circulation (11). Acute closed chest occlusion of a coronary artery under fluoroscopic techniques such as those used in the present study avoids these problems but requires the use of anesthesia. Although blood flow in the central ischemic zones of conscious, chronically instrumented dogs may exceed that in acute occlusion in open chest dogs (12), it is not clear whether the difference reflects induction of collateral circulation or the absence of anesthesia. Blood flow to the central ischemic zones in our study was consistently less than that reported in chronically instrumented dogs.

One disadvantage of eliciting reperfusion with a thrombolytic agent is that the timing of reperfusion cannot be as well controlled as it can by reflow induced by ligature release in open chest or chronically instrumented preparations. This may result in some variability in the duration of ischemia before reperfusion and therefore in infarct size. In our study, the onset of reperfusion, indicated by the onset of reperfusion arrhythmia, occurred within 30 minutes of initiation of streptokinase infusion in most dogs and always within 1 hour. Induction of reperfusion by dissolution of an occlusive thrombus was employed in part because of parallels to clinically induced reperfusion.

Tomographic assessments of perfusion with $H_2{}^{15}O$ have been shown to correlate closely with determinations of flow with radiolabeled microspheres (8,13). In previous studies, infarct extent expressed as the percent of left ventricular myocardium that accumulates less than 50% of ^{11}C-palmitate compared with that accumulated by normal myocardium was found to correlate with direct determinations of infarct size based on measurement of myocardial creatine kinase depletion and histologic study (9).

Determinations of regions at risk and of infarct extent by positron emission tomography are influenced by spatial limitations inherent in tomographic imaging, including effects of cardiac motion, partial volume effects and spillover of radioactivity (14–17). Despite these limitations, positron emission tomography exhibits several advantages compared with single photon imaging. Furthermore, assessment of regions at risk with $H_2{}^{15}O$ and delineation of infarct zones with ^{11}C-palmitate are subject to the same limitations in each group of dogs. Because positron emission tomography is noninvasive, it can be applied conveniently and safely in clinical investigations.

Enhanced salvage of myocardium by diltiazem. Diltiazem administered before thrombolysis resulted in a reduction of infarct size by 66% compared with values in dogs with persistent occlusion and a similar reduction of flow after coronary occlusion. The infarct extent was 46% less than that in dogs subjected to reperfusion alone without concomitant diltiazem ($p < 0.05$).

Calcium channel entry blockers appear to enhance recovery of ventricular performance associated with reperfusion (2). They appear to favorably alter the balance between myocardial oxygen supply and demand by improving myocardial perfusion in some circumstances, decreasing oxygen consumption of ischemic myocardium, or both. Nifedipine, given 30 minutes after the onset of ischemia, increases collateral blood flow in dogs and reduces myocardial creatine kinase depletion (18). Treatment with diltiazem maintains myocardial cellular integrity and adenosine triphosphate (ATP) stores and reduces accumulation of lactate and free fatty acids (19). It also improves the function of hypokinetic segments of ischemic myocardium (20). Both verapamil and diltiazem appear to protect myocardium when given before ischemia (21–23). Diltiazem alone or in combination with propranolol has been reported to enhance performance of reperfused myocardium (2,3). We elected to administer diltiazem 90 minutes after occlusion and 30 minutes before initiation of thrombolysis to determine whether effects of diltiazem on reperfusion injury could be differentiated from effects on injury resulting from ischemia alone.

Calcium channel entry blockers may decrease injury in ischemic myocardium subjected to reperfusion by inhibiting the rapid influx of calcium seen otherwise and implicated as being deleterious to cell membranes and mitochondria. They decrease the accumulation of calcium precipitates in mitochondria isolated from such tissue (24). Furthermore, they may decrease the extent of generation of potentially toxic oxygen free radicals by preventing conversion of xanthine dehydrogenase to xanthine oxidase (25,26).

Effects of diltiazem on myocardial perfusion after thrombolysis. Because calcium channel entry blockers are vasodilators, they may increase coronary collateral flow and blunt the "no reflow" phenomenon otherwise seen after reperfusion. In our study, however, beneficial effects of

diltiazem appeared to be independent of direct effects on myocardial perfusion, judging from the comparable restoration of perfusion measured 1 hour and 22 hours after thrombolysis induced by streptokinase in dogs with or without concomitant diltiazem. Flow to the endocardium in the centrally ischemic zones was affected most severely in both groups, but flow was diminished to some extent throughout the ischemic zone. The reduction of flow in reperfused myocardium 24 hours after coronary occlusion is consistent with results of other researchers. Higginson et al. (27) reported that despite initial restoration of flow to normal by reperfusion after 2 hours of ischemia, flow was less than 0.2 ml/min per g 24 hours later. The late "no-reflow" phenomenon may have been less severe in our study because of the richer collateral supply to myocardium in the anterior wall compared with collateral supply to the posterior papillary muscles evaluated by Higginson et al. (27). In addition, physiologic stress associated with thoracotomy in the Higginson study may have exacerbated the "no reflow" phenomenon.

Clinical implications. Results of this study suggest that diltiazem enhances salvage of myocardium subjected to reperfusion. They demonstrate the feasibility of utilizing positron emission tomography with tracers of flow and metabolism for assessment of adjunctive pharmacologic agents in the setting of clinical coronary thrombolysis. The results also suggest that calcium channel entry blockers are promising for enhancement of salvage of myocardium and that they merit exploration in patients subjected to coronary thrombolysis.

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