Absence of a cumulative deterioration of regional function during three repeated 5 or 15 minute coronary occlusions

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ABSTRACT Recurrent myocardial ischemia is commonly seen in patients with coronary artery disease, in patients undergoing cardiac surgery with intermittent cross-clamping, and experimentally when multiple occlusion preparations are used to test drugs. Our study was designed to investigate whether myocardial injury is cumulative after three sequential ischemic episodes. Sixteen dogs were instrumented with ultrasonic crystals to assess percentage of segmental shortening and percentage of wall thickening in ischemic and nonischemic regions. The left anterior descending coronary artery was occluded three times for 5 min in one group (n = 8) and three times for 15 min in another group of dogs (n = 8) and each occlusion was followed by 30 min of reflow. Blood flow was determined with microspheres before coronary artery occlusion in the 5 min group and during occlusion in the 15 min group and in all dogs 25 min into the first and third reperfusion periods. During the three reperfusion periods mean segmental shortening in the ischemic zone recovered to only 60.5 ± 8.7% (± SEM) of the preocclusion level (p < .003) in the 5 min occlusion group and to 36.9 ± 17.7% in the 15 min occlusion group (p < .01). Mean wall thickening recovered to 61.3 ± 16.6% (p < .06) in the 5 min group and to 48.6 ± 11.8% in the 15 min group (p < .004). There were no significant differences during the reperfusion phase when mean values for the ischemic segment after the second and third occlusion were compared with the data obtained after the first occlusion in either group. The degree and pattern of postischemic recovery was similar after each occlusion. In both groups during reperfusion blood flow in the previously ischemic zone tended to be lower than flow in the nonischemic zone. Infarction was absent, as assessed by tetrazolium staining, in both groups. The results show that a coronary artery occlusion as brief as 5 or 15 min leads to depression of endocardial contractile function in the reperfused zone, but that these alterations are not cumulative after three repetitive coronary artery occlusions.


RECURRENT MYOCARDIAL ISCHEMIA is a phenomenon commonly observed in both clinical and experimental cardiology. It occurs in patients with coronary artery disease who suffer from frequent anginal attacks as well as in those undergoing cardiac surgery with intermittent cross-clamping or repeated short-term occlusions of a coronary artery. Experimentally, many investigators rely on sequential preparations of occlusion to test drugs. However, little information exists as to whether myocardial injury is exacerbated by recurrent ischemic episodes. Previous experimental studies have shown that single brief occlusions of the coronary artery (5 to 15 min) cause prolonged abnormalities in regional function.1, 2 However, the myocardium will eventually recover completely and the term “stunned myocardium” was introduced to characterize this transitory depression.3

The effects of repeated ischemia on the still unrecovered (stunned) myocardium have not yet been described. This investigation was designed to determine regional function, blood flow, hemodynamics, and the development of infarction in the canine heart during three consecutive 5 and 15 min occlusions with intermittent 30 min periods of reperfusion. We sought to determine whether recurrent ischemia results in cumu-
lative myocardial damage with a subsequent stepwise decrease in regional function. Patients with coronary artery disease suffer from anginal attacks of various duration and number. We chose 5 as well as 15 min intervals of ischemia to mimic this clinical situation and to assess whether the duration of occlusion has further impact on the development of cumulative damage. Three repetitions were chosen to simulate the number of anginal attacks that a patient might sustain in 1 day.

Methods

Preparation. Mongrel dogs of both sexes weighing 13 to 32 kg were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and ventilated with a Harvard respirator. A micromanometer-tipped pressure transducer (Millar Instruments, Houston) was inserted into the left carotid artery of each dog to measure aortic pressure. A thoracotomy was performed through the fifth left intercostal space and the heart was suspended in a pericardial cradle. A second micromanometer-tipped pressure transducer was placed into the left ventricle via the left atrial appendage. A small segment of the left anterior descending coronary artery (LAD) was isolated just proximal to the first diagonal branch, approximately 2 cm from its origin.

Protocol. The LAD was occluded with an atrumatic Schwartz vascular clamp three times for 5 min in one group of dogs (n = 8) and three times for 15 min in another group (n = 8); in both groups each occlusion was followed by 30 min of reperfusion. Reperfusion was continued for a total of 180 min after the last occlusion so that staining with triphenyltetrazolium chloride (TTC) could be done later to determine if these were zones of irreversibly injured cells. At the end of the experiment the LAD was occluded again and the heart was injected with 30 ml of a 10% solution of monastral blue dye via the left atrium. The dye stains only the nonoccluded area blue and thus allows visualization of an unstrained ischemic "area at risk," which serves as a guide for sampling of tissue for regional blood flow. The hearts were then removed and the left ventricle cut into 5 to 6 mm transverse slices that were incubated for 15 min in a 1% solution of TTC at 30° to 32° C. 1-5

Regional function. One pair of ultrasonic crystals was implanted into a nonischemic region of the left ventricle and two pairs were implanted into a soon-to-be ischemic region (figure 1). The extent of the ischemic zone was identified by epicardial cyanosis after a 5 to 10 sec LAD occlusion. The two pairs of crystals were then placed well within this zone. The crystals for measurement of segmental shortening (SS) were positioned 1.5 to 2.0 cm apart, perpendicular to the major heart axis in the subendocardium. Another crystal was inserted diagonally into the endocardium of the soon-to-be ischemic zone and a specially designed disk-shaped crystal (2 mm in diameter; Dimonics, Boston, MA) was sutured to the epicardial surface at a location where the ultrasonic transit time was shortest, between the endocardial and epicardial crystals. Changes in the regional dimensions were measured on-line by continuous measurements of ultrasonic transit time between the crystals. The measured transit time was calibrated in steps of 1 μsec generated by a crystal controlled pulse generator. From the recorded signals the end-diastolic and end-systolic lengths (EDL, ESL) and the end-diastolic and end-systolic wall thicknesses (EDWT, ESWT) were identified by the beginning of the rapid rise in left ventricular (LV) pressure just before the isovolumetric contraction and the peak negative dp/dt, respectively, as previously described. 3 Percentage of SS was calculated to determine subendocardial circumferential contraction from the formula:

\[
\%SS = \frac{EDL - ESL}{EDL} \times 100
\]

and percentage of wall thickening (%WT) was calculated to determine transmural contraction from the formula:

\[
\%WT = \frac{ESL - EDL}{EDL} \times 100
\]

These values were then normalized to preocclusion values as previously described. 3

Tracings of four segments were analyzed for each time period and each pair of crystals and the mean derived from four contractions was then calculated. Measurements were taken before occlusion, just before release of each occlusion, and, after reperfusion, every 10 sec up to 1 min and subsequently every 5 min for 30 min.

Regional myocardial blood flow (RMBF). For determination of RMBF radioactive microspheres (2.0 × 10 6; 8 to 10 μm) labeled with 85Sr, 113Sn, or 144Ce were injected into the left atrium of each dog and a reference blood sample was drawn from the femoral artery at a rate of 15.3 ml/min starting 10 sec before injection of microspheres and lasting until 90 sec after injection. After the dogs were killed, zones for determination of RMBF were identified on two representative heart slices with the use of the "area at risk" in vivo and specimens were cut and divided into epicardial and endocardial halves (figure 2). The zones were designated as follows: zone A = central ischemic zone; zone B = zone approximately 5 mm within the area at risk in vivo; zone C = zone approximately 5 mm outside the area at risk in vivo; zone D = normal perfused zone.

Dissected pieces for RMBF determination were weighed and flow was calculated as previously described. 3 In dogs that underwent repeated 5 min occlusions, RMBF was determined before the first occlusion and 25 min into the first and third

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*Animals used in this study were maintained in accordance with the guidelines of the Committee on Animal of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council [DHEW publication No. (NIH) 78-23, revised 1978].
reperfusion periods. In dogs that underwent 15 min occlusions, RMBF was determined at the end of the first occlusion and at 25 min into the first and third reperfusion periods.

**Statistical analysis.** Hemodynamic, RMBF, and regional function measurements were analyzed by repeated-measures analysis of variance (ANOVA)\(^9\) with the GLM procedure in the Statistical Analysis System (SAS).\(^{10}\) For each hemodynamic outcome an F test was performed and a single contrast was tested comparing the response at baseline to the average response after intervention.

Data on regional function were grouped into the following four time periods for each occlusion: period I (values obtained just before release of each occlusion), period II (values from between 10 sec and 4 min of each reperfusion), period III (values from between 5 min and 25 min of each reperfusion), period IV (values at 30 min of reperfusion only). Values were expressed as a percentage of the preocclusion to control for the initial distance between the crystals. A test of the hypothesis of complete recovery was performed for each measurement of regional function by comparing the mean response in periods 3 and 4 averaging over the three occlusions to 100%. The measurements of function were further analyzed by a two-factor ANOVA with the two factors, period and occlusion, crossed with subject. Overall F tests were performed for differences between periods and differences between occlusions. Pairwise comparisons of periods and occlusions were performed with contrasts derived from the repeated-measures ANOVA, adjusting for multiplicity by Bonferroni’s method.\(^{11}\) All tests were performed at the .05 significance level. Data were summarized as means and SEMs for tabular and graphic presentation.

**Morphology.** At the end of the experiment, heart slices were incubated in triphenyltetrazolium to determine whether infarct was present. Biopsy specimens in vivo were available for electron microscopy in three dogs in the 5 min group. Specimens were obtained at 30 and 180 min after the third occlusion.

**Results**

An initial 19 dogs were used in this study. Three dogs in the 5 min occlusion group were excluded because of ventricular fibrillation upon the initiation of the first reperfusion. The present results are based on data from eight dogs in the 5 min occlusion group and eight dogs in the 15 min occlusion group.

**Hemodynamics (figure 2).** Changes from baseline in heart rate, LV end-diastolic pressure, and LV systolic pressure were not significant by repeated-measures ANOVA in either group. In the 5 min occlusion group, LV dp/dt fell significantly from 3600 ± 210 mm Hg·sec\(^{-1}\) before the first occlusion to an average value of 3275 ± 202 mm Hg·sec\(^{-1}\) after the first occlusion (p < .0001). In the 15 min occlusion group changes in LV dp/dt were not significant.

**RMBF (table 1).** In the 5 min occlusion group no significant changes in blood flows were observed when the flows in epicardium and endocardium before occlusion were compared with the flows obtained during the first and third periods of reperfusion. In the 15 min occlusion group, flow was significantly reduced during occlusion to 0.23 ± 0.05 ml·min\(^{-1}\)·g\(^{-1}\) (epicardium) and 0.16 ± 0.05 ml·min\(^{-1}\)·g\(^{-1}\) (endocardium, both p < .0001 when compared with the nonischemic zone). During the first and third reperfusion periods, flows in the previously ischemic zones A and B tended to be reduced in both groups, but this reduction did not reach statistical significance.

**Regional function in the 5 min occlusion group.** Upon each coronary artery occlusion, the EDL increased in the nonischemic region by 4% to 5% and in the ischemic region by 6% to 9% (figure 3, A). Each time, with the initiation of reflow, both values for EDL returned to the preocclusion level. EDWT of the ischemic zone decreased during the first occlusion only (figure 4, A).

Baseline values for SS before the first occlusion were 15.6 ± 1.6% in nonischemic and 18.5 ± 2.4% in ischemic segments. Values in nonischemic segments were slightly lower than in soon-to-be ischemic segments because the former were located closer to the base of the heart, which contracts less than the midventricular area. The baseline value for WT was 19.8 ± 6.4%. Changes in SS of the nonischemic region were minimal during and after the first and second occlusion. In all ischemic segments of the dogs in the 5 min occlusion group paradoxical systolic bulging and wall thinning were predominant during occlusion. SS changed to −5.0 ± 1.5% and WT to −6.4 ± 1.8% during the first occlusion. Upon release of each occlusion, active but reduced contraction was resumed in the reperfused zone within 10 to 20 sec (figures 3, A and 4, A). During the first 2 to 4 min of reperfusion, values for SS and WT were higher than during the remaining reflow phase. This relative elevation occurred at the same time as a slight increase in the EDWT and was significant when period II (10 sec to 4 min) was compared with periods III and IV (p < .01 for SS). During the three reperfusion periods mean SS in the ischemic zone (5 to 30 min) recovered to only 60.5 ± 8.7% of the preocclusion level (p < .003). At 30 min of reperfusion SS had recovered to 71.7 ± 9.8% of the preocclusion level after the first occlusion and to 65.7 ± 7.5% and 66.2 ± 9.2% after the second and third occlusions, respectively, and these values were not significantly different (figure 3, A). Mean WT recovered to an average of 61.3 ± 16.6% of the preocclusion level during the three reperfusion periods (p < .06). Recovery after the first occlusion was again not significantly different from the recovery after the second and third occlusions. Thirty minutes of reperfusion led to a recovery of 56.0 ± 10.9% of the preocclusion level after the first occlusion and to recoveries of 72.8 ± 31.1% and 63.5 ± 23.0% after the second and third occlusions. An original strip-chart recording of one representative dog is presented in figure 5, A.
FIGURE 2. Hemodynamic variables in the repeated 5 min (A) and 15 min (B) occlusion groups (mean ± SEM).
The WT and SS tracings from the ischemic segments 30 min into the second and third reperfusion periods were similar to the tracings from 30 min into the first reperfusion period.

**Regional function in the 15 min occlusion group.** The EDL in ischemic segments increased by approximately 15% above the preocclusion value during each occlusion ($p < .005$ for the first and second occlusion, $p < .01$ for the third occlusion) and remained significantly elevated during the first reperfusion period ($p < .05$ compared with preocclusion level), but not during subsequent reperfusions (figure 3, B). The EDWT in ischemic segments decreased upon each occlusion by approximately 10% (NS). During reperfusion the EDWT was not different from that before (figure 4, B).

Baseline values for SS before the first occlusion were $14.5 \pm 1.3\%$ in nonischemic and $16.6 \pm 1.9\%$ in ischemic segments and those for WT were $24.7 \pm 5.6\%$. Changes in SS in nonischemic segments were insignificant. As in the 5 min occlusion group, paradoxical systolic bulging and wall thinning were predominant during occlusion in all ischemic segments. SS changed to $-5.6 \pm 1.0\%$ and WT to $-8.8 \pm 3.1\%$ during the first occlusion. Upon release of each of the three occlusions, contraction resumed in all dogs. During the first 10 sec to 4 min (stage II) of each reperfusion period, SS and WT values were relatively higher than during the remaining reflow period ($p < .001$ when stage II is compared with stage III and IV for each reperfusion period). Segmental function then deteriorated after the initial period of relative hypercontraction and, in two of eight dogs, the initial contraction in ischemic segments was again replaced by paradoxical bulging (figures 3, B and 4, B). During the three reperfusion periods mean SS in the ischemic zone recovered to only $36.9 \pm 17.7\%$ of the preocclusion level ($p < .01$) and did not differ significantly among the three reperfusion periods. Thirty minutes into the first, second, and third reperfusion periods, mean SS in the ischemic zone had recovered to only $44.6 \pm 19.5\%$, $41.6 \pm 19.5\%$, and $41.5 \pm 18.3\%$ of the preocclusion levels, respectively ($p < .025$ when each value is compared with the preocclusion value; figure 3, B). Mean WT recovered during the three reperfusion periods to $48.6 \pm 11.8\%$ of the preocclusion level ($p < .004$). Thirty minutes after release of the first occlusion recovery of WT had reached $52.2 \pm 12.1\%$ of the preocclusion level and 30 min after release of the second and third occlusions WT had reduced $48.6 \pm 13.5\%$ and $38.2 \pm 12.8\%$, respectively. One hundred eighty minutes into the third reperfusion period both SS and WT had decreased further to $22.4 \pm 13.5\%$ and $11.9 \pm 10.8\%$ of the preocclusion value, respectively ($p < .005$). Three of eight dogs now showed paradoxical bulging. The overall level of recovery did not differ significantly between the first, second, and third 30 min periods of reperfusion. There were also no statistically significant differences when correspond-
ing stages were compared among the three reperfusion periods. Figure 5, B shows an original strip-chart recording from a typical dog. The tracings for SS and WT in the ischemic segment were almost identical at 30 min into the first, second, and third reperfusion periods. The nonischemic segment showed only minimal changes.

Histochemistry and electronmicroscopy. TTC staining showed no evidence of irreversibly damaged tissue in either group. Electromicrographs, prepared from biopsies obtained at 30 and 180 min after the third occlusion, were available in three dogs in the 5 min occlusion group. These showed abundant glycogen, homogenously dispersed nuclear chromatin and, in

**FIGURE 3.** The EDL increased upon each occlusion and %SS showed paradoxical systolic bulging. Recovery was incomplete but almost identical during the three reperfusion periods (mean ± SEM). A, Five minute occlusion group. B, Fifteen minute occlusion group.
one cell, intracellular vacuoles. A remarkable finding was the unfolding of most mitochondrial cristae, also described as an "energized twisted" or a condensed configuration.\textsuperscript{12, 13}

**Discussion**

In the present investigation, data obtained 30 min into the first reperfusion period confirm previous reports about prolonged functional abnormalities in the canine heart after single 5 to 15 min periods of ischemia.\textsuperscript{1, 2} Regional function remained severely impaired after the first occlusion in both groups. However, unlike previous investigators, we subjected the incompletely recovered (stunned)\textsuperscript{3} myocardium to a second and third ischemic episode of the same duration. Regional function was depressed to the same degree dur-

**FIGURE 4.** \%WT showed paradoxical systolic thinning during each occlusion. Like SS, WT values were not different during the three reperfusion periods in either the 5 min (A) or 15 min (B) occlusion group (mean ± SEM).
ing the second and third reperfusion periods as during the first in both groups. Hence, after one coronary occlusion, a second and third occlusion causes no further myocardial injury when intermittent reperfusion is allowed for 30 min.

It has previously been demonstrated that 40 min of uninterrupted ischemia results in myocardial necrosis in the dog. However, in the present study infarction was not observed either after three 5 min or three 15 min episodes of ischemia, and ultrastructural changes observed after three 5 min occlusions were similar to those previously described after one 5 min coronary occlusion. In contrast to our results, recently Geft et al. reported observing distinct areas of subendocardial necrosis and evidence of irreversible ultrastructural damage in three of 32 dogs subjected to 18 occlusions of 5 min and in seven of 24 dogs subjected to 18 occlusions of 15 min. In their study intermittent reperfusion was allowed for 15 min. Two reasons may account for the difference in results. First, the length of the reperfusion periods may be a determining factor in the development of cumulative injury. Second, after more than three but less than 18 occlusions, myocytes may reach a limit of ischemic tolerance.

Our results for regional function are consistent with those from a study by Weiner et al. who subjected

![Three successive 5 minute occlusions](image)

**FIGURE 5.** Original strip-chart recordings from a representative dog from the 5 min (A) and one from the 15 min (B) occlusion group. The nonischemic segment was unaffected by the repeated interventions. In the ischemic segment the tracings for segmental length and WT were similar during the first, second, and third reperfusion periods and hence a cumulative decline in regional function was not evident. Note that in A the calibration for the tracing of the ischemic segment length is shifted upwards for the second and third reperfusion periods.
dogs, instrumented with segment-length gauges, to three consecutive 20 min occlusions with 45 min of intermittent reperfusion. In this study ischemic segments also recovered to the same degree during each reperfusion period. In contrast, a study by Nicklas et al.\textsuperscript{18} showed that the EDL in ischemic segments, measured by ultrasonic crystals, increased progressively when canine hearts were subjected to eight to 16 coronary artery occlusions of 5 min, each followed by 10 min of intermittent reperfusion. The authors concluded that progressive expansion can be produced by repetitive brief coronary occlusions in the absence of myocardial necrosis. We found no progressive increase in EDL after three occlusions. The considerably larger number of occlusions followed by only very short periods of reperfusion in Nicklas’ study may account for the difference in results.

Our data are consistent with the hypothesis that two populations of cells may exist — one population that is susceptible to the stunning phenomenon and one population that is not. This hypothesis is supported by the fact that the susceptibility of myocytes to ischemia varies from cell to cell.\textsuperscript{19} The population of cells that were stunned by the first occlusion may not have resumed function after the first occlusion and it is likely that they remained equally stunned during subsequent occlusions and periods of reperfusion. It is likely that no additional cells from a second population of unstunned cells were affected by ischemia during subsequent occlusions.

There are several clinical implications of this study. Patients with coronary artery disease often have multiple episodes of angina. Further knowledge about the effects of recurrent ischemia on the myocardium is therefore of considerable clinical importance. Although 45 min of uninterrupted ischemia has been shown to result in irreversible damage in the canine heart\textsuperscript{14} we found no necrosis after three 15 min occlusions. Presumably a substantially larger number of ischemic episodes is required to cause irreversible cell injury. During cardiac surgery occlusion of a coronary artery could thus be repeated relatively safely two or three times without cumulative myocardial damage. Our results suggest that intermittent reperfusion between periods of aortic cross-clamping (ischemia) in the operating room may be important for preservation of myocardial tissue.

Sequential short occlusions are commonly used experimentally to test drugs. The results of the present investigation show that before second and third occlusions the functional baseline is depressed compared with before the first occlusion. However, the degree of functional recovery is similar during each reperfusion period after three consecutive occlusions.

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