Effects of Reperfusion on the Regional Contraction of Ischemic and Nonischemic Myocardium Following Partial Coronary Obstruction

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SUMMARY  In 14 dogs the effects on regional tension (Walton-Brodie gauges) and length (mercury-in-silastic) following 50% reduction (52.9 ± 2.1) in coronary flow for two hours and reperfusion afterwards for one hour were addressed. Within five minutes of partial coronary occlusion, ejection tension in the ischemic zone decreased to 36.3 ± 7.2% (P < 0.001) and total tension to 64.4 ± 5.7% of control (P < 0.001) while phasic segment length increased to 165.2 ± 16.3% of control. No further significant changes in regional tension or length were observed throughout the two hour period of partial occlusion. Ejection tension remained positive and segment length maintained systolic shortening during the ejection phase throughout the period of occlusion. Following reperfusion, ejection tension in the ischemic zone increased from 35.1 ± 5.9 to 87.0 ± 22.0% (P < 0.05) and total tension increased from 56.6 ± 5.4 to 70.2 ± 7.2% (P < 0.02) while segment length decreased from 149.3 ± 6.5 to 105.7 ± 5.7% (P < 0.001) within five to 15 min of reperfusion. The improvement in both regional tension development and segment length shortening was maintained throughout the one hour period of reperfusion. No significant changes were seen in the nonischemic zone. The present experimental study suggests that partial coronary occlusion producing a 50% reduction in coronary blood flow results in regional contractile changes. These changes are reversible at least twice as long as those following complete occlusion.

THE CONTRACTION ABNORMALITIES which occur following complete coronary occlusion have been extensively studied experimentally.1–4 However, it is well known clinically that patients with angina pectoris often exhibit asynergy on ventriculography without total occlusion in the coronary arteries subserving the asynergic zone.5–10 The advent of coronary bypass surgery has been a major stimulus to better understanding the potential of this procedure to improve contraction disorders in both acute and chronic coronary heart disease settings. Studies from our laboratory11 and others12–16 have demonstrated that the contraction impairment following total coronary occlusion is essentially irreversible after two hours and that reperfusion at this time is of either no value or deleterious. However, the effects of reperfusion as a function of time following partial reduction in coronary flow are incompletely understood. The present study was, therefore, undertaken to examine the effects of partial coronary occlusion and reperfusion on regional left ventricular contractile performance.

Methods

Experiments were performed in 14 dogs weighing 25–34 kg, anesthetized with sodium pentobarbital, 30 mg/kg/IV. The animals were ventilated with room air via an endotracheal tube connected to a Harvard respirator. A polyethylene tube was introduced through an external jugular vein for intravenous infusion and a stiff-bore catheter was introduced into the ascending aorta via a carotid artery for monitoring central aortic pressure. The heart was exposed through a left lateral thoracotomy through the fifth intercostal space and suspended in a pericardial cradle. A long segment of left anterior descending or left circumflex free of branches was exposed and mobilized. Coronary flow was measured using an electromagnetic flow probe (Micron MU1001-B) fitted properly to the diastolic diameter of the vessel. Zero flow references were obtained by transiently occluding the vessel proximal to the vessel probe site for a few seconds. Measurements over long periods of time revealed a satisfactory baseline stability. Two Walton-Brodie strain gauge arches were placed with deep sutures at 30–40% stretch, one in the area of left ventricle supplied by the left anterior descending and one in the area supplied by the left circumflex. A mercury-in-silastic segment length gauge (Park Electronics) was also sutured adjacent and parallel to the strain gauge arch in each zone. Standard ECG lead II was also monitored continuously throughout the experiment. All records were taken simultaneously on an Electronics for Medicine multichannel oscilloscopic recorder at paper speeds of 25 and 100 mm/sec. After obtaining control recordings of tension, segment length and blood pressure, partial coronary occlusion was carried out on either the left anterior descending or left circumflex in a random fashion to reduce coronary flow by approximately 50% (52.9 ± 2.1%) with a specially designed adjustable screw clamp. Simultaneous tension and segment length recordings were made at intervals of 5, 15, 45, 60, 90, and 120 min. At this point the clamp was removed and recordings were taken at the end of 5, 15, 30 and 60 min of reperfusion. The following variables were measured and interpreted as percent changes from control (before coronary occlusion) levels: 1) ejection tension; 2) total tension; and 3) phasic segment length.

At the end of each experiment, 2 cc of methylene blue dye was injected at systemic pressure immediately proximal to the site of the partial occluder to ascertain the proper positioning of the gauges and the area of myocardium stained was cut out and weighed. Coronary blood flow was

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47
expressed as ml/100g of myocardium. In addition, histologic sections were obtained and stained with phosphotungstic acid and hematoxylin and examined for evidence of myocardial ischemia.

Student's t-test for paired data was used for statistical analysis and results expressed as mean ± standard error of the mean (SEM).

Results

Partial Coronary Occlusion

Figure 1 demonstrates the characteristic qualitative changes in segment tension and length in the ischemic zone. Prior to coronary occlusion, the segment length curve shows pre-ejection shortening (a-b) and segment shortening during ejection (b-c). Following partial coronary occlusion, there is significant increase in pre-ejection shortening (a-b) but segment shortening persists (b-c).

The tension curve shows pre-ejection tension (a-b), ejection tension (b-c) and total tension (TT) prior to coronary occlusion. Following coronary occlusion, there is significant reduction in total tension (TT) as well as ejection tension (b-c). However, the upstroke of the tension curve during ejection (b-c) remains positive, though at a reduced level.

Ejection Tension

Quantitatively, ejection tension in the ischemic zone decreased to 36.3 ± 7.2% of control within five minutes of partial coronary occlusion (P < 0.001), but no significant further change was observed for the two subsequent hours of observation. At two hours ejection tension was 35.1 ± 5.9% (figs. 2 and 3).

Total Tension

Total tension decreased from 100 to 64.4 ± 5.7% within five minutes of 50% reduction in coronary flow (P < 0.001). There was a slight but insignificant decrease in total tension at the end of two hours of partial coronary occlusion (56.6 ± 5.4%) (figs. 2 and 3).
Phasic Segment Length

Phasic segment length increased from 100 to 165.2 ± 16.3% following partial coronary occlusion (P < 0.001). There was an insignificant decrease in phasic length to 149.3 ± 6.5% at the end of two hours (figs. 2 and 3).

There were no significant changes in the ejection tension, total tension, or segment lengthening in the nonischemic zone during the two hours of partial coronary occlusion (fig. 3).

Reperfusion

Ejection Tension

Ejection tension increased from 35.1 ± 5.9 to 87.0 ± 22.0% within 5 to 15 min of reperfusion (P < 0.05) (fig. 2). In none of the experiments was there any significant further increase in ejection tension after 30 min of reperfusion, and no difference in the ejection tension following five minutes (87.0 ± 22.0%) and one hour of reperfusion (71.2 ± 13.3%) was demonstrable.

Total Tension

Total tension similarly increased from 56.6 ± 5.4% to 70.2 ± 7.2% following five to 15 min of reperfusion (P < 0.02). There was no further significant change after one hour of reperfusion (70.1 ± 6.7%) (figs. 2 and 3).

Phasic Segment Length

Segment length decreased from 149.3 ± 6.5% to 105.7 ± 5.7% within 5 to 15 min of reperfusion (P < 0.001) and remained essentially unchanged throughout the subsequent two hour period of reperfusion (109.8 ± 5.4%) (figs. 2 and 3).

There was no significant change in the ejection tension, total tension, or phasic segment length in the nonischemic zone following reperfusion (fig. 4).

Figure 5 shows a typical experiment demonstrating the effect of extended partial coronary occlusion and reperfusion on segment length and tension in ischemic and nonischemic zones.

Comparison of nonischemic and ischemic zone myocardium following one hour of reperfusion revealed no significant difference in the segment length or ejection tension. However, total tension in ischemic zone (71.2 ± 13.3) was significantly decreased (P < 0.05) compared to nonischemic zone (100.2 ± 8.7).

Coronary Blood Flow

Prior to coronary occlusion, coronary flow was 70.2 ± 3.8 ml/100g. Following partial coronary occlusion, it decreased to 34.7 ± 2.1 ml/100g while at two hours it remained at 35.7 ± 1.9 ml/100g. Following five minutes of reperfusion, coronary flow immediately increased from 35.7 ± 1.9 to 97.4 ± 10.8 ml/100g (P < 0.001). The coronary flow then gradually decreased until it reached the pre-occlusion level of 73.4 ± 6.2 ml/100g after 15–30 min. No further significant changes were seen.

No significant histopathological changes were observed following two hours of partial coronary occlusion and reperfusion (fig. 6). There was no significant change in heart rate, systolic or diastolic blood pressure following partial coronary occlusion or reperfusion.

Discussion

The effects of total coronary occlusion on regional myocardial contractile characteristics have been carefully ex-
amined in our laboratory\(^7\) and others. In the central ischemic zone an immediate and precipitous decrease in pre-ejection tension and concomitant increase in pre-ejection length occurs. During ejection, tension also decreases abruptly and dramatically followed by the development of a characteristic negative slope which is accompanied by a change from segment shortening to lengthening.\(^7\) Thus, following complete coronary occlusion while the affected myocardium is still able to generate tension at a reduced level during isovolumic systole, it exhibits aneurysmal bulging during the ejection phase.

The results of the present study demonstrate that following partial reduction in coronary blood flow, the effects on regional contraction are both qualitatively and quantitatively different from complete occlusion.

As might be expected following 50\% reduction in coronary flow, the ejection and total tensions are decreased, and pre-ejection segment length increased to a lesser degree than with total occlusion (fig. 1). However, tension continues to develop during ejection while segment shortening persists during this phase of systole (fig. 1). Thus, aneurysmal bulging does not occur with this degree of coronary flow reduction. These observations are consistent with our previous findings\(^8\) and those of Forrester et al.\(^9\) who noted per-

**FIGURE 4.** Changes in segment length and tension in the nonischemic zone, following partial coronary occlusion and reperfusion. Barred line = mean ± SEM. None of the changes in the nonischemic zone was statistically significant.

**FIGURE 5.** A typical experiment showing the effect of partial coronary occlusion and reperfusion in the nonischemic and ischemic zones. Following 50\% reduction in coronary flow for two hours (panel B), the ischemic zone segment length curve shows significant increase in pre-ejection lengthening and persistence of segment shortening during ejection. The tension curve demonstrates marked decrease in total tension and preservation of ejection tension though at a reduced level. After one hour of restoration of coronary blood flow (panel C), segment lengthening decreases to control level and both the total tension and ejection tension improve markedly. The nonischemic zone shows only insignificant changes in segment length and tension either during coronary occlusion (panel B) or reperfusion (panel C).
sistance of systolic shortening with 50% reduction in coronary flow.

The results of reperfusion following extended periods of partial coronary occlusion are unknown. Studies from our laboratory and others have demonstrated that reperfusion does not restore contraction abnormalities beyond one hour of total coronary occlusion, and this is consistent with histologic studies which have indicated that irreversible cell necrosis takes place beyond 45 min of total occlusion. Kurg noted irreversible damage to the myocardium after 45 min of total occlusion by alteration in the myocardial H ion concentration, while Kane et al. noted marked impairment of mitochondrial function after 60 min of total occlusion. Sharma et al. showed histochemical and electron microscopic evidence of irreversible damage to the myocardial metabolism and ultrastructure after 90 min of total occlusion. Mathur et al. noted that even periods of reperfusion up to one week after two hours of total occlusion failed to prevent functional deterioration or mortality.

However, since the contractile characteristics of the central ischemic zone with partial occlusion are similar to the border zone following complete coronary ligation, the time course before irreversible damage may be more favorable. In fact, the results of the present study demonstrate that after two hours of partial occlusion, ejection tension improves from 35.1 ± 5.9% to 87.0 ± 22.0% while segment length decreases from 149.3 ± 6.5% to 105.7 ± 5.7% in 5 min (fig. 2). When evaluated in terms of regional length-tension relationships, earlier studies by Cotton and Bay and Nakhjavan et al. have demonstrated an increase in segment tension pari passu with an increase in segment length as would be expected from the Frank-Starling mechanism. However, in the present study, the increase in segment length is accompanied by a decrease in segment tension, suggesting that the ischemic myocardium is operating on a depressed length-tension curve (fig. 7). However, with 50% occlusion, reperfusion decreased segment length while increasing segment tension. This strongly suggests a return to a less depressed length-tension curve (fig. 7). In our previous study, reperfusion following total coronary occlusion resulted in a decrease in both segment tension and length, suggesting that altered compliance in the infarcted myocardium resulted in a shift of the length-tension relationship along the same depressed curve. The results of the present experimental study suggest that partial coronary occlusion resulting in a 50% reduction in coronary blood flow causes regional contractile changes which are reversible for at least twice as long a period as those following complete occlusion.

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Radioimmunoassay for Human Myoglobin

Initial Experience in Patients with Coronary Heart Disease

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SUMMARY A radioimmunoassay for human myoglobin has been used to study the serum myoglobin level in 13 normal individuals and 68 patients admitted to a Coronary Care Unit because of chest pain. Values in normal individuals ranged from 3 to 75 and averaged $25 \pm 23$ (SD) ng/ml. Thirty-two patients with myocardial infarction initially examined within 12 hours of the onset of chest pain all showed clear-cut elevations in serum myoglobin, peak values ranging from 200 to 5500 and averaging $1368 \pm 1357$ ng/ml. Seventeen patients with clinically atypical chest pain and no subsequent evidence of myocardial necrosis had myoglobin levels in the normal range, as did 11 of 19 patients with chest pain thought clinically to represent myocardial ischemia but no subsequent evidence of myocardial necrosis by conventional criteria. The final eight patients in the latter group showed mild elevations of serum Mb, peak values ranging from 102 to 280 and averaging $162 \pm 52$ ng/ml; the basis for these elevations remains to be clarified.

INTEREST IN MYOGLOBIN (Mb) as a potential marker of myocardial injury was stimulated in 1967 when Kagen reported the appearance of Mb in the urine of cardiac surgical patients in the immediate postoperative period. During the early 1970s several groups attempted to determine the frequency with which Mb appeared in the urine in the early stage of myocardial infarction. Results were variable, related in part to the sensitivity and specificity of the precipitin and hemagglutination inhibition procedures used to detect Mb, and perhaps also in part to an incomplete understanding of the renal handling of Mb. In 1973, Lwebuga-Mukasa and colleagues presented a preliminary report of a radioimmunoassay capable of quantitating Mb in the dog in the nanogram per milliliter range. Studies were performed in chronically instrumented animals, focusing on plasma rather than urinary Mb. The findings indicated that Mb appears in canine plasma 1–2 hours following coronary occlusion, peaks at approximately six hours and disappears at approximately 10 hours.

The development of a radioimmunoassay for human Mb was a logical next step but was hampered by an inability to radioiodinate human Mb with the conventional chloramine T reagent. This problem was circumvented in 1975 by three groups. Kagen and colleagues employed a micro complement fixation technique, while Stone and colleagues and Justy et al. reported successful radioimmunoassays for human Mb. The Stone group labelled human Mb using a new method of radioiodination described by Bolton and Hunter. This radioiodination technique has also been utilized subsequently by Rosano and colleagues for the preparation of labelled myoglobin and used to study myoglobin levels in patients with myocardial infarction. The resultant assay was able to detect serum Mb concentrations of only a few ng/ml and was initially applied to document elevations in serum Mb in the first few hours of myocardial infarction. The present study reports a further evaluation of myoglobinemia as an index of myocardial necrosis, using an alternate type of radioimmunoassay in which the conventional chloramine T procedure has been found suitable.

Methods

Purification of Human Mb

Human hearts were trimmed of fat and homogenized with an equal volume of cold isotonic saline (W/V). All operations were performed at 4°C thereafter. The homogenate was centrifuged at top speed (13,000 rpm) in a Sorvall refrigerated centrifuge (RC2B) with the large G-SA rotor for 30 minutes. The pellet was discarded and the super-

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