Depression of regional blood flow and wall thickening after brief coronary occlusions

GUY R. HEYNDRICKX, HANK BAIG, PAUL NELLENS, ISIDOOR LEUSEN, MICHAEL C. FISHEIN, AND STEPHEN F. VATNER
Departments of Cardiology and Physiology, State University of Gent, Belgium; Departments of Medicine and Pathology, Harvard Medical School and Peter Bent Brigham Hospital, Boston 02115; and New England Regional Primate Research Center, Southboro, Massachusetts 01772

HEYNDRICKX, GUY R., HANK BAIG, PAUL NELLENS, ISIDOOR LEUSEN, MICHAEL C. FISHEIN, AND STEPHEN F. VATNER. Depression of regional blood flow and wall thickening after brief coronary occlusions. Am. J. Physiol. 234(6): H653-H659, 1978 or Am. J. Physiol.: Heart Circ. Physiol. 3(6): H653-H659, 1978. — The effects of a 15-min coronary occlusion and subsequent reperfusion were investigated in conscious dogs previously instrumented for measurement of left ventricular pressure, dP/dt, regional wall thickening, electrograms, and myocardial blood flow. Coronary occlusion reduced overall left ventricular function only slightly but eliminated systolic wall thickening in the ischemic zone and reduced regional myocardial blood flow in the ischemic zone from 1.04 ± 0.04 to 0.27 ± 0.02 ml/min per g and the endo/epi flow ratio from 1.23 ± 0.04 to 0.44 ± 0.04, while S-T segment elevation increased from 1.1 ± 0.3 to 8.2 ± 0.9 mV. After release of the occlusion, S-T segment elevation disappeared within 1 min while reactive hyperemia in the previously occluded artery and a transient increase in cardiac diastolic wall thickness occurred and then subsided by 15 min. In contrast, systolic wall thickening and the endo/epi flow ratio remained significantly depressed for more than 3 h. Thus reperfusion after a 15 min coronary occlusion results in a prolonged period of reduced regional myocardial blood flow, particularly in the endocardial layers, which correlates with the prolonged depression of regional myocardial shortening and wall thickening.

METHODS

Twenty mongrel dogs weighing between 25 and 35 kg were anesthetized with intravenous sodium pentobarbital, 30 mg/kg. Through a thoracotomy in the fifth left intercostal space, a miniature pressure gauge (Konigsberg P22, Konigsberg Instruments, Inc., Pasadena, Calif.) was implanted through a stab wound in the apex of the left ventricle; a Doppler ultrasonic flow probe and a hydraulic occluder were implanted around the left anterior descending or circumflex coronary artery; pairs of miniature ultrasonic transducers were implanted on opposing sides of the left ventricular wall in the central ischemic zone to measure wall thickness and 1 cm apart, intramyocardially, to measure a segment length; and heparin-filled Tygon catheters (Norton Co., Plastics and Synthetics Division, Akron, Ohio) were implanted in the left atrium and descending thoracic aorta (eight dogs) (Fig. 1). The subendocardial crystal for wall thickness was introduced at an angle of 45°, so that the myocardium between the two ultrasonic transducers would not be impaired by injury or fibrosis. The epicar—

Depression of regional ischemic function can be due to edema of the myocardium, to pathological changes in the myocardial cells or vessels, or to biochemical alterations in the contractile proteins. The goal of this study was to investigate the mechanism of the prolonged depression of regional mechanical function. This was accomplished by examining responses of a) regional myocardial blood flow and b) wall thickening following reperfusion of the coronary artery after 15 min of occlusion. Using these techniques, we could determine if a) persistent regional ischemia or b) gross edema of the previously ischemic myocardium would explain the prolonged depression of regional function. In addition, morphologic studies were performed on samples of the previously ischemic myocardium to determine if structural changes had occurred. These experiments were conducted in conscious animals to eliminate the effects of general anesthesia and major surgery on systemic hemodynamics and in particular on myocardial contractility (8, 21, 22).

METHODS

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FIG. 1. The techniques utilized are shown schematically. Catheters were implanted in the left atrium and aorta to measure pressures and utilize the radioactive microsphere technique. A miniature pressure gauge was implanted in the left ventricle to measure pressure and dP/dt. Miniature ultrasonic transducers were implanted 1–2 cm apart to measure segment length and regional electrograms and across the ventricular wall to measure wall thickness. The endocardial wall-thickness transducer was implanted at an angle of 45° to avoid injury to the myocardium between the 2 transducers.

dial transducer was sutured in direct alignment with the subendocardial crystal and this procedure was verified by examination of the resultant electronic signal on an oscilloscope. Since regional blood flow measurements were made at six different times using four labeled microspheres, an additional 12 dogs were required; these were instrumented with catheters in the left atrium and thoracic aorta and with a flow probe and occluder around a coronary artery for measurement of overall and regional myocardial blood flow.

Calibration of the miniature pressure gauges was performed in vitro with a mercury manometer, as well as in vivo, against calibrated Statham P23dB strain-gauge manometers (Statham Instruments Division, Gould, Inc., Oxnard, Calif.) with use of the arterial and left atrial pressures as references. The position of the implanted gauge within the left ventricular cavity was confirmed at autopsy. An improved, ultrasonic transit-time dimension gauge (construction details are available from the authors) was used to measure left ventricular wall thickness and segment length in the ischemic zone of the myocardium (3, 11, 18, 20). It measures the transit time of acoustic impulses traveling at the sonic velocity of \(1.55 \times 10^5\) mm/s between two 5-MHz piezoelectric crystals. Calibration was performed using signals of known time duration from a calibrated pulse generator. A voltage proportional to transit time was recorded and calibrated in terms of crystal separation. In this manner, measurements of left ventricular wall thickening and segment length were continuously recorded. At a constant room temperature the drift of the instrument is minimal, i.e., less than 0.01 mm in 6 h, and the frequency response is flat to 60 Hz. Periodic calibration throughout the experiment eliminated any further drift in the electronics. The major feature of this measurement technique is its overall stability. This instrument was also adapted further to provide simultaneous measurements of the regional electrograms from the intramyocardial ultrasonic transducers (3).

Thus, the instrument was capable of measuring, simultaneously and from the same crystals, regional mechanical function in terms of wall thickening and segment-length shortening and electrophysiological function in terms of S-T segment elevation. These data were correlated with measurements of regional myocardial blood flow and morphologic studies of tissue taken from the same sites at which regional function was measured. The position of the miniature ultrasonic transducers was confirmed at autopsy.

Regional myocardial blood flow was measured by the radioactive-microsphere (3M Co., St. Paul, Minn.) technique (1). Microspheres were suspended in 0.1% Tween-80 to avoid the complicating influences of the surfactant (10). Microspheres were sonicated (ultrasonic bath, model DA 0950; Scientific Industries, Inc., Queens Village, N.Y.) for 5 min and mixed with a vortex agitator. Absence of microsphere aggregation was verified by microscopic examination. One to two million micro-
spheres (15 ± 3 μm) labeled with $^{51}$Cr, $^{85}$Sr, $^{147}$Ce, and $^{95}$Sr suspended in 10% dextran were injected through the catheter implanted in the left atrium for determinations of myocardial blood flow: during control, then 10–15 min after coronary occlusion, and finally 15 min, 30 min, and 60 min or 3 h after reperfusion. The sequence of the isotopes used was chosen at random. A reference sample of arterial blood was withdrawn beginning 10 s before injection of microspheres and continuing for 30 s after the injection was completed. After sacrifice of the animal, myocardial samples were weighed, placed in a gamma well counter (Searle Analytic, Inc., Lexington, Mass.) and counted for 10 min. The raw counts were then corrected for background activity and energy crossover and compared with the reference blood sample to obtain flow, expressed in milliliters per minute per gram tissue, according to the formula:

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\text{regional blood flow per gram} = \frac{\text{counts per gram tissue}}{\text{counts in reference blood}} \times \text{reference flow rate}
\]

Experiments were conducted 2–4 wk after operation. While the conscious, unsedated dogs rested quietly, control records of left ventricular pressure, rate of change of pressure (dP/dt), left ventricular wall thickness, segment length, arterial pressure, and heart rate were recorded along with the intramyocardial electrograms (Fig. 2). In all dogs control measurements were recorded for at least 1 h, and in four dogs control recordings were made for up to 4 h. As long as the animals remained quiet and in the basal state there were no significant changes in systemic or regional hemodynamics. After control measurements were recorded, including the first injection of microspheres, the coronary vessel was occluded by inflating the balloon occluder. Measurements were recorded continuously and the second microsphere injection was made 10 min after coronary occlusion at a time when hemodynamic parameters were stable. Reperfusion was carried out after 15 min of occlusion. The third and fourth injections of microspheres were performed selectively at 15 min, 30 min, 60 min, or 3 h after reperfusion. Animals were sacrificed with an overdose of pentobarbital 24 h after reperfusion, and myocardial samples obtained from the sites of crystal implantation were dissected into endo-, epi-, and mid-myocardial layers for regional blood flow determination. Samples from 10 hearts were incubated in triphenyltetrazolium chloride (TTC) for the gross demonstration of lactic dehydrogenase enzyme depletion, an indication of irreversible ischemic injury (6).

Sections were also fixed in 10% Formalin and multiple tissue samples from the anterior and posterior left ventricular free walls and papillary muscles were processed in a routine manner for histologic sectioning. Multiple sections (4–12) from each heart were stained with hematoxylin and eosin, periodic-acid–Schiff (PAS) stain for glycogen, and Masson’s trichrome stain for connective tissue (7). Morphologic evidence of 1) vascular changes, such as occlusion by platelet-fibrin thrombi and 2) acute ischemic myocardial injury were specifically investigated. Samples of myocardium from three dogs were also frozen immediately after the hearts were excised; frozen sections were stained for the histochemical demonstration of neutral fats (oil red O stain) and NADH-diaphorase (12).

Data were recorded on a multichannel tape recorder.
and played back on a multichannel direct-writing oscillograph at a paper speed of 100 mm/s. A cardiotachometer, triggered by the pressure pulse signal, provided instantaneous and continuous records of heart rate. Continuous records of dP/dt were derived from the left ventricular pressure signal with a Philbrick operational amplifier (Teledyne Philbrick, Dedham, Mass.) connected as a differentiator having a frequency response of 700 Hz. A triangular wave signal with a known slope (rate of change) was substituted for pressure to calibrate the differentiator directly. Average and SEM values were calculated and responses in each animal were compared by the paired t test (16).

RESULTS

Effects of Coronary Occlusion on Myocardial Function

Overall left ventricular function (Fig. 3). Coronary occlusion induced only slight changes in overall left ventricular function; left ventricular systolic pressure was not significantly affected, dP/dt fell from 2,990 ± 204 (SEM) to 2,690 ± 200 mmHg/s (P < 0.01), and heart rate rose from 92 ± 4 to 105 ± 4 beats/min (P < 0.05). By 15 min after reperfusion all values had returned to control and remained there for 24 h.

Wall thickness. During coronary occlusion end-diastolic wall thickness decreased from 10.25 ± 0.59 to 9.82 ± 0.59 mm while systolic wall thickening decreased from 1.53 ± 0.18 to -.05 ± .12 mm, indicating severe loss of systolic wall thickening (Figs. 2, 4). On reperfusion, end-diastolic wall thickness showed an immediate rebound above control to 11.17 ± 1.07 mm (Fig. 5). These changes were significant, P < 0.01. The time course of decay of increased end-diastolic wall thickness paralleled the reactive hyperemic response, i.e., by 15 min after reperfusion, end-diastolic wall thickness was not significantly different from control (Fig. 5). Systolic wall thickening also increased transiently upon reperfusion to 1.33 ± 0.23 mm, a level still slightly below preoclusion base line. Systolic wall thickening then decreased at 15 min to 0.78 ± 0.21 mm (P < 0.01) and stayed depressed for as long as 3 h (Fig. 5). Systolic wall thickening was not only depressed after reperfusion but also showed a marked delay in time-to-peak shortening (Fig. 4). For instance, at 15 min peak systolic wall thickening occurred 72 ± 8 ms later (P < 0.01) than it did prior to occlusion. This resulted in a marked change in configuration of the phasic waveform for wall thickness during recovery, with wall thickening persisting in early diastole. The time-to-peak wall thickening was no longer significantly delayed at 3 h after reperfusion, while the extent of systolic wall thickening was not significantly different from control at 24 h (Fig. 4).

Segment length. The changes observed in ischemiczone segment length were similar to those reported in a previous study with a similar protocol (3) and need not be reiterated. However, it is important to point out that the phasic waveforms for segment length showed changes reciprocal to those for wall thickness, including a prolonged shortening into early diastole which was reciprocal to the delayed wall thickening noted above.

Intramyocardial ECG

During the 15-min occlusion, S-T segment elevation in the ischemic zone rose from 1.1 ± 0.3 to 8.3 ± 0.9
Effects of Coronary Occlusion on Regional Myocardial Blood Flow

During coronary occlusion, regional flow fell from $1.04 \pm 0.04$ to $0.27 \pm 0.02$ ml/min per g. Flow to the subendocardial layers fell more than flow to the epicardial layers, resulting in a decrease in the endo/epi flow ratio from $1.23 \pm 0.04$ to $0.44 \pm 0.04$.

At 15 min, 30 min, and 60 min after reperfusion, when the reactive hyperemic response had subsided, transmural blood flow remained significantly depressed ($P < 0.05$) by $20 \pm 4\%$, $18 \pm 5\%$, and $11 \pm 3\%$, respectively (Fig. 6). The endo/epi flow ratio at 15 min, 30 min, 60 min, and 3 h after reperfusion was $1.06 \pm 0.08$, $0.83 \pm 0.10$, $0.95 \pm 0.06$, and $0.98 \pm 0.08$, respectively, values which were significantly lower ($P < 0.05$) than control.

Pathology

Examination of gross tissue slices stained with TTC revealed no evidence of ischemic damage to the myocardium. Histologic and histochemical studies also showed no ischemic damage. While occasional intramyocardial arterioles were congested with erythrocytes or contained fibrin deposits, no differences were observed in the samples from the anterior and posterior portions of the left ventricle. Platelet thrombi were not observed. In summary, morphologic studies by light microscopy showed no pathologic findings to explain the observed functional abnormalities after a 15-min coronary artery occlusion.

DISCUSSION

It has been demonstrated previously that reperfusion following short periods of coronary artery occlusion, i.e., of 5 and 15 min duration does not result in necrosis.
(4), but does result in prolonged depression of regional mechanical function as measured by segment length and velocity of myocardial fiber shortening (3, 23). The prolonged depression of regional function was surprising in view of the rapid return of the regional electrographic changes (1 min) and overall coronary flow (12 min) in the previously occluded vessel.

In the present study, the mechanism of the prolonged depression was examined by measurements of a) regional left ventricular wall thickening using the ultrasonic transit-time technique (3), b) regional myocardial blood flow using the radioactive-microsphere technique (1), and c) examination of histological sections. If the mechanism of the prolonged depression of function were related to gross edema developing in the reperfused tissue, as has been shown to occur with longer periods of ischemia (5), diastolic wall thickness should have remained elevated following reperfusion. On the other hand, if the mechanism were related to persistent ischemia, total flow or subendocardial flow should be depressed during the recovery period.

Regional wall thickening occurs during systole, when myocardial fibers shorten. Changes in wall thickness are thus complementary but opposite in direction to changes in segment length. Therefore, it was not surprising to find that systolic wall thickening of the ischemic segment was lost and systolic wall thinning was observed during occlusion (Figs. 2, 4). This has also been shown by others (2, 13, 19).

Upon reperfusion, diastolic wall thickness, increasing above control, showed a marked rebound phenomenon. The time course of this phenomenon paralleled the reactive hyperemic response, suggesting that the increase in end-diastolic wall thickness in this situation was due to an increase in the amount of blood volume in the myocardium during diastole. Diastolic wall thickness remained at preischemic control levels for the next 24 h, indicating that gross edema had not developed. While the technique used in this study is sufficiently precise to reflect consistent changes in dimensions of 0.05 mm, it may not be sufficiently sensitive to show that focal microscopic edema had not occurred. However, these findings are in agreement with Whalen et al. (24), who found slight increases in Na+ content but did not find cellular swelling of myocardial cells after 15 min of ischemia followed by 2 min of reflow in anesthetized dogs.

Systolic wall thickening showed two abnormalities after subsidence of the reactive hyperemic response. First, at 15 min after reperfusion, the time-to-peak systolic wall thickness was prolonged ($P < 0.01$), and second, the extent of systolic wall thickening was diminished, reflecting the prolonged depression of mechanical function. The prolongation of time-to-peak wall thickening resulted in persistent wall thickening during early diastole. A reciprocal delayed shortening was observed for segment-length recordings. This may reflect persistent effects of ischemia, which prolongs contraction to early diastole at a time when intraventricular pressure is no longer elevated. The time-to-peak wall thickening returned to normal after 1 h, but the extent of systolic wall thickening returned only after 24 h (Fig. 4), which is consistent with the concept that no permanent damage has occurred.

Another finding of the present study was that after recovery from the reactive hyperemic response, persistent ischemia was demonstrated in the central area subserved by the previously occluded vessel. Ischemia was reflected by reduced transmural flow in the previously ischemic segment at 15 min and as long as 1 h following reperfusion. The endo/epi flow ratio after release of the occlusion was also depressed up to 3 h. These findings could explain in part the depressed function observed up to 3 h following reperfusion. An alternative explanation could be that the decrease in regional function itself is due to certain damage to the contractile proteins that occurs during the period of ischemia. This damage, although completely reversible, takes a considerable time for recovery and is followed by a small reduction in flow in response to reduced oxygen demands.

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