Assessment of the Efficacy of Interventions to Limit Ischemic Injury by Direct Measurement of Intramural Carbon Dioxide Tension after Coronary Artery Occlusion in the Dog

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ABSTRACT Although numerous interventions have been shown to exert a salutary effect on the ischemic myocardium, the severity of ischemia generally has been measured by indirect techniques. In the present investigation the effect of ischemia on intramural carbon dioxide tension ($PmCO_2$) was measured directly in the open-chest, anesthetized dog with a mass spectrometer during repetitive 10-min coronary artery occlusions separated by 45-min periods of reflow; simultaneously, regional myocardial blood flow in the ischemic area was measured by ^{127}Xenon washout. In all dogs the increase in $PmCO_2$ from before to 10 min after the first occlusion ($\Delta PmCO_2$) exceeded that during subsequent occlusions. In those dogs not receiving an intervention (controls), $\Delta PmCO_2$ during the third occlusion was similar to that during the second occlusion. When propranolol, hyaluronidase, and nitroglycerin were administered to different groups of dogs before the third occlusion, each caused significantly smaller elevations in $\Delta PmCO_2$ than those occurring during the control second occlusion, and the combination of all three interventions induced the smallest increase in $\Delta PmCO_2$. Regional myocardial blood flow rose with hyaluronidase and was unchanged with propranolol, nitroglycerin, and the three drugs in combination. In contrast to these beneficial interventions, isoproterenol infused with the third occlusion caused a higher $\Delta PmCO_2$ than during the control second occlusion. It is concluded, first, that interventions that modify the severity of ischemia can be evaluated by measuring intramural carbon dioxide tension; second, that propranolol, hyaluronidase, and nitroglycerin reduce ischemic injury, whereas isoproterenol increases it; and third, that the combination of propranolol, hyaluronidase, and nitroglycerin exerts an additive beneficial effect on ischemia.

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INTRODUCTION

During the past few years a number of interventions have been shown to exert either a beneficial or a detrimental effect on the ischemic myocardium (1, 2). However, for the most part, these observations have been made with indirect methods of measuring the severity of ischemic injury, such as an analysis of electrocardiographic ST segment elevation. In an attempt to find a quantitative, direct technique for measuring myocardial ischemia, Khuri et al. (3), with the mass spectrometric measurement of intramural carbon dioxide tension ($PmCO_2$),[1] observed that $PmCO_2$ rises during ischemia, and recent studies have demonstrated that the magnitude of this rise in $PmCO_2$ after coronary occlusion corresponds closely with the severity of ischemic injury, as assessed histologically.[2] The present study was designed to use this direct measure of myocardial ischemia to investigate the effect of various

[1] *Abbreviations used in this paper*: $PmCO_2$, intramural carbon dioxide tension; RMBF, regional myocardial blood flow.

[2] Khuri, S. F., R. A. Kloner, L. D. Hillis, D. Tow, E. Barsamian, P. R. Maroko, and E. Braunwald. 1978. Intramural pCO_2: A reliable index of the severity of myocardial ischemic injury. Submitted for publication.

interventions on ischemia when applied individually and in combination during repetitive 10-min coronary artery occlusions in the dog. In addition, to provide an increased understanding of the mechanism of action of these interventions, their effect on regional myocardial blood flow was determined by the intramyocardial injection and subsequent washout of ^{127}Xenon.

METHODS

The studies were performed in 52 mongrel dogs of both sexes that weighed between 14 and 26 kg and anesthetized with pentobarbital sodium, 30 mg/kg i.v. Respiration was maintained through a cuffed endotracheal tube with a mechanical respirator (Harvard Apparatus Co., Inc., Millis, Mass.). Systemic arterial pressure was monitored continuously through a catheter inserted into the left common carotid artery with a Statham P23Db strain gauge (Statham Instruments, Inc., Oxnard, Calif.). Heart rate was recorded by a tachometer throughout each experiment on a polygraph (Grass Instrument Co., Quincy, Mass.). The left jugular vein was catheterized and used as a route of administration of fluids and drugs. The thorax was opened through the fifth left intercostal space and the heart suspended in a pericardial cradle, so that the anterior aspect of the left ventricle was well exposed. The midportion of the left anterior descending coronary artery was dissected free so that it could be occluded when desired.

Measurement of intramural carbon dioxide tension. The Medspec II mass spectrometer (Chemetron Medical Products, Chemetron Corp., Baltimore, Md.) was used to monitor $PmCO_2$, as previously described (3). In each dog two spectrometer probes (EXTC teflon catheters, Chemetron Corp.; or SpectraCaths, Sorenson Research Co., Salt Lake City, Utah) were placed in the left ventricular myocardium; they were inserted through a small nick in the epicardium and advanced gently until their sensing surfaces were entirely within the myocardium and their longitudinal axes approximately parallel to the epicardial surface. Each probe was secured with a single suture (3, 4). In 41 dogs both probes were placed in the area of distribution of the occluded left anterior discending coronary artery, as judged by its cyanosis. In the other 11 dogs, one probe was placed in this area and the other in an area remote from the occlusion. Carbon dioxide tension was measured continuously from each probe.

Measurement of regional myocardial blood flow (RMBF). In 43 of the 52 dogs RMBF was measured by quantifying the washout of the radioactivity of ^{127}Xenon injected into the myocardium directly adjacent to the spectrometer probe. At the desired time, 0.1 ml saline that contained $\cong$200 μCi of ^{127}Xenon was injected rapidly into the myocardium. The ^{127}Xenon washout curve was recorded on a rate meter connected to a strip chart recorder, and RMBF was calculated by plotting the washout values on semilog paper (5).

Experimental protocol. In all dogs three occlusions of the coronary artery were performed, each lasting 10 min, with a 45-min interval between occlusions for reflow. RMBF was measured during each occlusion. $PmCO_2$ was recorded continuously throughout each experiment. The dogs were assigned randomly to one of six groups. In group A (11 dogs), three successive coronary artery occlusions were performed without the administration of an intervention; therefore, these animals served as controls. Groups B-F were identical to the control group except that 10 min before the third occlusion they received one of several interventions: group B (eight dogs), propranolol, 2 mg/kg as an intravenous bolus; group C (nine dogs), hyaluronidase (Alidase, G. D. Searle & Co., Skokie, Ill.), 500 National Formulary units/kg as an intravenous bolus; group D (eight dogs), nitroglycerin (G. Pohl-Boskamp & Co., Holstein, West Germany.), 300 μg as an intravenous bolus followed by a continuous intravenous infusion of 3–5 μg/kg per min, the exact dose adjusted so that mean systemic arterial pressure declined by 20 mm Hg; group E (eight dogs), a combination of propranolol, hyaluronidase, and nitroglycerin, all at the doses listed above; and group F (eight dogs), isoproterenol, 0.25–0.50 μg/kg per min by continuous intravenous infusion begun 10 min before the third occlusion and continued throughout the occlusion, the exact rate of infusion adjusted so that heart rate increased by 30 beats/min.

After release of the third coronary artery occlusion, $PmCO_2$ was allowed to return to base line; then the dogs were sacrificed. The hearts were excised and dissected to assure that the entire sensing surface of each probe was completely within the myocardial wall. If the tip of the probe was found to have perforated the left ventricular endocardium, all its gas tension readings were discarded. In the 52 dogs (total of 104 probes), endocardial perforation occurred only in three instances. The thickness of the left ventricle in the area of the probe was measured, as was the depth of the probe within the myocardium. Of the 101 probes from which $PmCO_2$ was recorded, 65 were found in the middle third of the left ventricular wall; 22 were located in the epicardial third; and the remaining 14 were positioned within the endocardial third.

Data analysis. For each dog heart rate and mean systemic arterial pressure were recorded throughout each coronary artery occlusion. For each spectrometer probe the rise in $PmCO_2$ with each occlusion was assessed and expressed as $\Delta PmCO_2$, in millimeters Hg. Likewise, RMBF was measured 5 min after each occlusion, i.e., midway during the occlusion period. In each of the 41 dogs in which both spectrometer probes were placed in the ischemic area, the two values for $\Delta PmCO_2$ during each coronary artery occlusion were averaged. Each parameter was compared within each of the six groups during successive coronary artery occlusions. For each parameter an analysis of variance was performed to determine if some groups were different from others, after which individual groups were compared with each other with the Student's t test (6).

For all 52 dogs, a total of 90 probes was placed in the ischemic myocardium, $PmCO_2$ before the first coronary artery occlusion was 52.7±1.8 mm Hg, and with the occlusion it increased to 127.7±6.1 mm Hg ($\Delta PmCO_2$ = 75.0±5.0 mm Hg). During this first occlusion, $\Delta PmCO_2$ was 73.5±8.6 mm Hg for the 11 control dogs and 75.6±6.0 mm Hg for the 41 dogs that received an intervention. The second coronary artery occlusion caused a significantly lower ($P < 0.001$) rise in $PmCO_2$ ($\Delta PmCO_2$ = 54.1±3.6 mm Hg); for the 11 control dogs, $\Delta PmCO_2$ during this second occlusion averaged 53.5±7.6 mm Hg, whereas for the 41 treated dogs, it averaged 54.3±4.1 mm Hg. During the first coronary occlusion, heart rate for the 52 dogs was 150±3 beats/min, and during the second occlusion it was 144±3 beats/min ($P < 0.01$). The mean systemic arterial pressure during the first occlusion (113±3 mm Hg) was unchanged during the second occlusion (111±3, $P > 0.20$). RMBF in the ischemic area was 14.4±1.6 ml/100 g per min during the first occlusion and 13.8±1.3 ml/100 g per min during the second occlusion (n = 43, $P > 0.40$). In the 11 control dogs (without an intervention applied before the third occlusion), $\Delta PmCO_2$ and RMBF during the third occlusion (53.1± 7.5 mm Hg and 14.1±1.2 ml/100 g per min, respectively) were similar to the values obtained during the second occlusion (52.9±7.3 mm Hg and 13.9±1.7 ml/100 g per min, respectively). Because of the stability between the second and third occlusions (which also was noted in preliminary experiments), interventions were administered before the third occlusion,

and comparisons within each group of dogs were made between the second and third occlusions.

RESULTS

Changes in $PmCO_2$ in nonischemic tissue

In five groups of dogs (groups A-E), $PmCO_2$ in the myocardium remote from the area of ischemia did not change with coronary artery occlusion or with the administration of the intervention (n = nine dogs with nine probes). Furthermore, in these same groups, the administration of the intervention did not affect the base-line $PmCO_2$ in the area of distribution of the coronary artery to be occluded (n = 44 dogs with 76 probes). In contrast, in group F, the infusion of isoproterenol before the third occlusion caused an increase in $PmCO_2$ in both the myocardium remote from the area of ischemia (from 45.0±10.0 mm Hg before isoproterenol to 55.5±5.5 mm Hg during isoproterenol, n = two dogs with two probes) and within the area of distribution of the coronary artery to be occluded (from 49.0±5.5 to 61.2±5.3 mm Hg; n = eight dogs, $P < 0.05$).

Changes in $PmCO_2$ and ^{127}Xenon washout in ischemic tissue

Group A (controls). In the 11 dogs that served as controls, heart rate and mean systemic arterial pressure during the second and third occlusions were similar (Table I). The average RMBF during the second and third coronary artery occlusions was unchanged, as was $\Delta PmCO_2$ (Figs. 1 and 2, Table I). In the untreated dogs, therefore, the third coronary artery occlusion caused an identical rise in $PmCO_2$ and a similar decline in blood flow to the ischemic myocardium when compared to the second occlusion.

Group B (propranolol-treated). The administration of propranolol before the third coronary artery occlusion caused a marked decline in heart rate but no change in arterial pressure (Table I). RMBF was 12.1 ±3.8 ml/100 g per min during the second occlusion and 8.0±2.8 ml/100 g per min during the third occlusion (Table I). $\Delta PmCO_2$ with the second occlusion averaged 49.6±8.4 mm Hg, whereas during the third occlusion it fell to 28.5±6.2 mm Hg ($P < 0.01$) (Table I, Fig. 2). Therefore, despite no increase in RMBF in the ischemic tissue, propranolol reduced the rise of $PmCO_2$.

Group C (hyaluronidase-treated). The administration of hyaluronidase caused no significant change in heart rate or mean systemic arterial pressure (Table I). In contrast to the results with propranolol, hyaluronidase increased RMBF in the ischemic area from 11.4 ±3.1 ml/100 g per min during the second occlusion to 18.0±4.6 ml/100 g per min during the third occlusion ($P < 0.05$) (Table I). $\Delta PmCO_2$ with the second coronary artery occlusion was 59.9±9.6 mm Hg, and during the third occlusion it fell significantly to 49.9±7.4 mm Hg ($P < 0.05$) (Table I, Fig. 2). Therefore, hyaluronidase reduced the rise in $PmCO_2$ while simultaneously augmenting RMBF.

Group D (nitroglycerin-treated). The infusion of nitroglycerin caused a reduction in mean systemic arterial pressure (from 94±2 mm Hg to 76±2 mm Hg) but no change in heart rate (Table I). RMBF was similar during the two occlusions; $\Delta PmCO_2$ with the third occlusion (52.1±7.1 mm Hg) was significantly less ($P < 0.05$) than with the second occlusion (62.9±7.5 mm Hg) (Table I, Fig. 2).

Group E (propranolol-hyaluronidase-nitroglycerin combination). With the administration of the combination of agents, both heart rate and mean systemic arterial pressure declined (Table I). As with nitroglycerin and propranolol alone, RMBF did not change significantly from the second to the third occlusion (Table 1). $\Delta PmCO_2$ fell markedly from 54.1±11.7 mm

TABLE I

Effects of Two Consecutive 10-min Periods of Coronary Occlusion

Group	$\Delta PmCO_2$		RMBF		Heart rate		Mean arterial pressure	
	OCC 2	OCC 3	OCC 2	OCC 3	OCC 2	OCC 3	OCC 2	OCC 3
	mm Hg		*ml/100 g/min*		*beats/min*		*mm Hg*	
A (controls)	52.9±7.3	53.1±7.5	13.9±1.7	14.1±1.2	128±5	119±5	121±6	123±6
B (propranolol)	49.6±8.4	28.5±6.2*	12.1±3.8	8.0±2.8	142±7	109±5*	104±9	95±7
C (hyaluronidase)	59.9±9.6	49.9±7.4‡	11.4±3.1	18.0±4.6‡	143±5	140±6	105±3	105±3
D (nitroglycerin)	62.9±7.5	52.1±7.1‡	13.9±2.7	10.7±3.7	147±4	143±4	94±2	76±2‡
E (combination)	54.1±11.7	22.0±7.4*§	14.8±3.8	16.7±4.0	155±9	119±8*	112±7	89±5‡
F (isoproterenol)	45.1±9.1	67.6±7.5*	18.8±3.3	14.8±4.6	155±5	184±2*	127±9	102±8‡

All figures are mean±SEM. OCC, occlusion.
* $P < 0.01$ when compared to Group A.
‡ $P < 0.05$ when compared to Group A.
§ $P < 0.05$ when compared to Group B.

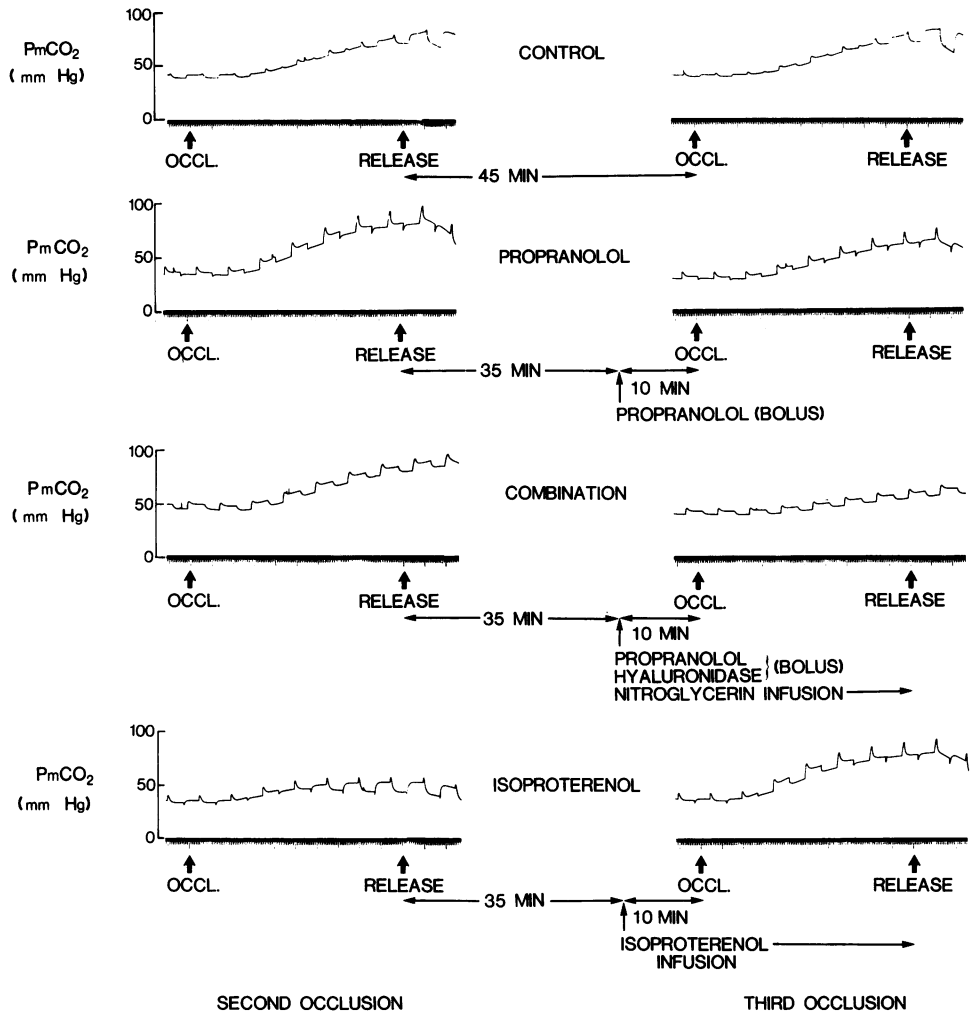

FIGURE 1 Examples of elevations in $PmCO_2$ with the second and third occlusions for several representative dogs. Note the following characteristics of each tracing: (*a*) there is a 2–3-min delay between the events occurring at the probe's sensing surface and the readout on the paper. Thus, the $PmCO_2$ begins to rise 2–3 min after occlusion and peaks 2–3 min after release. (*b*) The mass spectrometer is continuously analyzing the $PmCO_2$ in both probes, but at any one time only one probe is displayed. The machine cycles automatically every 45 s, and the points at which this cycling occurs are easily recognizable. In the top panel, tracings are shown from a control dog. Note that the rise in $PmCO_2$ with the third occlusion is similar to that during the second occlusion. The second panel is an example from a dog that received propranolol 10 min before the third occlusion. Note that the rise in $PmCO_2$ was blunted when compared to the second occlusion. In the third panel, tracings are displayed from a dog that received a combination of propranolol, hyaluronidase, and nitroglycerin before the third occlusion. Note the marked diminution in $\Delta PmCO_2$ with the third occlusion. Finally, in the bottom panel are tracings from a dog given isoproterenol with the third occlusion. $PmCO_2$ rose more with the third than with the second occlusion, demonstrating isoproterenol's detrimental influence on ischemia. OCCL., occlusion.

Hg during the second occlusion to 22.0±7.4 mm Hg during the third occlusion ($P < 0.01$) (Table I, Fig. 2). The reduction of $\Delta PmCO_2$ induced by the combination of agents was significantly greater ($P < 0.05$) than that caused by any of the agents administered individually (Fig. 3).

Group F (isoproterenol-treated). The administration of isoproterenol caused a significant increase in heart rate as well as a significant reduction in mean systemic arterial pressure (Table I). RMBF was unchanged from the second to the third occlusion (Table I). $\Delta PmCO_2$ increased from 45.1±9.1 mm Hg during the second occlusion to 67.6±7.5 mm Hg during the third occlusion ($P < 0.01$) (Table I, Figs. 1 and 2).

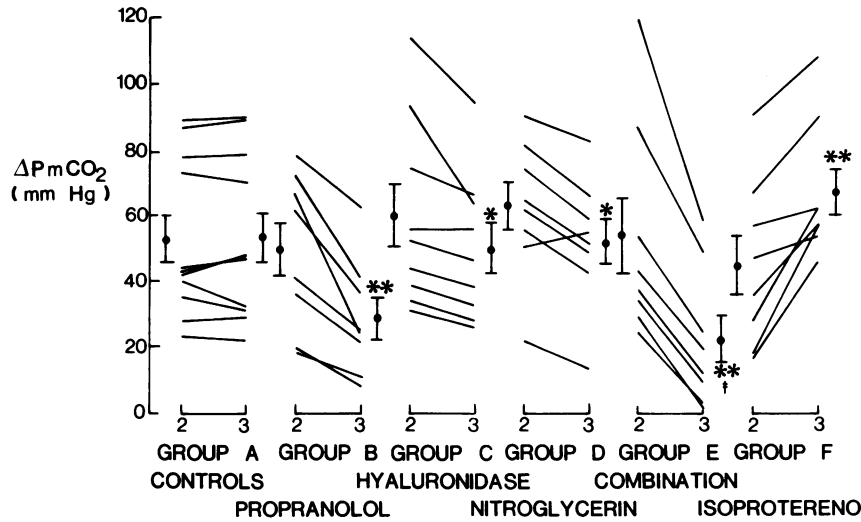

FIGURE 2 $\Delta PmCO_2$ during the second (2) and third (3) occlusions for the six groups of dogs. Each line represents the values from one dog. The mean±SEM during the two occlusions is shown on either side of the individual sets of data. In group A (controls), $\Delta PmCO_2$ with the two occlusions is similar. Group B (propranolol-treated), group C (hyaluronidase-treated), and group D (nitroglycerin-treated) all demonstrate a significant reduction of $\Delta PmCO_2$ with the third occlusion, and group E (combination therapy) shows a marked decline in $\Delta PmCO_2$. In contrast, group F (isoproterenol-treated) shows a significant increase in $\Delta PmCO_2$ with the third occlusion. $^*P < 0.05$, $^{**}P < 0.01$ when compared to group A; $\ddagger P < 0.05$ when compared to group B.

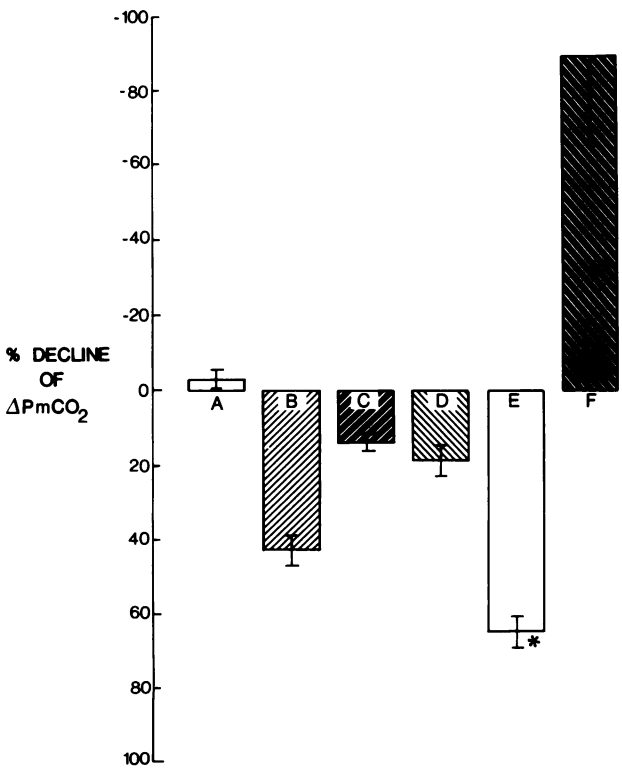

FIGURE 3 A comparison of the percentage decline in $\Delta PmCO_2$ from the second to the third occlusion for the six groups of dogs. The control dogs (group A) showed no significant change in $\Delta PmCO_2$ from the second to the third occlusion. Groups B, C, D, and E all showed significant reductions in $\Delta PmCO_2$ after drug administration; the most profound beneficial effect was seen in group E (combination therapy). In contrast, $\Delta PmCO_2$ rose with isoproterenol (group F), and, as a result, the percentage decline in $\Delta PmCO_2$ is expressed as a negative number. $^*P < 0.05$ compared to group B (propranolol-treated).

DISCUSSION

With the use largely of indirect methodology, the effect of numerous interventions on the extent of myocardial ischemia has been assessed (1, 2). Changes in the magnitude of epicardial or precordial ST segment elevation after coronary artery occlusion both in the experimental animal and in man have been used to show that beta adrenergic blockade (2, 7–9), hyaluronidase (10–12), and nitroglycerin (13–17) exert a salutary effect on ischemic injury, whereas isoproterenol exerts a detrimental influence on the ischemic myocardium (2, 18). More recently, the epicardial and precordial QRS complex has been shown to reflect accurately the extent of necrosis in the myocardium subjacent to the electrode; with this technique, hyaluronidase and propranolol have been shown to be beneficial (19, 20).

In the present study $PmCO_2$ was measured with a teflon membrane-mass spectrometer system (21). Previous studies with this technique have demonstrated that an increase in $PmCO_2$ after coronary artery occlusion is a sensitive indicator of myocardial ische-

mia, as assessed by intramyocardial ST segment elevation (3). More recent investigations have shown that the magnitude of rise of $PmCO_2$ during the 60 min after coronary occlusion corresponds closely, first, to the severity of injury, as assessed histologically, and second, to the reduction of regional myocardial blood flow.[2] At the same time, these studies have shown that changes in intramural oxygen tension with coronary occlusion are unreliable in the quantitation of the severity of ischemia. Therefore, in the present investigation the influence of various interventions on $PmCO_2$, but not intramural oxygen tension, was evaluated.

The accumulation of CO_2 within the myocardium reflects the balance between tissue CO_2 production and its clearance by regional blood flow (3). With ischemia, coronary blood flow and its associated delivery of oxygen are reduced drastically. As a result, the myocardium shifts from aerobic to anaerobic metabolism, with the resultant production of lactic acid (22). The increased concentration of hydrogen ion in the extracellular space accelerates the generation of CO_2 from HCO_3^-. As ischemia becomes more severe, there is an increase in the production of H^+ and, as a result, in the quantity of CO_2 that is produced; simultaneously, there is a reduction of CO_2 clearance. Consequently, the severity of myocardial ischemia is reflected in the elevation of $PmCO_2$.

In the present study RMBF was measured with the washout of ^{127}Xenon injected direction into the myocardium. This diffusible-indicator method of determining tissue blood flow was described by Kety et al. (23–25), who observed that the washout of a tracer injected intraarterially was proportional to tissue blood flow. More recently, to quantitate regional myocardial blood flow, Kety's technique has been applied to the intramyocardial injection and subsequent washout of a tracer material, most commonly ^{133}Xenon (26–28). Although the monoexponentiality of xenon washout curves is controversial (29), this method for measuring RMBF has been found to correlate with other methods of measuring coronary blood flow (26, 30, 31).

In the present study each animal was subjected to three successive 10-min coronary artery occlusions at 45-min intervals. In all animals the rise in $PmCO_2$ with the first occlusion was greater than during subsequent occlusions. This reduction of $\Delta PmCO_2$ is, at least in part, caused by, first, a decline in heart rate from the first to the second coronary occlusion and, second, a reduction of contractility in the ischemic tissue after the initial occlusion. Previous studies have demonstrated that brief periods of coronary artery occlusion result in prolonged (i.e., 3 h) depression of myocardial function in the ischemic zone (32). The fall of $\Delta PmCO_2$ is not caused by a lesser reduction in RMBF during the second and third occlusions compared to the first. The increases in $PmCO_2$ were similar with coronary artery occlusions subsequent to the first; that is, the control dogs (group A) demonstrated no change in $\Delta PmCO_2$ from the second to the third occlusion (Table I, Figs. 1 and 2). Similarly, RMBF was unchanged during these two occlusions (Table I).

In another investigation carried out in this laboratory, it was shown that the magnitude of rise of $PmCO_2$ ($\Delta PmCO_2$) is an excellent predictor of the severity of ischemic myocardial damage.[2] Specifically, it was found during a 60-min coronary artery occlusion, that the $\Delta PmCO_2$ occurring 10 min after occlusion, as well as the maximum $\Delta PmCO_2$ achieved during this period correlated well with the degree of histologic damage seen in 1-μm toluidine blue-stained sections, as reflected in clumping of nuclear chromatin, intermyofibrillar edema, and lifting of the sarcolemma off the myofilaments as well as with the reduction of regional blood flow. These observations support the use of $\Delta PmCO_2$ as an indicator of the severity of myocardial ischemic injury. However, this conclusion must be qualified, because in the present investigation we compared the differences in $\Delta PmCO_2$ between the second and third of three 10-min occlusions, whereas in the aforementioned study the $\Delta PmCO_2$ 10 min after the onset of a single 60-min occlusion was compared with the histologic findings at the end of that period. Nonetheless, despite this difference in experimental design, it should be pointed out that in the present study there were excellent correlations between: (*a*) the $\Delta PmCO_2$ during the first and second occlusions ($r = 0.93$, $P < 0.001$, $n = 52$); (*b*) the $\Delta PmCO_2$ during the second and third occlusions in the control dogs ($r = 0.99$, $P < 0.001$, $n = 11$); (*c*) the RMBF during the first and second occlusions ($r = 0.88$, $P < 0.001$, $n = 43$); and (*d*) the RMBF during the second and third occlusions in the control dogs ($r = 0.85$, $P < 0.01$, $n = 7$). Therefore, it appears reasonable to suggest that the severity of ischemia, as reflected in the RMBF and the $\Delta PmCO_2$ in the second and third 10-min occlusions would also correlate closely with the histologic damage after the 60-min coronary occlusion.

Propranolol has been shown to exert a beneficial effect on myocardial ischemia both in experimental animals and in man (2, 7, 9, 19, 33–37). Although most of these studies are based on indirect methods for measuring ischemic injury, in others the salutary effects of propranolol have been based on morphologic observations (34, 35, 37). In the present investigation it was observed that propranolol caused a marked decline in the elevation of $PmCO_2$ during ischemia without any increase in regional myocardial blood flow. Previous studies in anesthetized dogs have shown that propranolol causes either no change (38, 39) or a decline (40) in blood flow to the ischemic myocardium. In contrast, in the conscious dog with regional myocardial

ischemia, propranolol induces a redistribution of myocardial blood flow, with flow falling in normal zones and increasing in moderately and severely ischemic zones (41). The differences in the results obtained in these various studies are probably related to the wide variations in experimental design (conscious, chronically instrumented animals vs. anesthetized, acutely instrumented ones). Although there has been considerable debate about the mechanism by which propranolol reduced ischemic injury, the observation that it lessens the elevation of $PmCO_2$ without increasing regional blood flow indicates that it decreases CO_2 production in the ischemic tissue by reducing myocardial energy needs.

Hyaluronidase also has been shown to lessen ischemic injury with both indirect (10–12, 19, 42) and morphologic (43, 44) techniques. In the present study it reduced significantly the magnitude of carbon dioxide elevation during coronary occlusion. However, in contrast to propranolol, hyaluronidase acutely increased regional blood flow to the ischemic area, suggesting that it exerts its protective effect by augmenting collateral blood flow to the ischemic tissue. These findings are compatible with previous studies in our laboratory which have demonstrated that hyaluronidase prevents the decline in blood flow to the ischemic myocardium during the 6 h after coronary artery occlusion in the dog (45). Therefore, although both propranolol and hyaluronidase are beneficial in the presence of ischemia, they apparently exert their effects in different ways, because hyaluronidase increases and propranolol does not change regional myocardial blood flow. Interestingly, electron microscopic studies also have shown distinct ultrastructural differences in the ischemic myocardium after the administration of these two agents: propranolol induces preferential protection of the mitochondrial contents (46), whereas hyaluronidase primarily spares cytoplasmic glycogen granules (44).

Like propranolol and hyaluronidase, nitroglycerin has been shown to exert a beneficial effect on the ischemic myocardium (14–17), and in the present study it significantly lessened the rise in $PmCO_2$ after coronary occlusion (Fig. 2). Similar results of nitroglycerin administration have been reported in dogs with coronary artery constriction rather than total occlusion (47). Like propranolol, nitroglycerin did not significantly alter RMBF.

Isoproterenol has been shown to intensify ischemic injury in a number of experimental models (2, 18), and in the present investigation this detrimental effect was easily demonstrable. Interestingly, with isoproterenol, $PmCO_2$ increased slightly even without coronary artery occlusion, presumably reflecting an augmentation of myocardial energy demands and a significant decline in mean systemic arterial pressure (Table I). With occlusion, RMBF fell slightly (but not significantly), and carbon dioxide tension rose strikingly, reaching a much higher peak than had occurred during the previous occlusion (Figs. 1 and 2). The intensification of ischemia caused by isoproterenol appears to result from both an augmentation of myocardial metabolism secondary to the positive inotropic and chronotropic effects of the drug and the simultaneous decline in coronary artery perfusion pressure and, as a result, the failure of coronary blood flow to rise in response to the increased metabolic demands of the myocardium.

Although numerous interventions have been shown to exert a beneficial effect on ischemic injury, few studies have attempted to assess the effect of combinations of efficacious agents. After-load reduction, achieved either pharmacologically (with nitroglycerin or nitroprusside) or mechanically (with intraaortic balloon or external counterpulsation), exerts a beneficial effect on the ischemic myocardium when combined with phenylephrine (15, 48, 49); in addition, nitroprusside and external counterpulsation administered together are more effective in reducing ischemia than either intervention alone (50). In the dog with 3 h of coronary artery occlusion followed by reperfusion, a combination of intravenous propranolol, intraaortic balloon counterpulsation, and hypothermia exerted a remarkably salutary effect on ischemic injury. In fact, when the hearts of these animals were examined 7 d later, no evidence of necrosis was demonstrable (51). In the present study, propranolol, hyaluronidase, and nitroglycerin administered together markedly reduced the degree of ischemia, as reflected by much less elevation of the $PmCO_2$. In fact, the combined drugs exerted a significantly greater salutary effect than any one of them alone (Fig. 3).

The combination of propranolol, hyaluronidase, and nitroglycerin was administered because each of these agents exerts its beneficial effect on the ischemic myocardium through a mechanism different from the other two drugs. Specifically, propranolol reduces myocardial energy requirements; hyaluronidase augments local coronary blood flow; and nitroglycerin preserves flow through coronary collaterals (despite a decline in coronary artery perfusion pressure) while simultaneously reducing myocardial energy needs. The combination of these three agents caused a much more striking reduction in the severity of ischemia than did any one of them individually (Fig. 3).

In conclusion, the present investigation describes a technique for assessing the effect of interventions on the severity of ischemia. During successive coronary artery occlusions in the dog, the magnitude of rise in carbon dioxide tension, as measured with a mass spectrometer, allows for the measurement of ischemia. With this technique, propranolol, hyaluronidase, and nitroglycerin, all given alone, were shown to exert a

salutary effect on the ischemic myocardium, whereas isoproterenol was shown to have a detrimental influence. The beneficial effect of the combination of propranolol, hyaluronidase, and nitroglycerin was shown to be profound. With the mass spectrometer, the degree of ischemia and the effects on ischemia of various interventions can be assessed more accurately than has heretofore been possible.

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