Postischemic Myocardial "Stunning"

Identification of Major Differences Between the Open-Chest and the Conscious Dog and Evaluation of the Oxygen Radical Hypothesis in the Conscious Dog

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Recent studies suggest that oxygen-derived free radicals contribute to the pathogenesis of postischemic myocardial dysfunction (myocardial "stunning"). This concept, however, is predicated exclusively on results obtained in open-chest preparations, which are subject to the confounding influence of many unphysiological conditions. The lack of supporting evidence in more physiological animal models represents a major persisting limitation of the oxy-radical hypothesis of myocardial stunning. The goal of this study was to address two fundamental (and related) questions: 1) Does the open-chest animal model alter the phenomenon of myocardial stunning? 2) If so, how valid are the concepts, derived from such a model, regarding the pathogenetic role of oxy-radicals? In part 1 of the study, myocardial stunning after a 15-minute coronary occlusion was compared in 30 pentobarbital-anesthetized open-chest dogs and in 19 conscious dogs. For any given level of collateral flow during occlusion, the recovery of systolic wall thickening after reperfusion was markedly less in open-chest animals. In an additional group of five open-chest dogs, a close inverse relation was noted between body temperature and postischemic wall thickening, indicating that the recovery of the stunned myocardium in acute experiments may vary markedly depending on how temperature is controlled. Because of these major differences between open-chest and conscious dogs, the oxy-radical hypothesis needs to be tested in the latter model. Thus, in part 2 of the study, conscious unsedated dogs undergoing a 15-minute coronary occlusion were randomized to an intravenous infusion of either saline (19 coronary occlusions) or superoxide dismutase (SOD) plus catalase (CAT) (21 coronary occlusions). Despite the fact that the plasma levels of SOD and CAT declined rapidly after reperfusion, postischemic wall thickening was significantly greater in treated compared with control dogs throughout the first 6 hours of reflow. Thus, a brief (60-minute) infusion of SOD and CAT produced a sustained improvement of recovery of contractility. The magnitude of this beneficial effect was a function of the severity of ischemia: the lower the collateral perfusion, the greater the improvement effected by the enzymes. The accelerated recovery produced by SOD and CAT was not followed by any deterioration of contractility, suggesting that postischemic dysfunction is not a teleologically "protective" phenomenon. In conclusion, the severity of myocardial stunning is greatly exaggerated by the unphysiological conditions present in the barbiturate-anesthetized open-chest dog. However, antioxidant therapy does attenuate myocardial stunning in the conscious dog, indicating that oxygen radicals play a pathogenetic role that is independent of the abnormal conditions associated with the open-chest preparation. (*Circulation Research* 1991;69:731–747)

Recent studies[1–15] suggest that oxygen-derived free radicals contribute to the pathogenesis of postischemic myocardial dysfunction (myocardial "stunning"[16]). The validity of this concept, however, is limited by the fact that it is predicated exclusively on results obtained in barbiturate-anesthetized, open-chest preparations.[1–15] To our knowledge, no information is available regarding the effect of antioxidant therapy on myocardial stunning after brief (<20 minutes) ischemia in the conscious animal. Several investigators[17–28] have pointed out that observations in open-chest models may be con-

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Supported in part by National Institutes of Health grant HL-43151 and SCOR grant HL-42267. R.B. was a recipient of the Physician Scientist Award of the American College of Chest Physicians.

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Received April 12, 1990; accepted May 6, 1991.

founded by the effects of anesthesia, surgical trauma, lack of integrity of the chest wall, unphysiological hemodynamic conditions, excessive levels of circulating catecholamines, and other metabolic abnormalities. Certain conclusions derived from open-chest preparations have subsequently been found not to be applicable to conscious animals.[17,18,23,25,26,28] Furthermore, recent studies in conscious dogs[29,30] have shown that antioxidant therapy does not enhance recovery of function after prolonged (60–90 minutes) ischemia. In view of these considerations, the lack of supporting evidence in more physiological animal preparations represents a significant limitation of the oxy-radical hypothesis of myocardial stunning. Before results obtained in open-chest models can be extrapolated to humans, this hypothesis needs to be tested in awake animals.

Of course, the importance of repeating in conscious dogs experiments previously performed in open-chest dogs[1–15] depends on whether or not the postischemic recovery of function is significantly affected by the abnormal conditions associated with the latter model. Although it is generally agreed that the open-chest animal is not a physiological preparation, the extent to which this model distorts the specific phenomenon of myocardial stunning is not clear. One study[31] concluded that the time course of recovery of the stunned myocardium was similar in conscious and anesthetized dogs. Our experience, however, is equivocal. In our earlier work,[1,4,7,32,33] regional function recovered at a comparable rate in open-chest and conscious dogs, but in our more recent investigations,[5,6,9,11,12,14,15,34,35] the recovery in the open-chest model has been consistently less. Although we suspect that the difference is due to differences in body temperature in the acute preparation, we cannot resolve this apparent contradiction without additional research. If substantial differences do indeed exist between conscious and open-chest models, this would signify that myocardial stunning is altered by the unphysiological conditions inherent in anesthetized preparations, in which case the oxy-radical hypothesis (being entirely derived from these preparations) may not be applicable to conscious animals.

In summary, two fundamental (and related) questions pertaining to the oxy-radical hypothesis need to be addressed: 1) Does the open-chest animal model alter the phenomenon of myocardial stunning? 2) If so, how valid are the concepts, derived from such a model, regarding the pathogenetic role of oxygen radicals?

The present study was undertaken to address these questions. The first objective was to determine whether myocardial stunning is significantly affected by the conditions associated with the barbiturate-anesthetized open-chest preparation. To this end, the severity of postischemic dysfunction was compared in open-chest and conscious dogs undergoing the same duration of ischemia (a 15-minute coronary occlusion). The second objective was to test the oxy-radical hypothesis of myocardial stunning in the conscious, unsedated animal. To this end, the effect of superoxide dismutase (SOD) and catalase on recovery of contractile function was evaluated in chronically instrumented dogs undergoing a 15-minute coronary occlusion followed by 48 hours of reperfusion. SOD and catalase were selected because they are the most specific antioxidants available, so that a protective effect would provide cogent evidence for a pathogenetic role of oxygen metabolites. Observations were extended to 48 hours of reperfusion to determine whether any early improvement of function by antioxidant therapy was followed by deterioration. This was done because concern has been expressed that, by "forcing" the postischemic injured myocardium to contract, antioxidants may actually hinder the process of recovery and therefore delay the ultimate return of mechanical function to normal values.

Materials and Methods

A total of 84 dogs (45 open-chest and 39 conscious) were used for this study. The experimental preparations and techniques (both in open-chest and conscious dogs) have been previously described in detail.[1,4–7,9,11,12,14,15,33–37]

Studies in Anesthetized Dogs

Forty-five mongrel dogs of either sex (14–28 kg) were anesthetized with sodium pentobarbital (35 mg/kg i.v.), ventilated with room air, and instrumented as previously described.[1,4–7,9,11,12,14,15,34,35] Ventilatory parameters were adjusted on the basis of arterial blood gas determinations to maintain normal pH and P_{O_2}. Supplements of KCl (20 meq) were given intravenously to maintain plasma potassium concentration in the range of 4.0–5.0 meq/l.[5] The rate of fluid replacement was standardized at ~80 ml normal saline/hr.[9]

The mid left anterior descending coronary artery (LAD) was occluded for 15 minutes and then reperfused. Dogs were assigned to two groups. In group I (normothermic group), the esophageal temperature was kept at 37–38°C throughout the study by using a heating blanket and by covering the animals. This is the protocol that was used in recent studies from our laboratory[5,6,9,11,12,14,15,34,35] in which the postischemic myocardium exhibited little recovery and remained dyskinetic throughout the reperfusion phase. In group II (hypothermic group), no effort was made to control body temperature (i.e., no heating blanket was used, and the animals were not covered). This is the protocol that was followed in our earlier studies[1,4,7,32] in which the postischemic myocardium exhibited a significant recovery and became hypokinetic after reperfusion. To further assess the impact of temperature on postischemic dysfunction, after 4 hours of reflow the esophageal temperature in group II was increased by covering the dogs and by using heating blankets. Hemodynamic and wall thickening measurements

were repeated when the temperature had increased 2.8°C (5°F) (this required 85±8 minutes).

The purpose of studying group I was to determine whether the severity of myocardial stunning is exaggerated in open-chest dogs even when fundamental physiological variables (including temperature) are kept within the normal range. The purpose of studying group II was to determine whether the apparent discrepancy between our earlier[1,4,7,32] and later[5,6,9,11,12,14,15,34,35] studies in open-chest dogs is due to differences in body temperature.

The following exclusion criteria were established before the beginning of the investigation: 1) arterial Po_2<60 mm Hg, pH<7.36, or hematocrit<30%, 2) mean arterial blood pressure<60 mm Hg, 3) heartworm infestation, and 4) ventricular fibrillation during coronary occlusion or on reperfusion.

Studies in Conscious Dogs

Thirty-nine healthy mongrel dogs of either sex (20–30 kg) were instrumented as previously described.[33,36,37] Dogs were allowed to recover for a minimum of 7 days after surgery and were trained for at least 2 days to stand quietly in a sling.

On the day of coronary occlusion, treated dogs received a continuous infusion of human recombinant SOD and bovine catalase, starting 15 minutes before occlusion and ending 30 minutes after reperfusion (total doses: SOD, 16,000 units/kg; catalase, 55,000 units/kg). The enzymes were dissolved in normal saline and infused through a jugular venous line at a rate of 0.8 ml/min. Control dogs received equivalent volumes of normal saline. SOD (expressed in yeast cells[38]) was obtained courtesy of the Pharmacia–Chiron Partnership, Emeryville, Calif. (specific activity, 3,200 units/mg protein). Catalase (Sigma Chemical Co., St. Louis, Mo.) was purified from bovine liver (specific activity, 11,000 Sigma units/mg protein).

Throughout the study, dogs were studied in the awake, unsedated state while standing quietly in a sling. The LAD was occluded for 15 minutes and then reperfused. After at least 8 days from the first LAD occlusion, the same dogs underwent a second 15-minute coronary occlusion. At the time of the first coronary occlusion, dogs were randomly assigned to the treated or control group by tossing a coin; at the time of the second occlusion, they were assigned to the opposite group. Therefore, every dog that underwent two coronary occlusions contributed one occlusion to the treated group and one occlusion to the control group.

The plasma concentration of SOD was determined by radioimmunoassay; in addition, in four SOD-treated dogs, plasma SOD activity was measured using the cytochrome *c* assay.[39] Plasma catalase activity was determined according to the method of Aebi.[40] Specimens showing any visible hemolysis were not analyzed.

Techniques Used in Both Anesthetized and Conscious Dogs

Regional myocardial blood flow was determined by the radioactive microsphere technique[33] before coronary occlusion, 8–10 minutes after occlusion, and 1 hour after reperfusion. Regional myocardial function was assessed as systolic thickening fraction (i.e., systolic wall thickening/end-diastolic thickness×100) using a pulsed Doppler probe, as previously described.[1,4–7,9,11,12,14,15,33–37] The occluded coronary vascular bed was identified by a postmortem dual-perfusion technique.[26] Absence of irreversible damage was confirmed in all dogs by tetrazolium staining.[33]

Statistical Analysis

Data are reported as mean±SEM. Intragroup comparisons between baseline measurements and subsequent values were performed with the two-tailed Student's *t* test for paired data with the Bonferroni correction. Because postischemic recovery of function is determined by collateral flow,[33] the relation between these two variables was analyzed in both open-chest and conscious dogs using an exponential regression equation: $Y=100+P_1(e^{-P_2X}-e^{-P_2100})$, as detailed previously.[33]

Comparisons of open-chest and conscious dogs. Comparisons among the two open-chest groups and the conscious control group were performed by analysis of variance. If the *F* test showed an overall difference, comparisons between two groups were performed with the two-tailed Student's *t* test for unpaired data using the Bonferroni correction.

Intergroup comparisons in conscious dogs. The unpaired Student's *t* test was used to compare means between control and treated conscious dogs. Comparisons of thickening fractions between the two groups were made using a two-way analysis of variance with replication, whereby the effect of treatment was examined in different categories of collateral flow.[41] The advantage of this analysis is that it takes into account any difference in collateral flow between the two groups.

Results

Part 1: Comparison of Anesthetized and Conscious Dogs

There were two goals in part 1 of this study. The first goal was to determine whether the severity of myocardial stunning is exaggerated in open-chest dogs even when fundamental physiological variables (including temperature) are kept within the normal range. Accordingly, the anesthetized normothermic dogs (group I) were compared with the conscious dogs that received normal saline (controls). The second goal was to elucidate why the recovery observed in group I was markedly less than that observed in our earlier studies in open-chest dogs,[1,4,7,32] in which temperature was not controlled. Accordingly, the anesthetized normothermic dogs in group I

TABLE 1. Reasons for Excluding Dogs From Study

Reasons for exclusion	Anesthetized dogs	
	Group I (n=40)	Group II (n=5)
Death on induction of anesthesia	1	0
Arterial P_{O_2}<60 mm Hg	2	0
Mean arterial pressure<60 mm Hg	1	0
Ventricular fibrillation during instrumentation	1	0
Technical problems during instrumentation	1	0
Ventricular fibrillation during ischemia	1	0
Ventricular fibrillation on reperfusion	3	0
Total excluded	10	0

	Conscious dogs (n=39)		
	Before randomization	After randomization	
		Control	Treated
Technical problems during instrumentation	4	...	...
Postoperative death	2	...	...
Spontaneous occlusion of LAD during convalescence	3	...	...
Lack of dyskinesis during ischemia	...	2	1
Ventricular fibrillation on reperfusion	...	1	2
Total excluded	9	3	3

LAD, left anterior descending coronary artery. In group I, 10 (25%) of the 40 dogs anesthetized were excluded; in group II, none of the five dogs anesthetized was excluded; in the conscious group, 15 (38%) of the 39 dogs instrumented were excluded.

were compared with an additional group of anesthetized hypothermic dogs (group II).

Exclusions. After the exclusions, which are detailed in Table 1, the final analysis included 30 dogs in group I and five dogs in group II. The exclusions in the control group of conscious dogs are detailed in part 2 of "Results" (see below); the final analysis included 19 awake animals.

Arterial blood gases, hematocrit, and temperature. Arterial pH, P_{O_2}, and hematocrit were within physiological limits in groups I and II throughout the experimental protocol (pH, 7.38–7.42; P_{O_2}, 97–132 mm Hg; hematocrit, 40–45%). In group I, esophageal temperature remained stable at normal levels (between 37° and 38°C) until the end of the experiment (Table 2). In contrast, in group II, esophageal temperature declined progressively during the study and by 4 hours of reperfusion averaged 33.8±0.5°C. The measurements in conscious dogs are reported below (see part 2 of "Results").

Hemodynamic variables. In the open-chest dogs (groups I and II), hemodynamic variables did not change significantly from baseline values during the occlusion or the reperfusion phase, with the exception of peak positive left ventricular (LV) dP/dt, which exhibited a progressive deterioration in group I (Table 3). There was no significant difference between group I and group II at any time point during the protocol. However, when compared with the conscious dogs, the open-chest dogs in group I exhibited significantly higher heart rate, systolic arterial pressure, and rate–pressure product and significantly lower left atrial pressure and peak positive LV dP/dt (Table 3).

Occluded bed size and regional myocardial blood flow. The size of the occluded vascular bed averaged 17.7±1.2 g (21.1±1.1% of LV weight) in group I, 16.1±1.3 g (19.4±2.0% of LV weight) in group II, and 24.1±1.2 g (26.1±0.8% of LV weight) in conscious dogs. These values did not differ between groups I and II; however, in both groups I and II these values were significantly less (p<0.01) than in conscious dogs. There were no significant differences among group I, group II, and conscious animals with respect to blood flow in the LAD territory either during coronary occlusion or 1 hour after reflow (Table 4).

Regional myocardial function. Systolic thickening fraction in the nonischemic (control) region remained stable during the experiment in awake dogs but exhibited a modest deterioration in both anesthetized groups (Table 3).

Baseline systolic thickening fraction in the region to be rendered ischemic was similar in groups I and II and in conscious dogs (22.3±1.3%, 18.7±3.6%, and 21.6±1.6%, respectively). During coronary occlusion, however, the extent of paradoxical systolic thinning was significantly (p<0.001) greater in the anesthetized than in the conscious dogs (Figure 1, Table 3). After reperfusion, normothermic open-chest dogs (group I) exhibited little recovery of contractile function, so that 4 hours after restoration of flow the previously ischemic region was still dyskinetic (Figure 1, Table 3). In striking contrast, conscious dogs exhibited considerable recovery of contractile function, so that by 4 hours of reperfusion the previously ischemic region had regained almost 60% of baseline

TABLE 2. Esophageal Temperature in Anesthetized Dogs

	Baseline	Occlusion	Reperfusion					
			30 min	1 hr	2 hr	3 hr	4 hr	5.5 hr
Group I temperature (°C)	37.1 ±0.2	37.0±0.2	37.1±0.2	37.3±0.2	37.6±0.2	37.9±0.2	37.9±0.3	...
Group II temperature (°C)	37.1 ±0.9	36.1±0.6	35.2±0.6*†	34.4±0.4*†	33.6±0.3*†	33.9±0.4*†	33.8±0.5*†	36.6±0.5‡

Values are mean±SEM. Group I, normothermic open-chest dogs; Group II, hypothermic open-chest dogs. Measurements at 5.5 hours were taken after increasing temperature by 2.8°C over 4-hour values.

*p<0.001 vs. group I; †p<0.05 vs. baseline; ‡p<0.001 vs. 4-hour reperfusion.

TABLE 3. Hemodynamic Variables and Wall Thickening in Open-Chest and Conscious Dogs

			Reperfusion					
	Baseline	Occlusion	30 min	1 hr	2 hr	3 hr	4 hr	5.5 hr
HR (beats/min)								
Conscious	108±6	137±7*	112±6	112±6	112±6	116±6	111±5	...
Group I	156±4†	158±5‡	158±4†	164±4†	164±4†	167±4†	164±4†	...
Group II	175±14†	173±9§	163±5†	161±3†	153±8‡	150±9§	153±9†	173±10‖
SAP (mm Hg)								
Conscious	136±6	134±5	138±6	137±5	140±5	138±6	138±6	...
Group I	116±2†	113±3†	118±2†	118±3‡	118±3†	118±3‡	116±3†	...
Group II	128±7	121±3	128±4	121±2	114±5	123±6	123±6	129±3
RPP								
Conscious	14.6±0.9	18.4±1.3	15.3±1.0	15.4±1.2	15.6±1.1	16.0±1.1	15.2±1.0	...
Group I	18.1±0.6†	18.0±0.7	18.9±0.6‡	19.6±0.8‡	19.7±0.8‡	19.7±0.8‡	19.2±0.8‡	...
Group II	22.7±2.9‡	21.0±1.6	20.9±0.8§	19.5±0.6	17.6±1.5	18.8±1.8	18.7±1.2	22.4±0.8‖
LAP (mm Hg)								
Conscious	7.4±0.8	11.8±1.2*	7.9±0.8	7.6±0.7	7.1±0.6	7.4±0.7	6.8±0.7	...
Group I	3.0±3.0†	3.9±0.4†	3.2±0.4†	2.6±0.4†	2.6±0.3†	3.7±0.4	3.8±0.4	...
Group II	4.1±1.2	5.6±1.4§	4.4±0.9§	4.0±0.7§	3.2±1.2‡	3.2±1.2§	4.0±1.2	3.7±1.0
LV dP/dt_{max} (mm Hg/sec)								
Conscious	3,414±182	3,345±218	3,243±208	3,283±201	3,131±181	3,246±215	3,184±247	...
Group I	2,077±128†	1,824±145*†	1,950±152*†	1,976±170*†	1,874±170*†	1,841±160*†	1,765±151*†	...
Group II	2,192±518§	2,027±431§	2,109±376§	1,944±318‡	1,882±247‡	2,109±303§	2,219±286	2,082±215
ThF IZ (%)								
Conscious	100	−32±6	6±12	20±13	36±12	47±11	55±11	...
Group I	100	−76±8†¶	−49±11§¶	−23±12§¶	−24±12‡¶	−18±11†¶	−20±11†¶	...
Group II	100	−86±16†¶	−60±25§¶	−24±27¶	30±16¶	68±5¶#	67±12¶#	−53±18¶‖
ThF NIZ (%)								
Conscious	100	91±6	98±6	104±5	103±5	104±5	104±5	...
Group I	100	121±12	97±8	91±5	86±5*§	82±7*§	88±12	...
Group II	100	115±5	90±3	86±3	73±6§	62±5†	85±3	73±6

Values are mean±SEM. Group I, normothermic open-chest dogs; Group II, hypothermic open-chest dogs. HR, heart rate; SAP, systolic arterial pressure; RPP, rate–pressure product (heart rate×systolic blood pressure/1,000); LAP, mean left atrial pressure; LV dP/dt_{max}, maximal rate of left ventricular pressure rise; ThF IZ, systolic thickening fraction in the ischemic/reperfused zone (expressed as percent of baseline values); ThF NIZ, systolic thickening fraction in the nonischemic zone (expressed as percent of baseline values). Measurements at 5.5 hours were taken after increasing temperature by 2.8°C over 4-hour values. At baseline, systolic thickening fraction in the nonischemic (control) region was similar in groups I and II (15.2±1.0% and 11.1±1.6%, respectively), and in both groups it was significantly lower ($p<0.01$) than in the conscious dogs (19.8±1.2%).

*$p<0.05$ vs. baseline; †$p<0.001$, ‡$p<0.01$, and §$p<0.05$ vs. conscious dogs; ‖$p<0.05$ vs. 4-hour values; ¶$p<0.001$ vs. baseline; #$p<0.01$ vs. group I.

wall thickening (Figure 1, Table 3). The time course of recovery of contractile performance was also quite different: in awake animals wall thickening continued to improve up to 4 hours of reperfusion, whereas in group I the recovery ceased after 1 hour (Figure 1, Table 3). As a result, the distance between the two recovery lines increased from 1 to 4 hours of reflow, indicating that the distortion of myocardial stunning in the anesthetized model increases with the duration of reperfusion. The total postreperfusion deficit of function was calculated by measuring the area comprised between the thickening-versus-time line and the baseline (100% line) over the 4-hour period of reflow (Figure 1). This analysis revealed that the total postreperfusion deficit observed in conscious dogs was only 55% of that observed in open-chest dogs. Thus, the functional depression associated with the anesthetized model was almost twice that observed in the awake model.

Compared with group I, hypothermic open-chest dogs (group II) exhibited considerably greater recovery of contractility (Figure 2, Table 3). When the temperature of these animals was increased to values approaching those of normothermic dogs (group I), the contractile function of the stunned myocardium deteriorated to levels comparable to those observed in group I (Figure 2, Table 3). As illustrated in Figure 3, in group II small increases in temperature resulted in substantial decreases in wall thickening; overall, the decrement in thickening fraction averaged 43% of baseline values for each 1°C increment in temperature between 33.8°C and 36.6°C. Temperature had little effect on function in the nonischemic region (Figure 3, Table 3). Therefore, increases in temperature in this

TABLE 4. Regional Myocardial Blood Flow in Open-Chest and Conscious Dogs

		Ischemic zone			Nonischemic zone		
	n	Epi	Endo	Mean	Epi	Endo	Mean
Baseline BF (ml/min/g)							
Conscious	19	1.15±0.09	1.41±0.11	1.28±0.10	1.21±0.10	1.46±0.10	1.34±0.10
Group I	30	1.44±0.09*	1.14±0.08*	1.29±0.08	1.21±0.07	1.33±0.09	1.27±0.08
Group II	5	1.45±0.29	1.26±0.18	1.36±0.24	1.09±0.17	1.25±0.13	1.17±0.15
BF during occlusion (ml/min/g)							
Conscious	19	0.34±0.06†	0.16±0.04†	0.25±0.05†	1.43±0.11	1.69±0.12	1.56±0.11
Group I	30	0.28±0.05†	0.15±0.03†	0.21±0.04†	1.26±0.07	1.28±0.07‡	1.27±0.07*
Group II	5	0.23±0.07†	0.08±0.03†	0.16±0.05†	1.17±0.17	1.19±0.14	1.18±0.15
BF during occlusion (% of NZF)							
Conscious	19	27.8±5.5	11.1±2.8	18.7±3.9	...	...	...
Group I	30	23.6±4.4	12.6±2.5	18.1±3.4	...	...	...
Group II	5	21.8±8.6	8.4±3.8	14.8±5.3	...	...	...
BF 1 hr after reperfusion (ml/min/g)							
Conscious	15	1.24±0.13§	1.29±0.14†	1.27±0.13†	1.38±0.14	1.65±0.16	1.52±0.15
Group I	30	1.28±0.09	0.95±0.08†	1.12±0.08	1.10±0.09	1.19±0.09‡	1.15±0.09*
Group II	5	1.31±0.15	0.84±0.04	1.08±0.09	0.97±0.14	1.20±0.18	1.09±0.15

Values are mean±SEM. Group I, normothermic open-chest dogs; Group II, hypothermic open-chest dogs. Epi, epicardial flow; Endo, endocardial flow; Mean, mean transmural flow; BF, blood flow; NZF, simultaneous nonischemic zone flow. Blood flow to the ischemic zone during coronary occlusion is expressed both in absolute terms (ml/min/g) and as percent (% of NZF).

*$p<0.05$ vs. conscious dogs; †$p<0.001$ vs. corresponding value in the nonischemic zone by Student's t test for paired data; ‡$p<0.01$ vs. conscious dogs; §$p<0.05$ vs. corresponding value in the nonischemic zone by Student's t test for paired data.

range (~34–37°C) exerted a selective depressant effect on the mechanical function of the stunned myocardium.

Relation between recovery of function and collateral flow. As shown in Figure 4, the relation between wall thickening after reperfusion and collateral flow during occlusion was shifted to the right and downward in open-chest normothermic dogs (group I); that is, for any given level of collateral perfusion, wall thickening after reflow was less than in conscious dogs. These results indicate that the differences in the severity of myocardial stunning between anesthetized and awake dogs were independent of collateral flow.

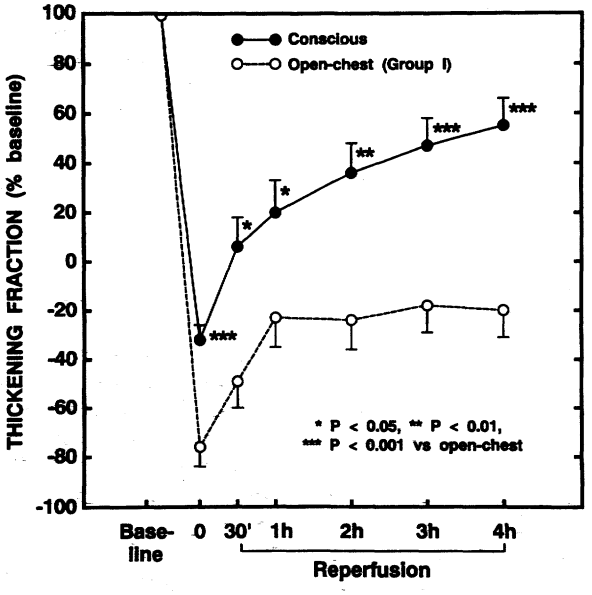

FIGURE 1. *Graph showing the systolic thickening fraction in the ischemic/reperfused region 5 minutes after coronary occlusion (O) and at selected times after reperfusion in conscious dogs (●, n=19) and in normothermic open-chest dogs (○, group I, n=30). Thickening fraction is expressed as percent of baseline values. Data are mean ±SEM.*

Part 2: Evaluation of the Oxy-Radical Hypothesis in Conscious Dogs

Exclusions. Of the 39 dogs instrumented, 15 (38%) were excluded for the reasons specified in Table 1. Of the remaining 24 dogs, 16 underwent two complete LAD occlusion–reperfusion sequences, whereas eight had only one complete LAD occlusion–reperfusion sequence. In these eight dogs, a second occlusion–reperfusion sequence could not be completed because of absence of appreciable dysfunction after the first coronary occlusion (three dogs), spontaneous occlusion of the LAD before the second occlusion (one dog), loss of the thickening signal from the ischemic zone (one dog), death before the second occlusion (one dog), and ventricular fibrillation after the second reperfusion (two dogs). The final analysis included 40 LAD occlusions (19 in the control group and 21 in the treated group), which were contributed by 24 dogs (16 dogs contributed to both the control and the treated group, three dogs contributed to the control group only, and five dogs contributed to the treated group only). In the remainder of this article, the term "control dogs" indicates the 19 conscious animals that underwent a coronary occlusion without treatment, whereas the term "treated dogs" denotes

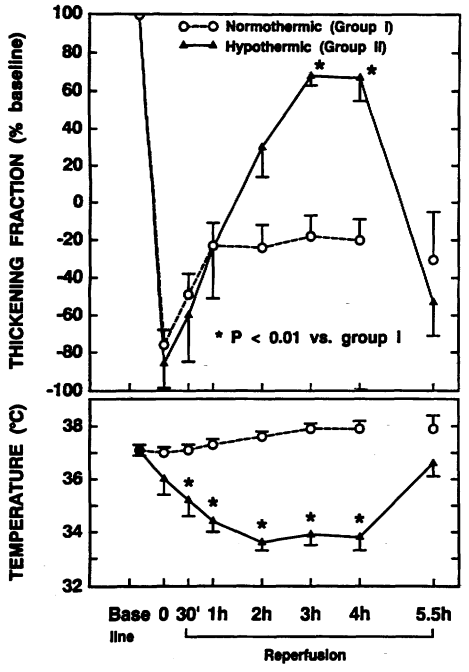

FIGURE 2. *Graphs showing the systolic thickening fraction in the ischemic/reperfused region* (top panel) *and esophageal temperature* (bottom panel) *5 minutes after coronary occlusion (O) and at selected times after reperfusion in normothermic open-chest dogs (group I)* (○, n=*30*) *and hypothermic open-chest dogs (group II)* (▲, n=*5*). *Thickening fraction is expressed as percent of baseline values. In group I, the esophageal temperature was kept between 37° and 38°C by using a heating blanket and by covering the animal; in group II, no effort was made to control esophageal temperature. After 4 hours of reperfusion, in group II the temperature was raised by 2.8°C by covering the animal and by using heating blankets (this required ~1.5 hours). Note that the increase in temperature was associated with a striking decrease in thickening fraction in the stunned myocardium. This decrease was not due to a spontaneous deterioration of the open-chest preparation after 4 hours of reperfusion because in eight dogs in group I thickening fraction remained stable between 4 hours (−32±24% of baseline) and 5.5 hours (−31±26%). The thickening fraction and the temperature in these eight dogs are represented by the isolated open circles at 5.5 hours of reperfusion. Data are mean±SEM.*

the 21 animals that underwent a coronary occlusion with SOD and catalase treatment.

Arterial blood gases, hematocrit, and temperature. On the day of coronary occlusion, arterial pH, P_{O_2}, hematocrit, and rectal temperature were within physiological limits in every dog. In the control group, the values were as follows: pH, 7.44±0.01; P_{O_2}, 86±2 mm Hg; hematocrit, 43±2%; temperature, 39.3±0.2°C. The values in the treated group were as follows: pH, 7.45±0.01; P_{O_2}, 86±2 mm Hg; hematocrit, 42±2%; temperature, 38.9±0.1°C (the range of normal rectal temperature in dogs is 37.8–39.9°C[42]).

Plasma SOD concentration and activity and catalase activity. The concentration of SOD and the activity of catalase in the arterial plasma are illustrated in Figure 5. The plasma activity of SOD (determined in four dogs) averaged 64.9±11.5 units/ml at 5 minutes of occlusion and 114.0±12.5 units/ml at 2 minutes, 117.9±23.3 units/ml at 20 minutes, and 68.1±8.5 units/ml at 1 hour of reperfusion.

Hemodynamic variables and regional myocardial blood flow. All measured variables (heart rate, systolic arterial pressure, rate–pressure product, left atrial pressure, LAD blood flow, and LV dP/dt) were similar in the two groups throughout the protocol (Table 5).

Occluded bed size and regional myocardial blood flow. Since most dogs contributed to both the control and the treated groups, the size of the occluded bed was virtually identical in the two groups: 24.1±1.2 g or 26.1±0.8% of LV weight.

During coronary occlusion, the average blood flow to the LAD territory was similar in control and treated groups (Table 6), but there was considerable individual variability (Figure 6). It has been previously demonstrated[33] that in the conscious dog the rate of contractile recovery after a 15-minute coronary occlusion is highly variable and that this variability is determined primarily by the severity of blood flow reduction during the antecedent ischemia. Accordingly, in an effort to analyze recovery of function in relatively homogeneous subgroups of animals, we partitioned dogs into subset I (collateral flow<10% of simultaneous nonischemic zone flow [NZF]), subset II (collateral flow between 10% and 30% of NZF), and subset III (collateral flow>30% of NZF) (Figure 7). (The average flows and sample sizes for each subset are specified in the legend to Figure 7.) It is recognized that any subdivision of animals on the basis of collateral flow is arbitrary. In the present study, the boundaries between the three subsets (10% and 30% of NZF) were retrospectively selected to obtain subgroups of roughly equivalent sizes. However, we also used other cutoff points such as 15% or 20% of NZF and found the results to be similar to those obtained by using 10% and 30% of NZF. Thus, the conclusions derived from the subset analysis are not contingent on the specific values of flow selected as cutoff points.

Regional myocardial function. Systolic thickening fraction in the nonischemic (control) region did not differ significantly between control and treated dogs at any time point during the protocol (Table 5). Baseline systolic thickening fraction in the region to be rendered ischemic was virtually identical: 21.6±1.6% in the control group and 21.7±1.6% in the group receiving SOD and catalase. During coronary occlusion, the extent of paradoxical systolic thinning was also similar in the two groups (Figure 8). After reperfusion, however, the recovery of contractile function was significantly enhanced in treated dogs at 1, 2, 3, 4, 5, and 6 hours (Figure 8).

Relation between recovery of function and collateral flow. As shown in Figures 6 and 9, the relation between wall thickening after reperfusion and collat-

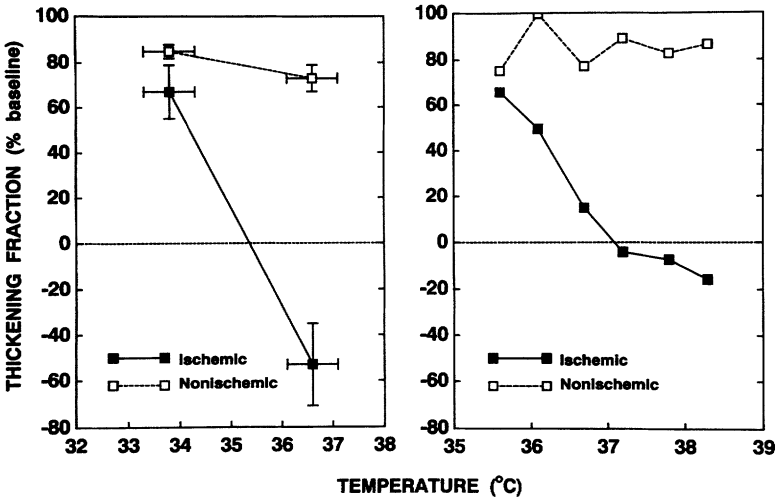

FIGURE 3. Left panel: *Graph showing the relation between the change in thickening fraction in the ischemic/reperfused region (vertical axis) and the change in esophageal temperature (horizontal axis) after 4 hours of reperfusion in five hypothermic open-chest dogs (group II). In these animals, temperature was raised by 2.8°C by covering the animals and by using heating blankets; this increase in temperature was associated with a striking decrease in thickening fraction in the stunned myocardium. The decrement in thickening fraction averaged 43% of baseline values for each 1°C increase in temperature between 33.8° and 36.6°C. Temperature had little effect on the nonischemic region. Therefore, the changes in temperature exerted a selective depressant effect on the mechanical function of the stunned myocardium. Data are mean±SEM.* Right panel: *Graph showing the relation between thickening fraction in the ischemic/reperfused region (■) and in the nonischemic region (□) and esophageal temperature in a dog in group II after 4 hours of reperfusion. In this dog, temperature was raised from 35.6°C at 4 hours of reflow to 38.3°C 82 minutes later, and measurements were repeated for every 0.6°C increase in temperature. Note that stepwise increases in temperature were associated with stepwise decreases in thickening fraction in the stunned myocardium but not in the nonischemic myocardium. Even small increases in temperature resulted in substantial decreases in wall thickening, indicating a sensitive coupling between body temperature and function in stunned myocardium.*

eral flow during occlusion was shifted to the left and upward in dogs treated with SOD and catalase; that is, for any given level of collateral perfusion, wall thickening after reflow was greater than in controls. These results indicate that the enhancement of functional recovery by antioxidant enzymes was still observed after the variability due to collateral flow was removed. Furthermore, the distance between the two regression lines was proportionately larger in the low-flow range (Figure 9), indicating that the beneficial effects of SOD and catalase were proportionately greater in dogs with lower levels of collateral perfusion.

This concept can be further appreciated when one examines recovery of function in different categories of collateral flow (Figure 7). It is apparent from Figure 7 that the effect of antioxidant enzymes was not uniform in the various ranges of ischemic flow. In subset I (collateral flow<10% of NZF), both the severity of myocardial stunning in control animals and the enhancement of contractile recovery in treated animals were greater than those observed in the entire group of dogs (Figure 7, top panel). In subset II (collateral flow=10–30% of NZF), the severity of myocardial stunning in the control group was less than that in subset I (Figure 7, middle panel). Although there was a trend for SOD and catalase to improve recovery of function, the differences did not achieve statistical significance. In subset III (collateral flow>30% of NZF), mild myocardial stunning was observed in control animals, and no significant improvement was noted with antioxidant enzymes (Figure 7, bottom panel). Even when subsets II and III were combined to increase the sample size, there were no significant differences in postischemic thickening fraction between control ($n=12$) and treated dogs ($n=10$). Thus, a significant improvement in contractility could be demonstrated only in dogs with low collateral flow and marked postischemic dysfunction (subset I).

Discussion

The present study demonstrates, for the first time, that myocardial stunning is significantly altered by the conditions associated with the barbiturate-anesthetized, open-chest dog model. Since the oxy-radical hypothesis is entirely derived from this model, the present findings underscore the importance of evaluating this theory in more physiological preparations.

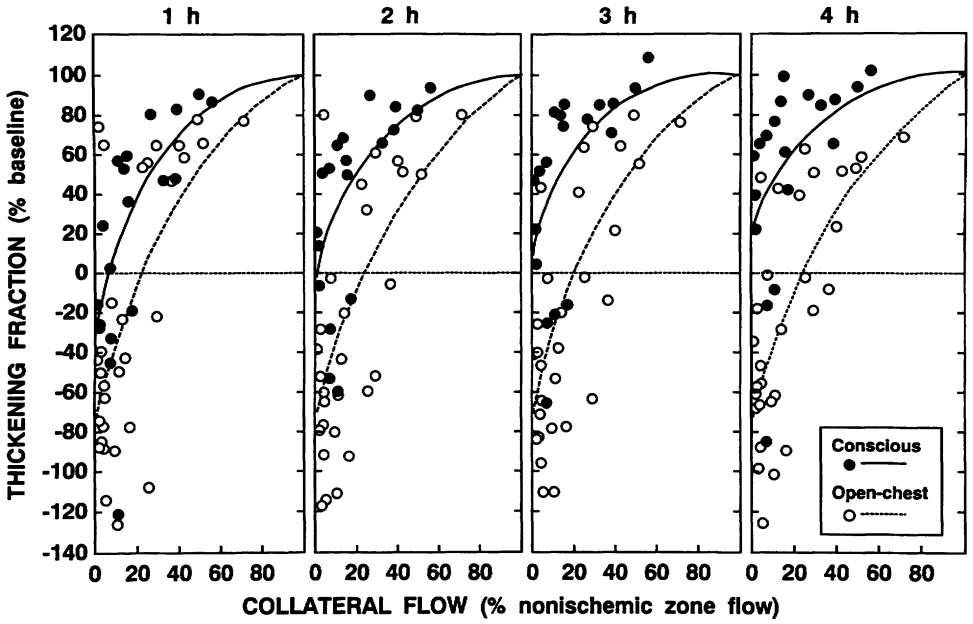

FIGURE 4. *Graphs showing the relation between mean transmural collateral blood flow to the ischemic region during coronary occlusion (horizontal axis) and systolic wall thickening at various intervals after reperfusion (vertical axis) in conscious dogs (●, n=19) and in normothermic open-chest dogs (○, group I, n=30). Collateral flow is expressed as percent of simultaneous nonischemic zone flow; thickening fraction is expressed as percent of baseline values. A previous study[33] demonstrated that the relation of postischemic wall thickening to ischemic flow is described best by an exponential regression equation (see text); therefore, the regression lines for the exponential expressions are illustrated in the figure. Note that the regression line was shifted to the right and downward in open-chest dogs, indicating that for any given level of collateral perfusion, wall thickening after reflow was less in these animals compared with conscious dogs. The distance between the two regression lines increased from 1 to 4 hours of reflow, indicating that the distortion of myocardial stunning observed in the anesthetized model increased with the duration of reperfusion. Statistical analysis demonstrated the regression lines to be significantly different; for example, the* y *intercept and the midpoint (i.e., the* y *value corresponding to* x=50*) were lower in anesthetized dogs at 2 hours* ($p<0.01$ *and* $p<0.05$*, respectively), 3 hours* ($p<0.01$ *and* $p<0.05$*), and 4 hours* ($p<0.001$ *and* $p<0.05$*). The exponential regression equations (written in simplified form[33]) for the conscious dogs were as follows: 1 hour,* $y=105.4-139.9\ e^{-0.033x}$ ($r=0.68$, $p<0.01$, $SEE=42.5$); *2 hours,* $y=105.2-109.2\ e^{-0.031x}$ ($r=0.62$, $p<0.01$, $SEE=39.0$); *3 hours,* $y=102.7-95.7\ e^{-0.036x}$ ($r=0.58$, $p<0.01$, $SEE=41.5$); *4 hours,* $y=102.2-82.5\ e^{-0.036x}$ ($r=0.51$, $p<0.05$, $SEE=42.3$), *where* x *is mean transmural blood flow during coronary occlusion (expressed as percent of simultaneous nonischemic zone flow) and* y *is the thickening fraction after reperfusion (expressed as percent of baseline). The equations for the open-chest group were as follows: 1 hour,* $y=125.2-205.6\ e^{-0.021x}$ ($r=0.73$, $p<0.001$, $SEE=47.1$); *2 hours,* $y=144.9-222.4\ e^{-0.016x}$ ($r=0.70$, $p<0.001$, $SEE=47.3$); *3 hours,* $y=138.2-208.9\ e^{-0.017x}$ ($r=0.72$, $p<0.001$, $SEE=42.9$); *4 hours,* $y=137.7-206.4\ e^{-0.017x}$ ($r=0.71$, $p<0.001$, $SEE=41.9$).

Differences Between Anesthetized and Conscious Dogs

Our results indicate that the severity of myocardial stunning is greatly exaggerated by the unphysiological conditions present in the open-chest dog, even when arterial blood gases, hematocrit, and body temperature are carefully kept within the normal range. For any given level of collateral perfusion, the recovery of mechanical function was markedly less in anesthetized animals. In fact, approximately half of the postischemic depression of contractility noted in this model was not observed in conscious dogs and thus appears to be model dependent. It seems probable that this striking depression of postischemic wall thickening in open-chest dogs was due to a combination of several factors, including anesthesia, surgical trauma, excessive myocardial oxygen demands, lack of integrity of the chest wall, and abnormal hemodynamic conditions.

It should be stressed that the purpose of this study was to determine whether myocardial stunning is altered by the open-chest preparation, not to elucidate the mechanism responsible for this effect. Whatever the cause of the exaggerated postischemic depression of contractility in open-chest dogs, the important point is that myocardial stunning in this preparation consists of two components of roughly similar sizes: one that is model dependent (not present in conscious dogs) and another that is model independent (also present in conscious dogs). Since the former component is quite large, it could distort the effect of antioxidant therapy, causing either "false-positive" or "false-negative" results. Antioxidants are uniformly effective in open-chest dogs,[1-15] but without additional data it is impossible to establish whether the antioxidant-sensitive component of myocardial stunning corresponds to the model-dependent or the model-independent component. If

TABLE 5. Hemodynamic Variables in Conscious Control and Treated Dogs

			Reperfusion					
	Baseline	Occlusion	30 min	1 hr	2 hr	3 hr	4 hr	5 hr
HR (beats/min)								
Control	108±6	137±7*	112±6	112±6	112±6	116±6	111±5	113±7
SOD+CAT	109±5	136±6*	114±7	109±6	115±7	110±5	109±6	110±6
SAP (mm Hg)								
Control	136±6	134±7	138±6	137±6	140±6	138±6	138±5	137±7
SOD+CAT	138±4	140±4	136±4	135±4	137±3	138±4	135±3	135±4
RPP								
Control	14.6±0.9	18.4±1.3	15.3±1.0	15.4±1.2	15.6±1.1	16.0±1.1	15.2±1.0	15.3±1.1
SOD+CAT	15.2±0.8	19.2±1.1	15.6±1.0	14.9±1.0	15.9±1.0	15.2±0.8	14.3±0.7	14.3±0.7
LAP (mm Hg)								
Control	7.4±0.8	11.8±1.2*	7.9±0.8	7.6±0.7	7.1±0.6	7.4±0.7	6.8±0.7	6.9±0.7
SOD+CAT	7.8±0.6	12.0±0.7*	7.2±0.5	7.0±0.7	6.2±0.5	6.3±0.6	6.1±0.5	6.7±0.5
LV dP/dt_{max} (mm Hg/sec)								
Control	3,414±182	3,345±218	3,243±208	3,283±201	3,131±181	3,246±215	3,184±247	3,243±217
SOD+CAT	3,305±146	3,233±149	3,106±151	2,984±138	3,324±140	3,324±160	3,331±169	3,233±113
ThF NIZ (%)								
Control	100	91±6	98±6	104±5	103±5	104±5	104±5	107±5
SOD+CAT	100	86±8	98±8	98±6	100±4	108±5	107±5	109±4

Values are mean±SEM. HR, heart rate; SOD, superoxide dismutase; CAT, catalase; SAP, systolic arterial pressure; RPP, rate-pressure product (heart rate×systolic blood pressure/1,000); LAP, mean left atrial pressure; LV dP/dt_{max}, maximal rate of left ventricular pressure rise; ThF NIZ, systolic thickening fraction in the nonischemic zone (expressed as percent of baseline values). Baseline measurements were taken 20 minutes before coronary occlusion (i.e., before administration of SOD and CAT). Baseline values for ThF NIZ were 19.8±1.2% in control and 21.2±1.6% in treated dogs.

*$p<0.01$ vs. baseline.

antioxidants attenuate only the model-dependent component of postischemic dysfunction, their efficacy could not be construed as evidence for a role of oxy-radicals in myocardial stunning; in this case, the oxy-radical hypothesis should be refuted. On the other hand, if antioxidants alleviate the model-independent component of postischemic dysfunction, their efficacy would support a pathogenetic contribution of oxygen metabolites. This critical distinction can only be made by evaluating the effect of antioxidant therapy in a model devoid of the confounding factors associated with the open-chest preparation. Hence the importance of the results obtained with SOD and catalase in awake dogs.

Effect of Antioxidant Enzymes in Conscious Dogs

This randomized study demonstrates that SOD and catalase significantly attenuate postischemic dysfunction in the conscious, unsedated dog. Despite the fact that the enzymes were essentially undetectable in the plasma by 2 hours of reperfusion, contractile performance was demonstrably enhanced for at least 6 hours after reflow. Thus, a brief infusion of SOD and catalase produced a sustained improvement in recovery of contractility. The present results further indicate that the magnitude of this beneficial effect is a function of the severity of ischemia: the lower the collateral perfusion, the greater the improvement effected by the enzymes. Finally, our observations show that the accelerated recovery produced by antioxidant therapy is not followed by any deterioration of contractility, suggesting that myocardial stunning is not a useful "protective" response of the heart to ischemic injury. Previous investigations[1-15] have demonstrated beneficial effects of antioxidants in open-chest animals. However, to our knowledge, this is the first study to suggest a role of oxy-radicals in

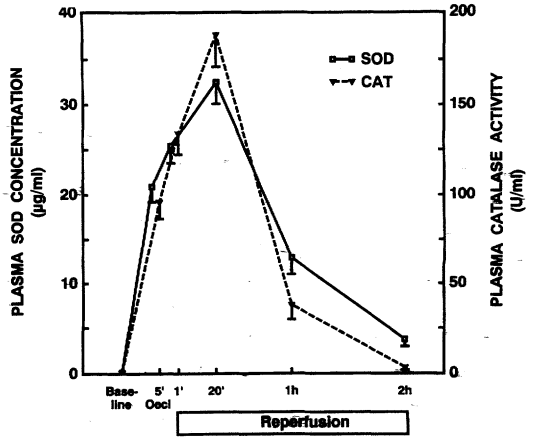

FIGURE 5. *Graph showing plasma superoxide dismutase (SOD) concentration and catalase (CAT) activity in conscious dogs treated with SOD and CAT (n=21). Illustrated are measurements obtained at baseline (before treatment), 5 minutes after coronary occlusion (Occl), and at selected times after reperfusion. Data are mean±SEM.*

TABLE 5. Continued

Reperfusion		
6 hr	24 hr	48 hr
110±5	108±5	107±5
106±5	110±4	110±4
127±5	137±5	129±5
133±3	137±4	120±4
14.2±1.0	14.9±1.1	14.1±0.8
14.3±1.0	15.0±0.7	13.9±0.8
7.5±0.8	7.3±0.8	6.4±0.7
6.1±0.5	7.5±0.5	7.1±0.5
3,146±263	3,223±175	3,360±194
3,313±145	3,298±126	3,206±129
106±4	107±4	103±4
105±6	110±4	102±3

the pathogenesis of postischemic myocardial dysfunction after brief (<20 minutes) ischemia in the conscious animal. The present findings significantly expand our understanding of myocardial stunning by suggesting a pathogenetic role of oxygen metabolites that is independent of the abnormal conditions associated with the open-chest dog model. Therefore, despite the large differences between anesthetized and awake preparations, it appears that the oxy-radical hypothesis derived from acute studies[1–15] is applicable to the conscious animal.

The use of two LAD occlusions was necessitated by practical considerations dictated by the cost of experiments in conscious dogs and by the sample sizes needed to achieve sufficient statistical power. Even when the analysis was restricted to the first LAD occlusion, however, the recovery of function in treated dogs was still enhanced compared with that in control dogs (e.g., at 6 hours, thickening fraction averaged 90.6±5.5% of baseline in SOD and catalase–treated animals [n=11] versus 66.7±7.8% in controls [n=13], $p<0.05$).

The time course of the protective effects of SOD and catalase deserves further comment. The difference in wall thickening between treated and control dogs became significant at 1 hour of reperfusion (Figures 7 and 8); thereafter, this difference persisted up to 6 hours and actually became greater (Figures 7 and 8). It should be noted that the plasma levels of SOD and catalase fell rapidly after the infusion was discontinued and were virtually undetectable by 2 hours (Figure 5). Hence, the effect of the enzymes persisted for several hours after they disappeared from the circulation, indicating that SOD and catalase produce a *sustained* protection that does not require their continuing presence. The time course of the protective effects of SOD and catalase in conscious dogs is distinctly different from that observed in open-chest preparations, in which the improvement of function by the enzymes is generally maximal at 1 and 2 hours of reperfusion and then remains unchanged or decreases.[1–3,10] These differences may be due to the abnormal conditions in open-chest animals which, as shown above, cause the time course of recovery to be distorted.

Relation Between Severity of Ischemia and Efficacy of Antioxidants

The relation between severity of ischemia and efficacy of antioxidant therapy for myocardial stun-

TABLE 6. Regional Myocardial Blood Flow in Conscious Control and Treated Dogs

		Ischemic zone			Nonischemic zone		
	n	Epi	Endo	Mean	Epi	Endo	Mean
Baseline BF (ml/min/g)							
Control	19	1.15±0.09	1.41±0.11	1.28±0.10	1.21±0.10	1.46±0.10	1.34±0.10
SOD+CAT	21	1.09±0.07	1.34±0.10	1.22±0.08	1.16±0.09	1.14±0.08	1.29±0.09
BF during occlusion (ml/min/g)							
Control	19	0.34±0.06*	0.16±0.04*	0.25±0.05*	1.43±0.11	1.69±0.12	1.56±0.11
SOD+CAT	21	0.37±0.06*	0.18±0.04*	0.27±0.05*	1.40±0.08†	1.63±0.08†	1.52±0.07†
BF during occlusion (% of NZF)							
Control	19	27.8±5.5	11.1±2.8	18.7±3.9	...	...	...
SOD+CAT	21	27.5±4.9	10.9±2.5	18.5±3.6	...	...	...
BF 1 hr after reperfusion (ml/min/g)							
Control	15	1.24±0.13‡	1.29±0.14*	1.27±0.13*	1.38±0.14	1.65±0.16	1.52±0.15
SOD+CAT	14	1.06±0.06	1.14±0.09*	1.10±0.07*	1.16±0.10	1.41±0.09	1.29±0.09

Values are mean±SEM. Epi, epicardial flow; Endo, endocardial flow; Mean, mean transmural flow; SOD, superoxide dismutase; CAT, catalase; NZF, simultaneous nonischemic zone flow. Blood flow to the ischemic zone during coronary occlusion is expressed both in absolute terms (ml/min/g) and as percent (% of NZF).

*$p<0.001$ vs. corresponding value in the nonischemic zone; †$p<0.05$ vs. corresponding value before occlusion; ‡$p<0.05$ vs. corresponding value in the nonischemic zone.

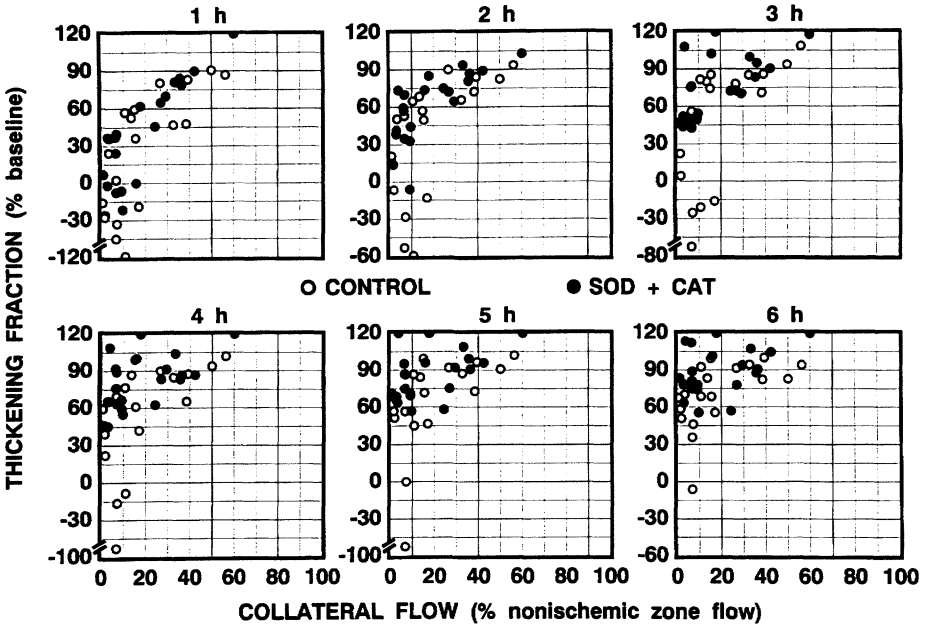

FIGURE 6. *Graphs showing the relation between mean transmural collateral blood flow to the ischemic region during coronary occlusion (horizontal axis) and systolic wall thickening at various intervals after reperfusion (vertical axis) in control (○, n=19) and superoxide dismutase plus catalase (SOD+CAT)-treated (●, n=21) conscious dogs. Collateral flow is expressed as percent of simultaneous nonischemic zone flow; thickening fraction is expressed as percent of baseline values. Note that the relation between collateral flow and wall thickening is nonlinear and that treated dogs exhibit greater wall thickening than controls in the low-flow range but tend to overlap with controls in the higher flow range.*

ning is unknown. Theoretically, antioxidants could be more efficacious either in animals with mild ischemia or in those with more severe ischemia, depending on the relative importance of free radical–mediated processes in these two settings. In either case, the regression lines of the wall thickening versus collateral flow relations (Figure 9) would be shifted to the left and upward by antioxidant treatment, but in the former case, this shift would be relatively greater in the high-flow range, whereas in the latter case, it would be relatively greater in the low-flow range.

The present study supports the latter possibility. SOD and catalase produced the maximal improvement of function in the dogs with the lowest collateral flows during LAD occlusion (Figure 7); as collateral flow increased, the improvement of function decreased (Figures 7 and 9), even at times (e.g., 1 or 2 hours) when function in the high-flow range was still significantly depressed (Figures 7 and 9) and thus could have been significantly improved by effective therapy. On the basis of these results, it appears that the intensity of the oxy-radical–mediated damage responsible for stunning depends on the severity of the antecedent ischemic insult: the more severe the ischemia, the more pronounced the subsequent oxy-radical injury, and thus the more effective the antioxidant therapy.

This paradigm has pathophysiological, methodological, and therapeutic implications. From a pathophysiological standpoint, it implies that the mechanisms responsible for free radical–induced damage in the stunned myocardium are activated by ischemia and that the intensity of such activation is determined by the intensity of flow reduction. From a methodological standpoint, studies of antioxidants in models of myocardial stunning should probably be restricted to animals with relatively low collateral perfusion, since it is in these animals that both oxy-radical–mediated injury and antioxidant-induced protection are maximal. From a therapeutic standpoint, patients prone to develop the most severe postischemic dysfunction might benefit the most from antioxidant therapy. In addition, any intervention that improves perfusion during ischemia would be expected to attenuate myocardial stunning after reperfusion.

Is Myocardial Stunning a Protective Phenomenon?

The accelerated recovery effected by SOD and catalase was not followed by any deterioration of contractile function. The opinion is frequently expressed that myocardial stunning is not a form of injury but rather a protective response of the heart to the ischemic insult, whereby energy is diverted from contractile activity to cellular reparative processes. If this hypothesis is correct, then interventions that prevent myocardial stunning could actually be detrimental; by "forcing" the postischemic "convalescent" myocardium to contract, these interventions would be expected to delay the ultimate return of mechanical function to normal values, so that an early improvement of function could be followed by a deterioration. Our results do not support this view. Wall thickening was consistently greater in treated dogs up to 6 hours of reperfusion and was still greater (albeit not significantly) at 24 hours (Figures 7 and 8); at 48 hours of reperfusion, wall thickening was back to baseline levels. The fact that the early attenuation of postischemic dysfunction did not produce subsequent unfavorable effects on contractility suggests that myocardial stunning is not a teleologically protective phenomenon. This conclusion agrees with a previous investigation[43] in which inotropic

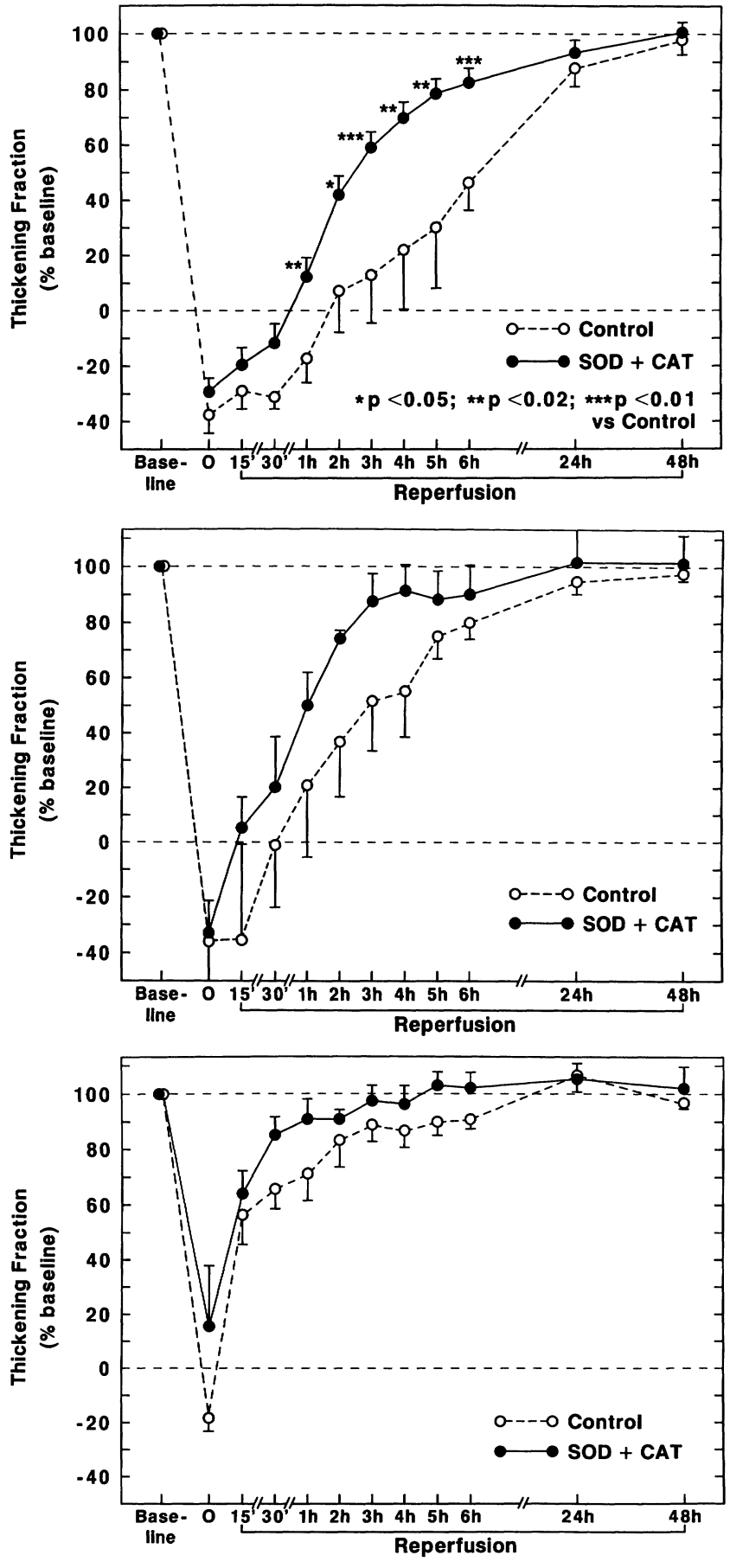

FIGURE 7. *Graphs showing the systolic thickening fraction in the ischemic/reperfused region 5 minutes after coronary occlusion (O) and at selected times after reperfusion in subset I* (top panel), *subset II* (middle panel), *and subset III* (bottom panel) *of the conscious dogs. Control dogs are represented by the dashed line with open circles; superoxide dismutase and catalase (SOD+CAT)-treated dogs are represented by the continuous line with solid circles. Thickening fraction is expressed as percent of baseline values. Data are mean±SEM. Subset I was formed by dogs with collateral flow <10% of nonischemic zone flow; subset II included animals with collateral flow between 10% and 30% of nonischemic zone flow; subset III consisted of dogs with collateral flow >30% of nonischemic zone flow. In subset I, transmural ischemic zone flow averaged 0.07±0.02 ml/min/g in controls* (n=7) *and 0.09±0.01 ml/min/g in treated animals* (n=11) *(4.1±1.1% and 5.9±0.9% of nonischemic zone flow, respectively). In subset II, ischemic zone flow was 0.25±0.04 ml/min/g in controls* (n=7) *and 0.31±0.03 ml/min/g in treated dogs* (n=5) *(15.7±2.0% and 23.0±2.6% of nonischemic zone flow, respectively). In subset III, ischemic zone flow averaged 0.52±0.06 ml/min/g in controls* (n=5) *and 0.63±0.04 ml/min/g in treated dogs* (n=5) *(43.2±4.2% and 41.6±4.9% of nonischemic zone flow, respectively).*

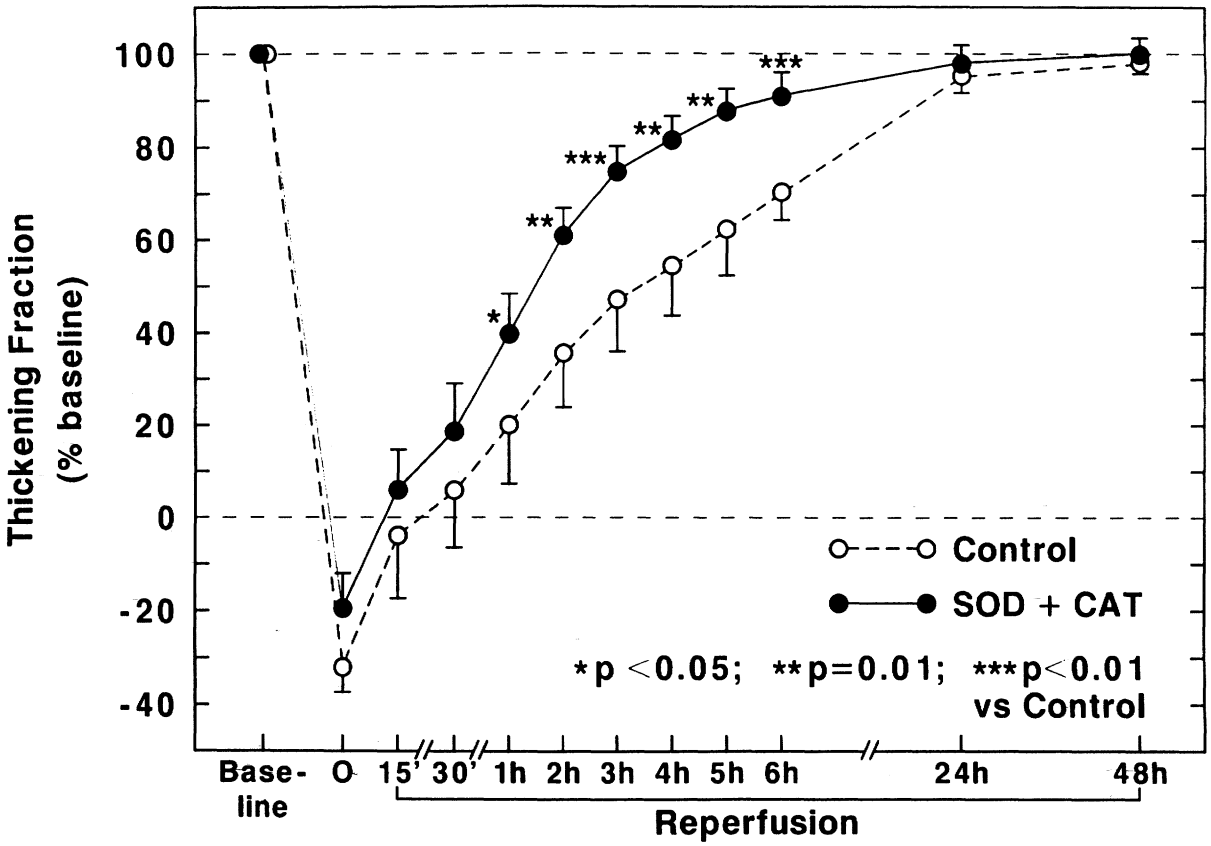

FIGURE 8. *Graph showing the systolic thickening fraction in the ischemic/reperfused region 5 minutes after coronary occlusion (O) and at selected times after reperfusion in control* (○) *and superoxide dismutase and catalase (SOD+CAT)-treated* (●) *conscious dogs. Thickening fraction is expressed as percent of baseline values. The data pertain to all of the conscious dogs studied (controls,* n=*19; treated,* n=*21). Data are mean±SEM.*

stimulation failed to affect the initial recovery of regional function after a 15-minute coronary occlusion in open-chest dogs. Our study expands these previous findings[43] by examining the problem in a conscious animal model and by assessing the entire time course of recovery.

Comparison With Previous Studies

The present findings are seemingly in conflict with those of Preuss et al,[31] who reported that recovery of segment shortening was not significantly different in open-chest versus conscious dogs after 5, 10, or 15 minutes of coronary occlusion and 3 hours of reperfusion. The reason for this discrepancy is not readily apparent. Since in the Preuss study collateral flow was not measured, differences in severity of ischemia cannot be ruled out. Furthermore, after a 15-minute coronary occlusion and 1 hour of reperfusion, the recovery of function in that study tended to diverge, with the conscious dogs showing an improvement and the anesthetized dogs a deterioration; as the authors point out,[31] it is possible that a difference may have become apparent if the experiments had been carried out for longer periods of time.

Influence of Temperature on Myocardial Stunning

The poor recovery of function observed in open-chest dogs (group I) agrees with our recent results[5,6,9,11,12,14,15,34,35] but contrasts with our earlier experience.[1,4,7,32] These contradictions need to be resolved before one can unequivocally conclude that there are major differences between anesthetized and conscious dog models of myocardial stunning. Accordingly, we investigated the role of temperature (group II dogs).

A striking finding of these studies was that the stunned myocardium is extremely sensitive to variations in temperature. A close inverse relation was noted, whereby small increases in body temperature resulted in large decreases in postischemic wall thickening that were not accounted for by hemodynamic changes. (The modest increments in heart rate and rate–pressure product associated with the increase in temperature [Table 3] were clearly insufficient to explain the dramatic decrement in function [Figures 2 and 3].) This relation was not observed in the nonischemic region and thus appears to reflect a selective effect of temperature on the stunned myocardium.

These observations in group II explain the apparent discrepancy between the results in group I, in

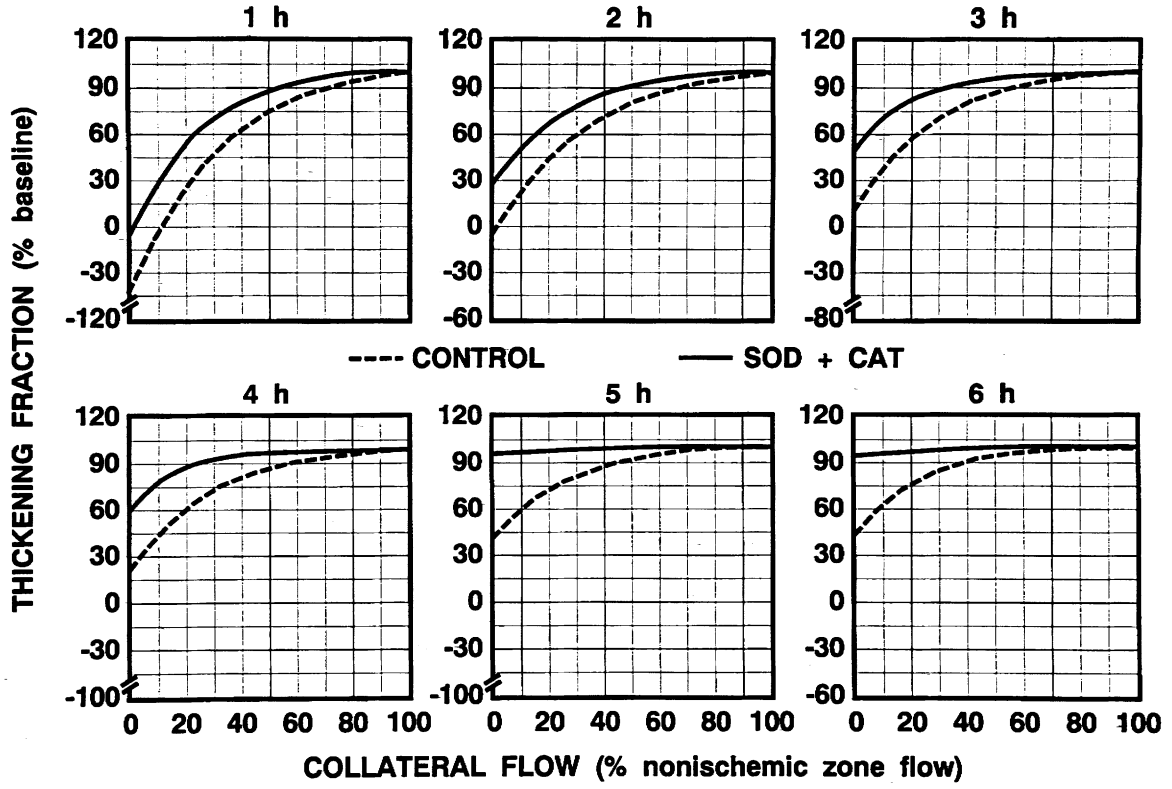

FIGURE 9. *Graphs showing the relation between mean transmural collateral blood flow to the ischemic region during coronary occlusion (horizontal axis) and systolic wall thickening at various intervals after reperfusion (vertical axis) in control (dashed line, n=19) and superoxide dismutase and catalase (SOD+CAT)-treated (continuous line, n=21) conscious dogs. Collateral flow is expressed as percent of simultaneous nonischemic zone flow; thickening fraction is expressed as percent of baseline values. The regression lines pertain to the data shown in Figure 6. A previous study[33] demonstrated that the relation of postischemic wall thickening to ischemic flow is best described by an exponential regression equation (see text); therefore, the regression lines for the exponential expressions are illustrated in the figure. Note that the regression line was shifted to the left and upward in dogs treated with SOD+CAT, indicating that for any given level of collateral perfusion, wall thickening after reflow was greater in these animals compared with controls. Furthermore, the distance between the two regression lines was proportionately larger in the low-flow range, indicating that the enhancement of recovery by SOD+CAT was proportionately greater in dogs with lower levels of collateral perfusion. The exponential regression equations (written in simplified form[33]) for the control group were as follows: 15 minutes,* $y=133.3-182.5\ e^{-0.017x}$ *(*$r=0.75$, $p<0.001$, $SEE=37.6$*); 30 minutes,* $y=110.6-161.0\ e^{-0.027x}$ *(*$r=0.82$, $p<0.001$, $SEE=30.9$*); 1 hour,* $y=105.4-139.9\ e^{-0.033x}$ *(*$r=0.68$, $p<0.01$, $SEE=42.5$*); 2 hours,* $y=105.2-109.2\ e^{-0.031x}$ *(*$r=0.62$, $p<0.01$, $SEE=39.0$*); 3 hours,* $y=102.7-95.7\ e^{-0.036x}$ *(*$r=0.58$, $p<0.01$, $SEE=41.5$*); 4 hours,* $y=102.2-82.5\ e^{-0.036x}$ *(*$r=0.51$, $p<0.05$, $SEE=42.3$*); 5 hours,* $y=101.8-68.4\ e^{-0.036x}$ *(*$r=0.46$, $p<0.05$, $SEE=40.3$*); 6 hours,* $y=100.9-54.6\ e^{-0.041x}$ *(*$r=0.59$, $p<0.01$, $SEE=22.1$*), where* x *is mean transmural blood flow during coronary occlusion (expressed as percent of simultaneous nonischemic zone flow) and* y *is the thickening fraction after reperfusion (expressed as percent of baseline). The equations for the treated group were as follows: 15 minutes,* $y=124.0-162.2\ e^{-0.019x}$ *(*$r=0.83$, $p<0.001$, $SEE=22.8$*); 30 minutes,* $y=107.4-142.5\ e^{-0.030x}$ *(*$r=0.81$, $p<0.001$, $SEE=28.9$*); 1 hour,* $y=103.6-109.7\ e^{-0.034x}$ *(*$r=0.81$, $p<0.001$, $SEE=23.9$*); 2 hours,* $y=101.3-72.6\ e^{-0.040x}$ *(*$r=0.74$, $p<0.001$, $SEE=19.2$*); 3 hours,* $y=100.4-51.4\ e^{-0.050x}$ *(*$r=0.61$, $p<0.001$, $SEE=20.7$*); 4 hours,* $y=100.1-42.3\ e^{-0.066x}$ *(*$r=0.53$, $p<0.01$, $SEE=20.4$*); 5 hours,* $y=100.2-26.7\ e^{-0.049x}$ *(*$r=0.44$, $p<0.05$, $SEE=20.8$*); 6 hours,* $y=100.1-21.5\ e^{-0.055x}$ *(*$r=0.36$, $p<0.1$, $SEE=22.9$*).*

which thickening fraction remained at −20–30% of baseline values throughout reperfusion, and our earlier experiments,[1,4,7,32] in which thickening fraction recovered to an average of 30–40% of baseline after 4 hours of reperfusion. There has been no change in our experimental protocol except that in the earlier studies[1,4,7,32] we did not make any effort to maintain body temperature at normal levels, so that the dogs became progressively hypothermic with time. The observations in group II confirm our suspicion that the differences in postischemic recovery of contractility between our earlier and present experiments are due to differences in temperature. In addition, these observations are of conceptual interest and also have methodological implications, because they emphasize the importance of carefully controlling temperature in open-chest preparations of myocardial stunning. It should be noted that the purpose of this analysis was to reconcile our earlier and present results, not to elucidate the mechanism for the effect

of temperature. The latter is a complex issue that will require further extensive investigation.

Conclusions

In conclusion, there are major differences between the barbiturate-anesthetized and the conscious dog model of myocardial stunning. Since the oxy-radical hypothesis is predicated exclusively on data obtained in the former model, it needs to be tested in awake animals. The present results indicate that oxygen metabolites contribute to postischemic dysfunction in the absence of the confounding factors associated with the open-chest state, and thus should help remove an important limitation of the oxy-radical hypothesis of myocardial stunning.

Acknowledgments

We thank Jennifer S. Pocius and Alejandro Tumang for their excellent technical assistance, the Pharmacia–Chiron Partnership for generously supplying human recombinant SOD, Denise Dawson and Andrea Gruebele for performing the assays of SOD and catalase, and William LaCour, Karen Robins, and Valerie R. Price for their expert secretarial assistance.

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KEY WORDS • oxygen radicals • postischemic myocardial dysfunction • coronary reperfusion • superoxide dismutase • catalase