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N-2-Mercaptopropionylglycine Improves Recovery of Myocardial Function After Reversible Regional Ischemia

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Myocardial reperfusion after reversible regional ischemia is known to result in delayed recovery of contractile function, but the mechanism responsible for this phenomenon remains unclear. We examined the ability of N-2-mercaptopropionylglycine, a synthetic thiol compound with oxygen free radical scavenging properties, to attenuate postischemic dysfunction in open chest dogs undergoing a 15 minute occlusion of the left anterior descending coronary artery followed by 4 hours of reperfusion. Treated animals received an infusion of N-2-mercaptopropionylglycine (50 mg/kg per h) for 4 hours starting 15 minutes before coronary occlusion. Collateral flow, as determined with radioactive microspheres after 10 minutes of ischemia, was 0.07 ± 0.01 ml/min per g (mean ± SE) in both control (n = 20) and treated (n = 13) groups. The occluded vascular bed, as determined by postmortem perfusion, averaged 26.1 ± 1.2% of the weight of the left ventricle in control and 29.6 ± 1.3% in treated animals.

Systolic wall thickening (an index of regional function) was assessed with an epicardial pulsed Doppler probe. The two groups exhibited comparable systolic thickening under baseline conditions and similar degrees of dyskinesia during ischemia. Nevertheless, recovery of function (expressed as percent of baseline) was considerably greater in the treated dogs at 1 hour (44.6 versus 12.8%, $p = 0.05$), 2 hours (64.0 versus 31.6%, $p < 0.02$), 3 hours (77.1 versus 36.7%, $p < 0.01$) and 4 hours of reperfusion (75.0 versus 40.0%, $p < 0.05$).

Thus, N-2-mercaptopropionylglycine produced a significant and sustained improvement in recovery of contractile function after a brief episode of regional myocardial ischemia. These results suggest that oxygen free radicals play a significant role in myocardial postischemic dysfunction. Because N-2-mercaptopropionylglycine is potentially available for oral prophylactic therapy, these results have significant therapeutic implications.

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Myocardial reperfusion after reversible ischemia is associated with delayed recovery of contractile function in experimental models of coronary artery occlusion (1,2). Recent observations strongly suggest that such myocardial "stunning" (3) also occurs in humans, and may be of considerable significance in various clinical settings of transient ischemia followed by reperfusion (4–6). The ability to modify this postischemic dysfunction, however, has been limited by a lack of understanding of its pathophysiology.

A growing body of evidence implicates oxygen free radicals as mediators of reperfusion injury in a variety of organ systems (7–11). We have recently demonstrated (12) that the oxygen free radical scavengers superoxide dismutase and catalase improve recovery of function after a brief episode of coronary artery occlusion. Because superoxide dismutase and catalase eliminate the superoxide anion and hydrogren peroxide, respectively, these results suggest that oxygen free radicals play an important role in the pathogenesis of postischemic dysfunction. The application of these enzymes to patients with coronary artery disease, however, is at present limited by several factors, including the lack of previous clinical utilization and the need for parenteral administration. The demonstration that a free radical scavenger is effective in improving postischemic contractile function would provide additional evidence to support the role of oxygen free radicals in postischemic dysfunction, and would also furnish important data relevant to potential clinical applications.

N-2-mercaptopropionylglycine is an oxygen free radical scavenger (13–16) that has been used clinically in Europe

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and Japan in the treatment of several disorders linked to abnormal free radical production (17–19). It can be administered orally and can theoretically scavenge both intracellular and extracellular oxygen free radicals because of its ability to cross the cell membrane. Its potential value in the setting of ischemia and reperfusion is suggested by a recent report (16) in which its use was associated with a significant decrease in infarct size in a canine preparation of 90 minutes of coronary occlusion followed by reperfusion. The purpose of the present study was to determine whether N-2-mercaptopropionylglycine enhances recovery of contractile function after a reversible ischemic insult. Accordingly, we used a canine model of 15 minutes of coronary artery occlusion followed by reperfusion in which improved recovery of function was previously observed with the use of superoxide dismutase and catalase (12). This duration of ischemia was selected because it does not result in myocardial cell necrosis (2,20), but nevertheless does produce delayed recovery of function (1–3).

Methods

This study was performed in accordance with the guidelines of the Committee on Animals of Baylor College of Medicine and with the Guiding Principles in the Use and Care of Animals approved by the American Physiological Society.

Instrumentation. Mongrel dogs of both sexes weighing 15 to 31 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated and ventilated with room air through a Harvard ventilator. Ventilatory variables were adjusted on the basis of arterial blood gas determinations to maintain normal pH and satisfactory oxygenation. The left pleural cavity was entered through the fifth intercostal space and the pericardium was incised. The left anterior descending coronary artery was isolated and encircled with a snare at a site distal to the first major diagonal branch, to produce an ischemic zone encompassing approximately one-quarter of the left ventricle. A Doppler flow velocity probe was placed around the artery distal to the site of occlusion. Polyethylene catheters were inserted through the left carotid artery into the aorta and through the left atrial appendage into the left atrium, and connected to Statham P23Db pressure transducers. An 8 French Millar pressure transducer was introduced into the left ventricle through an apical stab wound. A Doppler ultrasonic wall thickening probe (21) was positioned over the portion of the left ventricle to be rendered ischemic, and a second probe was placed on the posterior left ventricular wall to serve as a control. The Doppler probe consists of a 4 mm ultrasonic crystal bonded to a 1.5 cm diameter fabric disk impregnated with Silastic. The disk was secured to the epicardial surface with interrupted 6-0 Prolene sutures placed 0.5 to 1 mm into the myocardium, thus producing only minimal trauma. Aortic pressure, left ventricular pressure, first derivative of left ventricular pressure (dP/dt), left atrial pressure, left anterior descending blood flow velocity, myocardial thickening and lead II of the electrocardiogram were recorded on an eight channel Gould-Brush recorder (200 series).

Protocol. Treated dogs received a continuous infusion of N-2-mercaptopropionylglycine (Sigma Chemical Co.), 50 mg/kg per h, in normal saline solution for 4 hours starting 15 minutes before coronary occlusion. Control dogs received saline solution alone. The infusion was administered directly into the left ventricle through the Millar catheter at a rate of 25 ml/h. The left anterior descending coronary artery was occluded for 15 minutes and then reperfused. Restoration of flow was confirmed by noting the immediate reactive hyperemic response detected by the coronary flow velocity probe. No attempt was made to resuscitate animals that developed ventricular fibrillation during ischemia or reperfusion. Hemodynamic and wall thickening data were obtained before and after 5 minutes of ischemia, and at 1, 2, 3 and 4 hours of reperfusion. At the conclusion of the study, the dogs were given heparin (6,000 U) followed by a lethal dose of potassium chloride, and the hearts were excised.

Quantification of occluded vascular bed. A postmortem dual perfusion technique was used to determine the size of the occluded coronary vascular bed (12). Two cannulas were inserted immediately proximal and distal to the site of the previous coronary artery occlusion. The distal left anterior descending coronary bed was perfused with normal saline solution. The proximal left anterior descending and circumflex arteries were perfused retrogradely with a 0.5% solution of Monastral blue dye in saline, while digitally occluding the left coronary ostium. The two vascular beds were perfused simultaneously for 2 minutes at equal physiologic pressure (100 mm Hg). The heart was then cut into 1.0 cm slices in a plane parallel to the atrioventricular groove, and all atrial, valvular and right ventricular tissue was excised. The slices were incubated in a 1% solution of triphenyltetrazolium chloride for 20 minutes at 38°C to verify the absence of irreversible injury. This agent stains only viable myocardium containing dehydrogenase dark red (22). The portion of the left ventricle supplied by the previously occluded coronary artery (occluded coronary bed) was identified by the absence of Monastral blue dye, and separated from the rest of the left ventricle. Both components were weighed to determine occluded bed size as a percent of the left ventricle.

Regional myocardial blood flow. Regional myocardial blood flow was determined by the radioactive microsphere technique (23) 10 minutes after coronary occlusion. Microspheres 15 $\pm$ 3 μm in diameter (Dupont Co.), labeled with scandium-46, strontium-85, tin-113 or cerium-141, were obtained as 1 mCi of nuclide suspended in 10 ml of 10% dextran containing Tween 80. After 2 minutes of vigorous

mixing on a vortex agitator, approximately 2 million spheres were suspended in 3 ml of normal saline solution and injected into the left atrium over 10 seconds, after which the catheter was flushed with an additional 10 ml of saline. Beginning 15 seconds before and continuing for 2 minutes after injection, a reference blood sample was withdrawn from the aorta with a Harvard pump at a constant rate of 4.05 ml/min. After postmortem perfusion and triphenyltetrazolium chloride staining, four transmural samples (1.0 to 1.5 g) were obtained from both the occluded and the nonoccluded beds. To avoid admixture of ischemic and nonischemic tissue, ischemic samples were obtained at least 1 cm inside the margin of the unstained region. The radioactivity of the tissue and reference blood samples was determined with a sodium iodide crystal well counter. Regional myocardial blood flow was calculated by standard methods (23) and expressed as milliliters per minute per gram.

Regional myocardial function. Regional myocardial function was assessed using a 10 MHz pulsed Doppler ultrasound wall thickening probe. Theoretical and experimental validation of this technique has been previously published (21,24). The pulsed Doppler technique utilizes a single epicardial crystal to determine systolic myocardial wall displacement by digitally integrating the velocity of myocardial layers passing through the range gated sample volume. A representative example of the wall thickening tracings obtained with this probe is illustrated in Figure 1. The beginning and end of systole were determined from the onset of the upstroke of the left ventricular pressure tracing and the peak negative dP/dt, respectively. Systolic thickening fraction was calculated by dividing net systolic thickening by end-diastolic wall thickness as determined by the range gate depth. Net systolic thickening was defined as the maximal systolic increase in wall thickness from the end-diastolic value (25). When paradoxical wall thinning persisted for 50% or more of systole, the maximal extent of wall thinning was substracted from wall thickening (25). Thickening fraction determined by the Doppler method correlates closely with thickening fraction measured using two ultrasonic transit-time crystals (21,24). The use of the Doppler probe is advantageous, however, in that the single epicardial crystal eliminates the trauma of intramyocardial crystal insertion.

Statistical analysis. Data are expressed as group means ± SEM. The Student's unpaired *t* test was used to compare mean values between control and treated animals, and a probability value of less than 0.05 was considered statistically significant.

Results

Study animals. Of the 43 dogs initially instrumented, 4 from each group died of ventricular fibrillation immediately after reperfusion. The protocol was discontinued in one control dog because coronary artery occlusion failed to produce dyskinesia, and in one treated dog because occlusion produced a marked decrease in global function such that nonischemic zone contractile function decreased to 50% of that obtained under baseline conditions, with continued deterioration after reperfusion. Analysis of data was thus carried out in 20 control and 13 treated dogs. Triphenyltetrazolium chloride staining was uniformly present in the

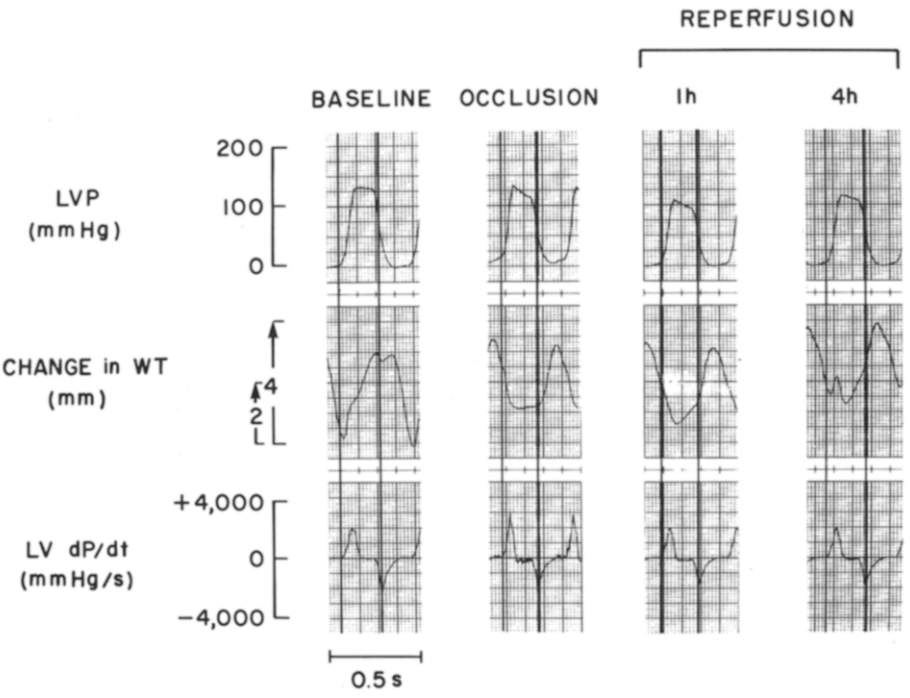

Figure 1. Representative wall thickening tracings from one control animal. Systolic wall thickening present under baseline conditions was replaced by paradoxical thinning (dyskinesia) during left anterior descending artery occlusion. Dyskinesia persisted at 1 hour of reperfusion, with minimal recovery at 4 hours. From **top** to **bottom:** left ventricular pressure (LVP), change in wall thickness (WT) in the left anterior descending artery territory and left ventricular dP/dt. **Vertical lines** indicate the beginning and end of systole. Because the Doppler probe measures changes in wall thickness rather than absolute wall thickness, the position of the signal on paper bears no relation to absolute wall thickness.

Table 1. Hemodynamic Variables in Control (n = 20) and Treated (n = 13) Dogs Before, During and After a 15 Minute Occlusion of the Left Anterior Descending Coronary Artery

			Reperfusion			
	Baseline	Ischemia	1 h	2 h	3 h	4 h
HR						
Control	135 ± 4	138 ± 4	129 ± 5	126 ± 5	120 ± 5	115 ± 6
MPG	135 ± 4	136 ± 4	136 ± 5	136 ± 6	136 ± 5*	132 ± 5*
MAP						
Control	109 ± 3	105 ± 4	100 ± 3	99 ± 3	99 ± 3	102 ± 4
MPG	105 ± 3	89 ± 5†	92 ± 3	100 ± 4	99 ± 5	99 ± 4
RPP						
Control	16.7 ± 0.7	16.4 ± 0.7	15.0 ± 0.8	14.6 ± 0.8	13.6 ± 0.7	13.1 ± 0.8
MPG	16.3 ± 0.6	14.6 ± 0.7	15.3 ± 0.8	16.6 ± 1.1	16.6 ± 1.2*	15.9 ± 1.0*
LAP						
Control	4.2 ± 0.3	6.3 ± 0.4	4.6 ± 0.4	3.5 ± 0.3	3.5 ± 0.3	3.6 ± 0.3
MPG	4.6 ± 0.3	5.4 ± 0.5	3.2 ± 0.3‡	3.2 ± 0.3	3.9 ± 0.4	4.0 ± 0.5

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.02$ versus control. Values are mean ± SE.

HR = heart rate (beats/min); LAP = mean left atrial pressure (mm Hg); MAP = mean arterial blood pressure (mm Hg); MPG = N-2-mercaptopropionylglycine; RPP = rate-pressure product (heart rate × systolic blood pressure/1,000).

previously ischemic myocardium, confirming the absence of infarction in all animals.

Systemic hemodynamics (Table 1). There were no significant differences between control and treated groups with respect to baseline heart rate, mean arterial blood pressure, rate-pressure product or mean left atrial pressure. The mean arterial pressure was slightly lower in the treated dogs during the 15 minutes of coronary occlusion (89 versus 105 mm Hg), but was similar to the control group throughout reperfusion. During reperfusion the heart rate was slightly greater in the treated group but the difference reached statistical significance only at 3 and 4 hours. This difference was further reflected in the greater rate-pressure product in the treated group during reperfusion.

Occluded bed size and regional myocardial blood flow (Table 2). The two groups were closely comparable with respect to both occluded bed size and coronary collateral flow. Occluded bed size was 26.1 ± 1.2% of the left ventricular weight in the control group and 29.6 ± 1.3% in the treated group. Transmural ischemic zone blood flow was 0.07 ± 0.01 ml/min per g in both groups. Blood flow to the nonischemic region was also similar: 1.16 ± 0.09 ml/min per g in control and 0.97 ± 0.08 ml/min per g in treated dogs.

Regional myocardial function. Systolic thickening fraction in the control (nonischemic) region remained similar in the two groups throughout the study (Table 3). Baseline systolic thickening fraction in the territory to be rendered ischemic averaged 32.8 ± 1.8% in the control and 32.1 ± 1.8% in the treated dogs. Thickening fraction remained virtually unchanged (32.9 ± 2.4%) after the initial 10 minutes of drug infusion. The results of serial measurements of systolic thickening expressed as a percentage of baseline are displayed in Figure 2. The two groups did not differ with respect to the extent of systolic thinning (dyskinesia) during ischemia. Nevertheless, recovery of function after reperfusion was consistently greater in the animals treated with N-2-mercaptopropionylglycine. After reperfusion, thickening fraction (expressed as percent of baseline) was 12.8 ± 11.3% in control versus 44.6 ± 9.8% in treated dogs ($p = 0.05$) at 1 hour, 31.6 ± 9.8 versus 64.0 ± 6.1% ($p < 0.02$) at 2 hours, 36.7 ± 11.2 versus 77.1 ± 5.3% ($p < 0.01$) at 3 hours and 40.0 ± 11.5 versus 75.0 ± 4.4% ($p < 0.05$) at 4 hours.

Table 2. Occluded Coronary Bed Size and Regional Myocardial Blood Flow

	Occluded Bed Weight	Ischemic Zone Flow	Nonischemic Zone Flow
	Left Ventricular Weight (%)	(ml/min per g)	(ml/min per g)
Control (n = 20)	26.1 ± 1.2	0.07 ± 0.01	1.16 ± 0.09
MPG (n = 13)	29.6 ± 1.3	0.07 ± 0.01	0.97 ± 0.08
p Value	NS	NS	NS

Values are mean ± SE. MPG = N-2-mercaptopropionylglycine.

Table 3. Nonischemic Zone Systolic Thickening Fraction

			Reperfusion			
	Baseline	Occlusion	1 h	2 h	3 h	4 h
Control (n = 20)	23.4 ± 1.3	23.7 ± 1.2	22.0 ± 1.0	19.6 ± 0.8	19.5 ± 1.0	19.1 ± 0.7
MPG (n = 13)	22.4 ± 1.8	22.0 ± 2.1	20.6 ± 1.9	20.1 ± 1.7	19.8 ± 1.7	19.4 ± 1.7
P Value	NS	NS	NS	NS	NS	NS

Values are mean ± SE. MPG = N-2-mercaptopropionylglycine.

Discussion

This study demonstrates that the free radical scavenger N-2-mercaptopropionylglycine produces a significant and sustained improvement in recovery of myocardial function after reversible regional ischemia. A 15 minute coronary occlusion was selected because it does not result in myocardia necrosis in the dog (2,20,26,27). This was confirmed in the present study with the use of triphenyltetrazolium chloride staining. The ability of this enzyme-mapping technique to delineate irreversible injury within hours of the onset of ischemia, particularly when followed by reperfusion, has been previously demonstrated (28,29). Thus, persistent postischemic dysfunction was observed in this study in the absence of irreversible damage, a finding consistent with previous observations in similar models (1,2).

Several variables that might influence postischemic dysfunction were examined, but there were no differences between control and treated dogs that could account for the beneficial effect observed. The two groups were virtually identical with respect to regional function under baseline conditions and collateral blood flow during coronary occlusion. The size of the occluded vascular bed was slightly greater in the treated group, a factor that if anything would be expected to have an adverse rather than favorable effect on recovery of function. The mean arterial pressure was slightly lower in the treated dogs during the 15 minutes of ischemia, but the rate-pressure product was similar in the two groups. The similarity of the rate-pressure product during coronary occlusion in control and treated dogs indicates that the two groups were comparable with respect to myocardial oxygen demands during the ischemic phase. The difference in mean arterial pressure was not apparent during the reperfusion interval. Therefore, the enhanced myocardial performance as detected by wall thickening in the treated group at 1, 2, 3 and 4 hours of reperfusion cannot be ascribed to reduced afterload. The heart rate was slightly higher in the treated group during the latter part of the reperfusion phase which would be expected to be deleterious rather than beneficial. Thus, N-2-mercaptopropionylglycine exerted a significant beneficial effect that was sustained even during the late phase of reperfusion when the rate-pressure product was greater in the treated group.

Postulated mechanisms of postischemic dysfunction. The cause of the persistent contractile dysfunction after a brief episode of myocardial ischemia unassociated with necrosis remains unknown. Adenosine triphosphate levels decrease rapidly after coronary occlusion (30). Although there appears to be a parallel between delayed replenishment of adenosine triphosphate stores and delayed recovery of contractile function after reperfusion (30,31), recent investigations (32–34) have failed to substantiate high energy phosphate depletion as the primary cause of persistent postischemic dysfunction. Other mechanisms that have been postulated include a defect in the transfer of adenosine triphosphate from the mitochondria to their site of utilization (35), damage to contractile proteins (2) or disruption of sympathetic neural responsiveness (36). We (12) have pre-

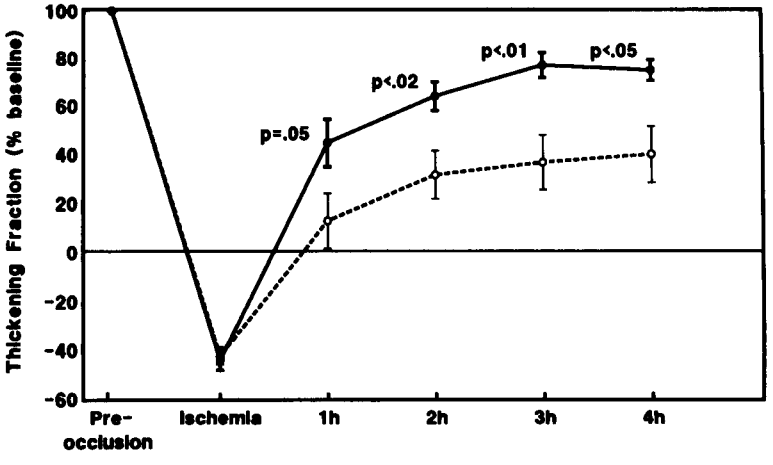

Figure 2. Recovery of regional function in the left anterior descending coronary artery territory after 15 minutes of occlusion in control **(dashed line)** and treated **(solid line)** groups.

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viously shown that administration of the oxygen free radical scavengers, superoxide dismutase plus catalase, improves recovery of function after a 15 minute coronary occlusion. The present study provides further evidence to support the concept that oxygen free radicals play a significant role in the pathogenesis of persistent contractile abnormalities after reversible regional ischemia.

Oxygen free radicals in myocardial reperfusion injury. Oxygen free radicals have been identified as mediators of cell injury in a wide range of pathologic conditions (37–39). Increasingly, interest has focused on the role of free radical production in the setting of myocardial ischemia and reperfusion (9,39). Jolly et al. (40) demonstrated that the combination of superoxide dismutase and catalase reduces myocardial infarct size in a canine model of 90 minutes of coronary artery occlusion followed by reperfusion. The administration of free radical scavengers has produced improved recovery of global left ventricular function after prolonged hypothermic global ischemia (41–43). In addition, Burton (44) recently reported that superoxide dismutase improves contractile performance after 1 hour of reperfusion in an isolated, perfused rabbit septal preparation subjected to 1 hour of ischemia. Our present data extend these observations to the dysfunction that occurs after regional myocardial ischemia unassociated with necrosis in the intact normothermic animal. Thus, our results suggest that even a brief coronary occlusion can lead to the generation of toxic oxygen metabolites in sufficient quantity to affect postischemic contractility. Confirmation of the role of free radicals in myocardial ischemia and reperfusion awaits the availability of a reliable method of measuring free radical production in vivo.

Many biologic processes are known to result in the production of oxygen free radicals (45,46). Nevertheless, the mechanism of increased free radical production in tissues subjected to ischemia and reperfusion remain speculative. Both intracellular and extracellular sources have been postulated. Ischemia-induced mitochondrial damage may result in impaired oxygen metabolism with concomitant free radical production when relatively high concentrations of oxygen are restored at the time of reperfusion (37). Another potential source of free radicals is the enzyme xanthine oxidase, which, in the presence of oxygen, catalyzes the conversion of hypoxanthine to xanthine with simultaneous generation of superoxide anion (39). While one substrate (hypoxanthine) accumulates during ischemia as a result of adenosine triphosphate degradation, the other (molecular oxygen) is provided by reperfusion, which could theoretically result in a burst of superoxide production. The evidence regarding the significance of xanthine oxidase in myocardial reperfusion injury is, however, contradictory (47–49). A potential extracellular source of free radicals is the activated neutrophil, which is known to produce superoxide anion, hydrogen peroxide and the hydroxyl radical (50). It has been demonstrated that neutrophil depletion results in a significant decrease in myocardial infarct size (51), and that activated leukocytes can disrupt sarcoplasmic reticulum calcium transport through an oxygen radical mechanism (52). Because N-2-mercaptopropionylglycine readily crosses the cell membrane (53), it may protect from oxygen free radicals generated in both the intracellular and extracellular space.

N-2-mercaptopropionylglycine. The antioxidant N-2-mercaptopropionylglycine is a synthetic thiol compound that scavenges the superoxide anion and, possibly, the hydroxyl radical (13,14,16). In addition, the drug is able to substitute as a sulphydryl donor for glutathione (53,54), an important intracellular antioxidant that is significantly depleted by hypoxia and reperfusion (55). Glutathione functions as the hydrogen donor for glutathione peroxidase, a protective enzyme that eliminates hydrogen peroxide and inactivates lipid peroxides (55). Many of the deleterious effects of free radicals are produced through lipid peroxidation of cellular and subcellular membranes, with resulting changes in membrane structure and function. N-2-mercaptopropionylglycine may, therefore, exert its protective effect both through the direct elimination of oxygen free radicals and through prevention of lipid peroxidation (56).

Therapeutic benefit has been demonstrated with the use of this agent in the treatment of rheumatoid arthritis (19) and senile cataracts (17), two disorders that have been linked to abnormal free radical production. In addition, studies in several animal models (15) have demonstrated its efficacy in ameliorating the side effects of radiation, a process whose damaging effects are largely mediated through free radical formation (45). N-2-mercaptopropionylglycine is readily absorbed after oral administration (53) and is generally reported to be a well tolerated drug (17,19).

Clinical implications. Although caution is obviously warranted in extrapolating experimental data to humans, the results of this study may have therapeutic implications. Transient myocardial ischemia occurs in a variety of clinical settings: unstable or variant angina, cardiac transplantation, open heart surgery with cardioplegic arrest and patients with acute myocardial infarction undergoing thrombolytic therapy. Restoration of myocardial blood flow in these situations may be associated with persistent, albeit reversible, depression of contractile function (3–6). Our results indicate that such dysfunction may be effectively reduced with N-2-mercaptopropionylglycine. Because of the potential benefit to be derived from such an oral prophylactic agent, further investigation of N-2-mercaptopropionylglycine appears warranted.

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