Nitroprusside Attenuates Myocardial Stunning Through Reduced Contractile Delay and Time (44489)

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Abstract. **We hypothesized that myocardial stunning would be reversed through increased cyclic GMP caused by nitroprusside, and that this would be accomplished through a decreased proportion of regional work during diastole. Hearts were instrumented to measure left ventricular pressure, and regional myocardial mechanics were recorded using a miniature force transducer and ultrasonic dimension crystals in eight open-chest anesthetized dogs. Following baseline (CON), the left anterior descending coronary artery (LAD) was occluded for 15 min, followed by a 30-min recovery (STUN). Then intracoronary LAD infusion of sodium nitroprusside (NP) (4 μg/kg/min) was begun. The time delay (msec) to regional shortening increased significantly from 18 ± 13 to 73 ± 13 following stunning, but was reduced to 49 ± 18 by NP. Total regional work (g*mm/min) at baseline (1368 ± 401 CON) was unchanged with stunning (1320 ± 333 STUN), but reduced (961 ± 240) following NP. Time to peak force development (msec) increased significantly with stunning from 284 ± 13 (CON) to 333 ± 11 (STUN), but was reduced to 269 ± 12 following NP. The percentage work during systole was reduced from 96% ± 2% (CON) to 77% ± 7% (STUN), but returned to 98% ± 1% with NP. Regional O_2 consumption was unaffected by either treatment. Cyclic GMP was unchanged by stunning (2.9 ± 0.3–2.9 ± 0.4 pmol/g) but increased significantly with NP (4.6 ± 0.6). These data indicated that regional myocardial stunning could be attenuated by nitroprusside, which increased cyclic GMP, decreased contractile delay, increased the proportion of work done during systole, and reduced time of shortening.**

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Following brief myocardial ischemia, there is often mechanical dysfunction characterized by reduced systolic work and delayed shortening (1, 2). Myocardial stunning does not usually reduce local oxygen consumption (1, 3). Earlier work from our laboratory demonstrated regional mechanical asynchrony with much of the local work occurring after ventricular systole without metabolic derangement (1). In addition, these mechanical abnormalities can be temporally eliminated by the addition of inotropic agents, such as isoproterenol (1, 4, 5). This led to suggestions that part of the problem of myocardial stunning was associated with calcium handling or excitation-contraction coupling (6–9). These data implied that all inotropic agents would reduce myocardial stunning.

We have recently reported that reducing myocardial cyclic GMP using methylene blue resulted in increased segment force of contraction, but significant increases in the shortening delay and the degree of stunning (2). Nitric oxide increases cyclic GMP levels in the myocardium through activation of guanylate cyclase (10). This reduces cardiac and isolated myocyte function, contractile duration, and metabolism (10, 11). This may or may not be related to blockade of L-type calcium channels in the myocardium (12, 13). It could also be related to changes in cyclic AMP through effects of the cyclic GMP–affected cyclic AMP phosphodiesterases or changes in protein phosphorylation (10, 14). We thought that increasing cyclic GMP with nitric oxide might reduce myocardial stunning by reducing contractile

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duration, thus increasing the portion of local cardiac work during systole.

The current study was undertaken to test the hypothesis that increasing nitric oxide and myocardial cyclic GMP could reverse myocardial stunning. We used an intracoronary infusion of sodium nitroprusside to increase nitric oxide and cyclic GMP in a canine model of myocardial stunning caused by ischemia and reperfusion. We further hypothesized that nitric oxide would lead to a reduction in contractile duration, which would reduce the degree of myocardial stunning. This could increase the percentage of local myocardial work performed during systole in the stunned myocardium. We found that increases in cyclic GMP resulted in a reversal of mechanical stunning.

Materials and Methods

All animals used in this study were maintained in accordance with the guidelines of our Institutional Animal Care and Use Committee and the National Research Council's *Guide for the Care and Use of Laboratory Animals* (1996). Eight mongrel dogs were used in an acute anesthetized open-chest preparation.

A 20-gauge angiocatheter was placed percutaneously into a peripheral vein for administration of fluids and anesthetic. General anesthesia was induced using a bolus injection of intravenous sodium pentobarbital (30 mg/kg), followed by supplements as needed to maintain surgical anesthesia. The animals were endotracheally intubated and ventilated on a Bennett MA-1 volume ventilator (Bennett Respirator Products, Santa Monica, CA). An angiocatheter (16G) was placed in the left femoral artery for systemic arterial blood gas sampling. Arterial blood gas samples were obtained throughout the experiment and analyzed for pH, PO_2 and PCO_2 on a Radiometer ABL-330 blood gas analyzer (Radiometer America, Cleveland, OH). Hemoglobin and O_2 saturation were measured on a Radiometer OSM-2 oximeter (Radiometer America). Appropriate ventilation parameters were adjusted to maintain eucapnea and physiologic pH.

A thoracotomy was performed at the left fifth intercostal space, and the heart was suspended in a pericardial cradle. Catheter-tipped micromanometers (Millar TC500; Millar Instruments Inc., Houston, TX) were placed in the left ventricle *via* an apical stab wound, and in the descending thoracic aorta, *via* advancement from the right femoral artery. These were used to monitor left ventricular blood pressure, ventricular dP/dt_{max}, and systemic blood pressure. The first or second diagonal branch of the left anterior descending (LAD) coronary artery was isolated and cannulated with an angiocatheter (22G) for intracoronary infusion of sodium nitroprusside. A branch of the anterior interventricular coronary vein was cannulated using an angiocatheter (22G) for collection of coronary venous blood. An ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the proximal LAD for measurement of blood flow, and a silk snare was used to provide intermittent complete LAD coronary artery occlusion.

A pair of 5 MHz ultrasonic dimension crystals was implanted in an area of the left ventricular free wall supplied by the LAD in the direction of the short axis, 6 mm deep and $\approx$ 10 mm apart. A miniature force transducer (Warren Research Products, Charleston, SC) was sutured at an adjacent site, at the same depth as the pair of dimension crystals, to measure simultaneous segment force changes. The force transducer was connected to a Wheatstone bridge, which was balanced and calibrated before each experiment. A second pair of ultrasonic dimension crystals was similarly implanted in the circumflex region of the heart, to serve as a regional control.

Lead II electrocardiogram, along with global hemodynamic and regional functional measurements (segment length and force), were monitored continuously on a Gould V1000 digital display and recorded on a multichannel electrostatic recorder (Gould ES1000; Gould Inc., Valley View, OH). Data were digitized at a sampling frequency of 200 Hz over 10-sec periods (Data Translation DT2801) and acquired on a microcomputer for analysis. The digitized data were analyzed using the algorithm of ensemble beat averaging. This resulted in a single representative beat, free of random noise, from which all calculations were performed. Measured parameters included aortic blood pressure, left ventricular blood pressure, segment force, and segment length. Calculated parameters included heart rate, maximum first derivative of the left ventricular pressure (LV dP/dt_{max}), peak force, and percentage segment shortening. Coronary vascular resistance was calculated by dividing mean arterial pressure by normalized coronary blood flow. Delay times were measured from the beginning of ventricular systole to the beginning of shortening. Total regional myocardial segment work per minute was calculated by determining the area under the force-length loop over the cardiac cycle and multiplying it by the heart rate. This was accomplished by multiplying each measured value for force by its corresponding change in length and integrating all positive values over the interval of the averaged heart beat (15). The systolic portion of the local segment work was determined for the systolic portion (start of ventricular pressure rise to closure of aortic valve) of the cardiac cycle only.

Transmural myocardial biopsies were obtained for determination of cyclic GMP levels. These were obtained from both the LAD (experimental) and circumflex (control) regions at least 1 cm from the implanted regional functional measurement devices. Biopsies were obtained using a true-cut biopsy needle and immediately frozen in liquid nitrogen. No significant bleeding occurred during this procedure. The heart was excised at the completion of the experiment and frozen in liquid nitrogen for later analysis.

Following instrumentation, the animal was allowed to stabilize before baseline measurements were obtained. Baseline hemodynamic and functional recordings were then made, and control myocardial biopsies as well as arterial

and coronary venous blood gas samples were obtained. The LAD coronary artery was then occluded *via* the snare such that ultrasonic blood flow measurements reached zero. This LAD occlusion was allowed to continue for 15 min, at which point the occlusion was released and animals were given 30 min to reach a new steady state. Stunned (STUN) data collection was then obtained, including global and regional hemodynamic and functional measurements, blood gas samples, and transmyocardial biopsies. Following acquisition of these data, intracoronary infusion of sodium nitroprusside was begun at 4 μg/kg/min. Following 10 min of nitroprusside infusion, nitroprusside (NP) data collection and biopsies were obtained. The nitroprusside infusion was then halted, and functional data were obtained after 10 min.

Regional myocardial oxygen extraction was calculated by multiplying the difference between arterial and venous percentage O_2 saturation times the hemoglobin times 1.36 ml O_2/100 ml blood. Regional myocardial oxygen consumption was calculated as the product of the oxygen extraction and normalized blood flow.

To determine cyclic GMP, biopsy samples were warmed to 0°C and homogenized in ethanol using a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY) placed in an ice bath. The homogenate was centrifuged at 30,000*g* for 15 min in a Sorvall RC-5B centrifuge (Newtown, CT). The supernatant was recovered, and the pellet was resuspended in 1 ml of 2:1 ethanol:water and centrifuged as before. The combined supernatants were evaporated to dryness in a 60°C bath under a stream of nitrogen gas. The final residue was dissolved in 1.5 ml of assay buffer (0.05 *M* sodium acetate, pH 5.8, containing sodium azide). Cyclic GMP levels were then determined using a radioimmunoassay (Amersham Corporation, Arlington Heights, IL). This assay measures the competitive binding of ^{125}I-cyclic GMP to a cyclic GMP specific antibody. After construction of a standard curve, cyclic GMP levels were determined directly from activity counts in picomoles/gram of wet tissue weight.

A repeated measure analysis of variance (ANOVA) was used to determine whether there were differences in hemodynamic or blood gas variables at the various experimental time points. This analysis was also used to determine differences between time and regions for myocardial O_2 consumption, cardiac function, cyclic GMP, and coronary blood flow indices. Duncan's *posthoc* procedure was applied to assess the significance of differences. In all cases a value of $P < 0.05$ was accepted as significant. All values are expressed as the mean ± standard error of the mean (mean ± SEM).

Results

Table I presents global hemodynamic data during the experiment. Stunning of the area of left ventricular free wall perfused by the left anterior descending (LAD) coronary artery resulted in no significant changes in heart rate, systemic, or left ventricular blood pressure. Global left ventricular contractility as measured by left ventricular dP/dt_{max} was slightly, though not significantly, depressed by both stunning and nitroprusside infusion. Intracoronary infusion of nitroprusside caused significant systolic, diastolic, mean, and left ventricular hypotension, compared with both control and stunned animals. Neither stunning nor nitroprusside affected heart rate or coronary vascular resistance. Arterial blood gases and pH were not affected by the experimental protocol and were maintained within the normal range.

Regional myocardial functional and O_2 supply/consumption parameters and cyclic GMP data are presented in Table II. Regional percentage shortening was not altered in the LAD area or in the control circumflex region by stunning or nitroprusside. In addition, LAD force was unaffected by these conditions. Total regional segment work/min was unaffected by stunning. Decreases in total work caused by nitroprusside were not significant. Stunning increased end diastolic length in the LAD region (10.7 ± 0.8–11.5 ± 0.7 mm), whereas nitroprusside restored end diastolic length (10.7 ± 0.7). Neither stunning nor nitroprusside had any significant effect on LAD blood flow, O_2 extraction, or O_2 consumption. Stunning had no effect on the level of cyclic GMP, whereas administration of intracoronary nitroprusside resulted in a nearly two-fold increase in the LAD level of cyclic GMP.

Figure 1 graphically depicts the effects of stunning on regional shortening. This figure presents an ensemble beat, averaged from 10 sec of data, from the LAD region of an animal. Prior to coronary occlusion (CONTROL), regional segment shortening occurred simultaneously with the be-

Table I. Effect of Regional Myocardial Stunning and Local Infusion of Nitroprusside on Global Hemodynamic Parameters

	Control	Stun	Nitroprusside
Heart rate (beats/min)	139 ± 3	141 ± 4	137 ± 6
Systolic blood pressure (mm Hg)	128 ± 5	121 ± 6	93 ± 10[a,b]
Diastolic blood pressure (mm Hg)	103 ± 5	93 ± 6	72 ± 9[a,b]
Mean blood pressure (mm Hg)	115 ± 5	104 ± 7	79 ± 10[a,b]
Left ventricular pressure (mm Hg)	122 ± 5	114 ± 7	90 ± 8[a,b]
Left ventricular dP/dt_{max} (mm Hg/sec)	2769 ± 247	2115 ± 127	1989 ± 319

Note. Values are means ± SEM.
[a] $P < 0.05$ vs Control.
[b] $P < 0.05$ vs Stunned.

Table II. Effect of Regional Myocardial Stunning and Local Infusion of Nitroprusside on Regional Myocardial Mechanics, O_2 Supply/Consumption Parameters and Cyclic GMP

	Control	Stun	Nitroprusside
LAD shortening (%)	12.1 ± 2.1	11.7 ± 1.8	9.2 ± 1.2
Circumflex shortening (%)	6.8 ± 0.7	7.1 ± 0.7	7.1 ± 0.6
LAD force development (g)	9.4 ± 1.4	10.3 ± 1.4	8.0 ± 1.3
LAD total segment work (g * mm/min)	1368 ± 401	1320 ± 333	961 ± 240
LAD O_2 consumption (ml O_2/min/100g)	6.94 ± 0.90	6.11 ± 0.91	5.84 ± 0.52
LAD O_2 extraction (ml O_2/100 ml)	8.50 ± 1.26	9.20 ± 0.91	8.13 ± 0.81
LAD blood flow (ml/min/100g)	82 ± 7	69 ± 6	71 ± 6
LAD vascular resistance (mmHg/ml/min/100g)	1.49 ± 0.16	1.59 ± 0.15	1.13 ± 0.11
Cyclic GMP (pmol/g)	2.89 ± 0.33	2.93 ± 0.35	4.56 ± 0.59[a,b]

Note. Values are means ± SEM.
[a] $P < 0.05$ vs Control.
[b] $P < 0.05$ vs Stunned.

ginning of the upstroke in the left ventricular pressure curve. After regional myocardial stunning caused by LAD coronary occlusion and release (REPERFUSION), there was a delay in the onset of regional shortening with respect to left ventricular pressure development. The intracoronary infusion of nitroprusside largely reversed these changes, as graphically depicted (NITROPRUSSIDE).

Stunning resulted in a significant increase in time delay in the onset of regional shortening, which was reversed by the nitroprusside infusion. This is graphically depicted in Figure 2. In the control circumflex region, there were no significant differences in time to onset of shortening between control (71 ± 50 msec), stunning (63 ± 28), and nitroprusside administration (69 ± 26). The time to peak shortening was increased with stunning (223 ± 7–280 ± 11 msec) and was restored with nitroprusside (218 ± 7). Similarly, the time to peak force development increased significantly with stunning (284 ± 13–333 ± 11 msec) and was completely restored by nitroprusside (269 ± 12). Percentage work performed during systole (calculated as systole work divided by total work times 100) was significantly decreased by myocardial stunning, whereas nitroprusside completely reversed this decrease (Fig. 3).

Ten min after cessation of the nitroprusside infusion, mean arterial pressure (91 ± 12 mmHg) returned toward control values. Functional timing parameters returned toward the pre-nitroprusside stunning values in the LAD region. The delay until the onset of shortening increased significantly to 76 ± 4 msec. The time to peak shortening (227 ± 4 msec) and time to peak force (283 ± 11 msec) also increased.

Discussion

The current findings demonstrated partial reversal of myocardial stunning following ischemia and reperfusion through the intracoronary administration of sodium nitroprusside. Reversal of stunning was affected through reversal of contractile delay, an increase in the proportion of work done during systole, and reduction of time of shortening. This was despite a lack of significant change in local shortening, force, and total work in the stunned region after

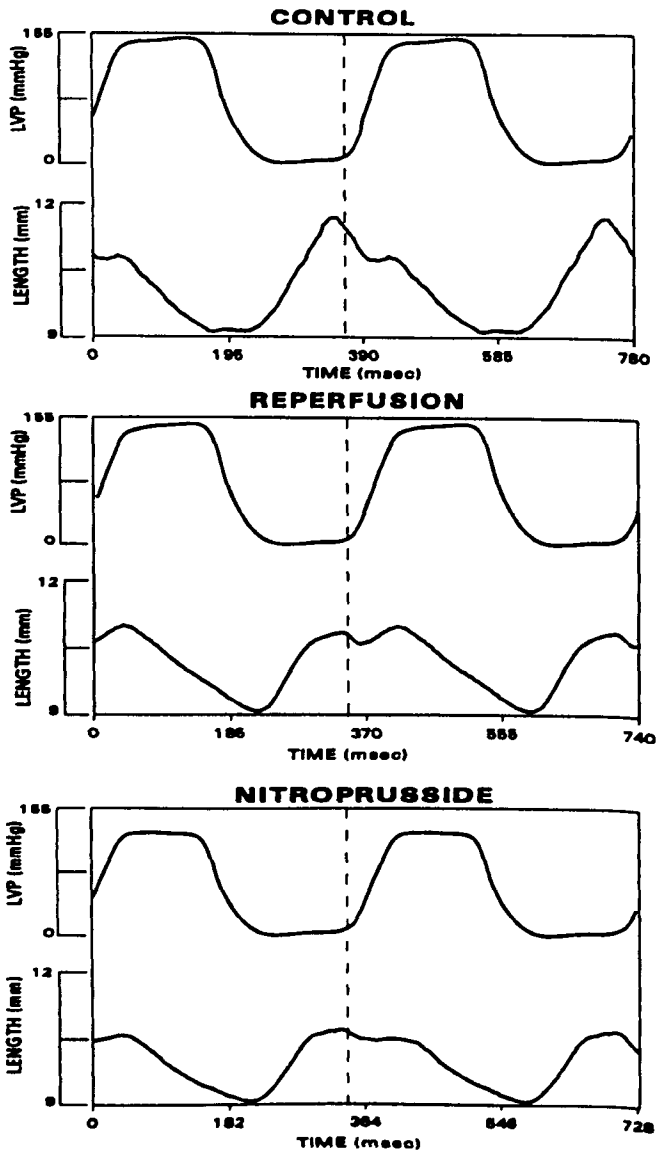

Figure 1. Effect of myocardial stunning (REPERFUSION) and intracoronary nitroprusside (NITROPRUSSIDE) administration on time course of LAD segment shortening (LENGTH) and left ventricular pressure (LVP) development versus baseline (CONTROL). The dashed line represents the beginning of ventricular systole. Note that LAD segment shortening is delayed only during reperfusion.

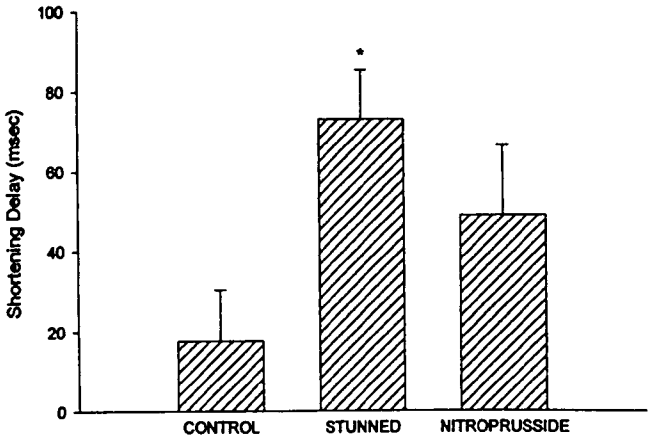

Figure 2. Effect of myocardial stunning and intracoronary nitroprusside administration on time delay (msec) of regional (LAD) shortening ($n = 8$). $^*P < 0.05$ vs control.

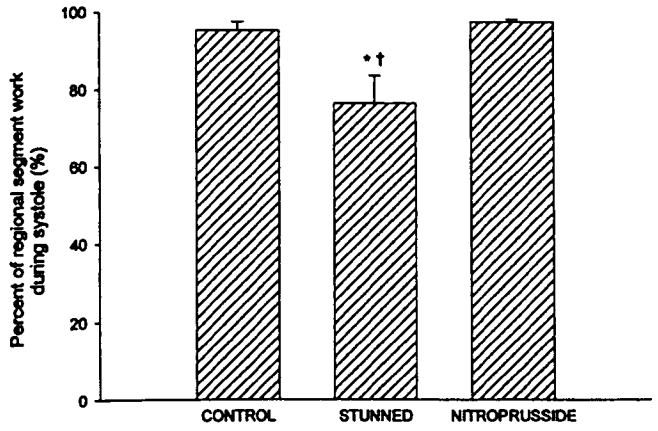

Figure 3. Effect of myocardial stunning and intracoronary nitroprusside administration on percentage of local segment work performed during systole (%), (100* systole segment work/total segment work) ($n = 8$). $^*P < 0.05$ vs control. $\dagger P < 0.05$ vs nitroprusside.

nitroprusside. These mechanical effects appeared to be related to the increased intracellular cyclic GMP from sodium nitroprusside. This report demonstrated a reversal of myocardial stunning in the presence of an agent that does not increase inotropy.

Myocardial stunning has been defined as ischemia-induced mechanical dysfunction of the myocardium that persists following reperfusion, in the absence of irreversible damage such as necrosis (8, 16). Major components of myocardial stunning include a time course, which persists significantly longer than that of the inciting ischemia, as well as reversibility of functional deficits. The mechanical depression of myocardial contractility in myocardial stunning exists in the absence of significant metabolic derangement (5). In the current study, we found that myocardial stunning produced almost no changes in total segment work or local O_2 consumption. However, there was a significant time delay in the beginning of shortening and an increase in the total time of shortening. This led to an increase in the proportion of the total segment work performed during diastole. Delays in shortening and relaxation have been reported previously (1, 17).

Stunned myocardium still retains the ability to respond to inotropic stimulation with increased contractility and increased oxygen consumption (1, 2). In stunning, there is an uncoupling of oxidative metabolism from mechanical contraction that has been termed the "O_2 consumption paradox" (3). Several mechanisms have been proposed to explain stunning. These include the generation of oxygen free radicals, sarcoplasmic reticulum dysfunction leading to excitation-contraction uncoupling, and calcium overload (4, 6, 8, 16). Experimental evidence exists to demonstrate that stunning can be alleviated by inotropic interventions (1, 5, 16, 18). These agents either increase intracellular calcium levels or calcium sensitivity.

The intracoronary administration of methylene blue to stunned hearts resulted in decreased cyclic GMP and significant increases in peak force and contractility (2). However, methylene blue led to increased contractile delay and systolic bulging and a reduction in systolic regional work, increasing the degree of myocardial stunning. These findings suggested that although depressed contractility is present in stunned myocardium, it is not the primary cause of mechanical dysfunction (2). With exacerbation of myocardial stunning seen in the presence of methylene blue, which decreased cyclic GMP, the current study aimed to test whether supplementation of cyclic GMP using nitroprusside would attenuate stunning.

The second messenger cyclic GMP exerted both negative inotropic and metabolic effects on the heart. Some evidence exits to suggest that the negative inotropic effects of cyclic GMP are mediated through inhibition of L-type calcium channels (13). Recent work in our laboratory suggests that cyclic GMP functional effects may not be mediated directly *via* L-type calcium channels (12), though several authors have demonstrated an inverse relationship between cyclic GMP and intracellular Ca^{2+} levels (19, 20). The negative inotropic effects of cyclic GMP could also be related to changes in cyclic AMP through effects of the cyclic GMP–affected cyclic AMP phosphodiesterases or changes in protein phosphorylation (10, 14, 19). It has also been suggested that nitric oxide can reduce myocardial O_2 consumption through direct effects on mitrochondrial respiration (21, 22). We felt that manipulation of myocardial cyclic GMP concentrations might affect the myocardial response to stunning through one of these mechanisms.

Our findings confirmed the achievement of regional myocardial stunning through transient ischemia and reperfusion. Significant increases in the time delay to maximal regional shortening and time to maximal developed force were seen, in addition to a significant decrease in percentage of left ventricular work during systole. However, each of these parameters was reversed in the presence of nitroprusside infusion following stunning. This was accompanied by an increase in the myocardial concentration of cyclic GMP. These data and our previous publication (2) showed that

reducing cyclic GMP worsened the degree of myocardial stunning and suggested that the level of cyclic GMP may affect the degree of myocardial stunning. However, Ehring *et al.* (23) found no effect of nitric oxide synthase blockade with NG-nitro-L-arginine methylester (L-NAME) on the degree of myocardial stunning. This may be related to the minimal effects of endogenous nitric oxide production on myocardial function, O_2 consumption, and cyclic GMP levels under basal conditions (24, 25).

We demonstrated a reversal of regional functional myocardial stunning following intracoronary nitroprusside administration. Most studies demonstrated that stunning could be alleviated by positive inotropic interventions (1, 5, 16, 18). These studies also showed a reduction in the time of contraction. We used nitroprusside-induced generation of nitric oxide to increase myocardial cyclic GMP in stunned myocardium and found it caused a reversal of mechanical stunning in terms of decreased time delay of regional shortening, reduction of time to maximal developed force and time of relaxation. Thus, despite not greatly affecting total regional work, nitroprusside caused a shift in the total work done into the period of ventricular systole. There have been several reports that nitric oxide may protect against the late effects of myocardial stunning (26, 27). However, this is the first report that nitroprusside reverses early stunning. We did not find any metabolic effect of administration of nitroprusside after stunning. This differs from some reports of reduction in myocardial O_2 consumption with nitric oxide (21, 22). This difference may be related to the stunning.

Both L-type calcium channel blockers and agents that increase local adenosine levels have been claimed to have beneficial effects on myocardial stunning (9, 28–30). Some evidence has suggested that this protection might be related to reduced calcium levels. However, protection was not observed under all circumstances. In general, the doses employed did not significantly depress myocardial function. This is somewhat similar to our reported effects of nitroprusside, where local function was not significantly depressed. One possible mechanism by which increased cyclic GMP levels may cause attenuation of myocardial stunning is through decreased intracellular Ca^{2+} concentrations, resulting in reversal of myocardial stunning related to intracellular calcium overload.

In summary, the current findings indicate that regional myocardial stunning caused by transient ischemia and reperfusion can be reversed through nitroprusside administration that increases cyclic GMP, decreases contractile delay, increases the proportion of work during systole, and reduces time of shortening. These findings demonstrate a reversal of stunning using an agent that does not increase myocardial function. Thus, both agents that increase cyclic GMP and positive inotropes have been shown to attenuate myocardial stunning through decreased duration of contraction and a shift in the proportion of local segment work performed during systole.

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