MYOCARDIAL STUNNING: A THERAPEUTIC CONUNDRUM

Dobutamine and pyruvate are two inotropic agents with different mechanisms of action. Although both agents alter postischemic myocardial dysfunction, their potential metabolic effects in the setting of in vivo myocardial stunning have not been addressed. In this study, the effects of dobutamine and pyruvate on systolic wall thickening, myocardial phosphorylation potential index, interstitial fluid adenosine level, and myocardial oxygen consumption in in vivo stunned porcine myocardium were assessed. Stunning was induced with a 10-minute occlusion of the left anterior descending coronary artery. After 30 minutes of reperfusion, pigs were treated with either intravenous dobutamine (10 μ g/kg per minute) or intracoronary pyruvate (1 ml/min, 150 mmol/L solution, pH 7.4). Infusion of both agents resulted in a marked improvement in regional systolic wall thickening. The dobutamine effect, however, produced a marked increase in myocardial oxygen consumption and was associated with an increase in interstitial adenosine caused by myocardial de-energization, because the myocardial phosphorylation potential index ratio decreased from 0.17 ± 0.02 to 0.09 ± 0.02 ($p < 0.05$). In contrast, pyruvate enhanced myocardial energy status, because the myocardial phosphorylation potential index ratio increased from 0.20 ± 0.03 to 0.55 ± 0.08 ($p < 0.01$). These experimental findings suggest that under certain circumstances the use of β -receptor agonists to treat myocardial stunning may be suboptimal, if not undesirable. Further investigation is warranted to determine the optimum therapy for the stunned heart. (*J THORAC CARDIOVASC SURG* 1995;110:1391-1401)

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Reversible myocardial contractile dysfunction after a short period of ischemia is referred to as myocardial stunning. Although difficult to quantify, it is known that myocardial stunning occurs after coronary thrombolysis and heart operations.¹ Stunned myocardium can persist for hours to days, thus inotropic support is often needed during this critical period, particularly when there is an associated

limitation in myocardial reserve. Although the underlying mechanisms are still a topic of controversy, substantial evidence suggests that myocardial stunning may be related to ischemic calcium overload,² impaired calcium handling by the sarcoplasmic reticulum,³ and reduced calcium sensitivity of contractile proteins during reperfusion.⁴ These pathophysiologic changes provide the basis for current inotropic therapy with agents such as dobutamine and dopamine, which increase contractility by increasing intracellular calcium levels.

Although dobutamine increases contractility in the stunned myocardium, other effects associated with its administration may not be beneficial. The inotropic effect of dobutamine has been reported to be associated with low energy efficiency (oxygen-wasting effects) in isolated and normal human hearts.^{5,6} This effect may be exacerbated in stunned myocardium because sarcoplasmic reticulum Ca^{2+} adenosinetriphosphatase (ATPase) activity is re-

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duced in the postischemic heart.³ In addition, β -adrenergic agonists further reduce myofibril calcium sensitivity, which is thought to be depressed in stunned myocardium.⁴

The inotropic effect of dobutamine is associated with increased myocardial adenosine release in *in vivo* experiments,⁷ which suggests that treatment with this agent reduces the oxygen supply versus demand ratio. Increased adenosine release is inversely related to myocardial phosphorylation potential, which monitors the Gibbs free energy change of ATP hydrolysis.⁸ Gibbs free energy serves as the driving force for energy-dependent ATPases, such as the sarcoplasmic reticulum Ca^{2+} -ATPase, which has the highest energy demand. Myocardial phosphorylation potential is primarily determined by the $[\text{ATP}]/[\text{ADP}] \cdot [\text{P}_i]$ ratio, where brackets indicate the free intracellular concentration of these metabolites (ADP, adenosine diphosphate; P_i , inorganic phosphate). Because of the difficulty of directly measuring tissue free ADP content, the myocardial phosphorylation potential index ($\text{CrP}/\text{Cr} \cdot \text{P}_i$) ratio has been accepted as an index of myocardial phosphorylation potential (CrP, creatine phosphate; Cr, creatine; P_i , inorganic phosphate).^{9,10} Because dobutamine increases interstitial adenosine concentration, it is possible that dobutamine decreases phosphorylation potential. Although norepinephrine has been reported to decrease phosphorylation potential in isolated guinea pig hearts,¹⁰ the effect of dobutamine on phosphorylation potential in *in vivo* stunned myocardium has not been determined.

Pyruvate has also been shown to be an effective positive inotropic agent in normal and stunned myocardium.^{10,11} Pyruvate is an excellent energy substrate that exerts its inotropic effects, not by receptor activation, but presumably by altering cytosolic or mitochondrial redox potential.¹⁰⁻¹² The combination of enhancing myocardial phosphorylation potential and contractility may be valuable in the treatment of stunned myocardium, which may have impaired energy metabolism. The purpose of this study was to evaluate the different effects of dobutamine and pyruvate in *in vivo* stunned myocardium and to assess their effects on myocardial phosphorylation potential.

Methods

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals"

prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Animal preparation. Domestic pigs of either sex weighing 25 to 35 kg were used. Anesthesia was induced with ketamine (30 mg/kg, intramuscularly), followed by sodium pentobarbital (15 mg/kg, intravenously through an ear vein). Anesthesia was then maintained with sodium pentobarbital (10 to 15 mg/kg per hour). A tracheostomy was done and the lungs were mechanically ventilated with a mixture of room air and 100% O_2 . Tidal volume, respiratory rate, and percent oxygen in the inspired air were adjusted to maintain normal arterial blood gas and pH values. Core body temperature was monitored with an esophageal temperature probe and maintained at 38.0° to 39.0° C with a heating pad. The right femoral artery was cannulated for withdrawal of samples for blood gas determinations and for monitoring blood pressure. The femoral vein was cannulated for the infusion of fluids and for the maintenance of anesthesia.

The anterior chest wall was removed, the pericardium incised, and the heart suspended in a pericardial cradle. Left ventricular pressure was measured with a Millar catheter (Millar Instruments Inc., Houston, Tex.) placed through the ventricular wall in the apical region. Changes in left ventricular pressure with respect to time (dP/dt) were determined by differentiation of left ventricular pressure. In preparation for coronary artery occlusion, a 2 cm portion of the proximal region of the left anterior descending coronary artery (LAD) was dissected and a modified 24-gauge angiographic catheter was inserted into the LAD distal to the occluder with the tip facing downstream. Coronary blood flow was measured with an ultrasonic flow probe (Transonic Systems Inc., Ithaca, N.Y.) placed around the proximal portion of the LAD distal to the occluder.

Regional ventricular function. Systolic wall thickening (SWT) was used as an indicator of regional ventricular function. Sonomicrometric measurements of left ventricular wall thickness were made with pairs of piezoelectric crystals. One of the crystals was inserted at a 45-degree angle into the endocardium (ideally this crystal ends up situated in the innermost endocardial layer) and the second crystal, which is incorporated into a Dacron fabric patch, was sewn onto the epicardium at the location indicating shortest distance between the two crystals. The position of the crystal in the ischemic area was verified by a 40-second occlusion of the LAD. Pairs of crystals were placed in both the LAD and left circumflex coronary artery perfused beds, the latter area serving as a nonischemic time control.

Cardiac microdialysis. Interstitial fluid adenosine, inosine, and hypoxanthine levels were measured with a microdialysis fiber developed in this laboratory.⁷ The dialysis fiber has a 2.0 cm exchange window and molecular cutoff size of 2000 dalton. Dialysis fibers were placed in the LAD and left circumflex coronary artery perfused beds. The dialysis fiber was perfused at 2 $\mu\text{l}/\text{min}$ with Krebs buffer (gassed with 5% CO_2 and 95% N_2 to minimize O_2 delivery). Samples were collected over a 10-minute period, except during ischemia when two 5-minute samples were collected. Samples were stored at

-80°C before analysis. Dialysate purine concentrations were determined by high-performance liquid chromatography as previously described.⁷

Determination of myocardial tissue metabolites. To determine tissue ATP, creatine phosphate, creatine, and inorganic phosphate levels, biopsy samples were taken by means of a rapid-freezing biopsy drill (Alko Diagnostics Co., Holliston, Mass.).¹³ The biopsy system consisted of an air-driven drill, a 2 mm diameter bore, and a vacuum line at -20 mm Hg pressure to cut and pull a 20 to 60 mg transmural myocardial sample into a reservoir of isopentane (approximately -100°C) precooled by liquid nitrogen, such that the sample is cut and frozen in less than 1 second. Immediately after the biopsy sample was obtained, the reservoir was checked to verify that the tissue plug was frozen in the isopentane. Only samples that were cut and frozen instantly in the isopentane were used for analysis. Frozen samples were stored at -80°C until they were processed. The first biopsy sample was taken in the apical region to avoid altering blood supply to the proximal region where the second biopsy sample was taken. The distance between the biopsy sites was approximately 1 cm.

Frozen samples were trimmed to a size of approximately 30 mg, rapidly weighed, then homogenized in 2.0 ml 0.3N ice-cold perchloric acid solution with use of a Teflon rod (Glas-Col Instruments, Terre Haute, Ind.). The acid homogenates were centrifuged for 10 minutes at 10,000 rpm (approximately 9200 g), and 1.5 ml of the resulting supernatant was neutralized with 1N KOH. The pH was adjusted to 5.5 to 6.0 with a pH electrode, which was rinsed between samples with deionized distilled water. After 30 minutes on ice, the potassium perchlorate was removed by centrifugation and the supernatant stored at -80°C .

Creatine phosphate and ATP were measured by a modified ion pair reverse-phase high-performance liquid chromatography method as described by Cordis.¹⁴ Neutralized extracts were injected into a C_{18} reverse-phase column and eluted with a linear gradient of 100% buffer A (75 mmol/L KH_2PO_4 plus 2 mmol/L tetrabutylammonium hydrogen sulfate, pH 5.5) to 40% buffer B (50% acetonitrile) in 15 minutes at a flow rate of 2.0 ml/min. Peaks were quantified by peak area with the use of MAXIMA 820 software (Waters Instruments, Milford, Mass.). Myocardial adenine nucleotide contents were expressed as micromoles per gram wet weight.

Inorganic phosphate was determined by a standard enzymatic method as previously described by Bünger and associates.¹⁵ This method is based on the stoichiometric phosphorylation of glycogen by inorganic phosphate by means of glycogen phosphorylase to yield glucose-1-phosphate, which is then converted to glucose-6-phosphate. The oxidation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase results in the reduction of the oxidized form of nicotinamide-adenine dinucleotide phosphate; the increase in absorbance difference at 340 nm caused by the formation of the reduced form of nicotinamide-adenine dinucleotide phosphate is directly proportional to the amount of inorganic phosphate present. Freeze-thawing of extracts, which is known to cause nonenzymatic creatine phosphate breakdown, was limited

to 1 to 2 cycles to minimize artifact increases in creatine and inorganic phosphate levels.

Creatine content was determined by a standard enzymatic method described by Bünger and associates.¹⁵ Creatine kinase in the presence of ATP catalyzes the conversion of creatine to creatine phosphate and ADP, which is then converted to ATP and pyruvate in the presence of phospho(enol)pyruvate and pyruvate kinase. Pyruvate is converted to lactate via lactate dehydrogenase; the oxidation of nicotinamide-adenine dinucleotide phosphate to the oxidized form of nicotinamide-adenine dinucleotide and the resulting decrease in absorbance at 340 nm is proportional to the amount of creatine.

General protocol. After instrumentation, the preparation was stabilized for a minimum of 30 minutes. In the animals ($n = 14$) in which dialysis fibers were used, 90 minutes of equilibration was allowed after fiber insertion to allow dialysate purine levels to stabilize. After baseline hemodynamic and metabolic data were obtained, myocardial ischemia was induced by 10 minutes of complete LAD occlusion. Lidocaine (3 mg/kg) was given intravenously before the clamp was released to reduce the occurrence of ventricular fibrillation. After 30 minutes of reperfusion, either intracoronary sodium pyruvate (1 ml/min, 150 mmol/L, pH 7.4) or intravenous dobutamine (10 $\mu\text{g/kg}$ per minute) was infused for 10 minutes and hemodynamic effects were recorded. The treatment was then terminated and the animals were monitored for an additional 20 minutes.

Series 1. In this series of experiments, the effects of dobutamine ($n = 7$) and pyruvate ($n = 7$) on hemodynamics and interstitial purines were assessed. Baseline hemodynamic measurements and dialysate purine samples were collected during the 10 minutes immediately preceding coronary occlusion. Two 5-minute dialysate samples were taken during ischemia, and reperfusion samples were collected every 10 minutes for 1 hour of reperfusion. A small catheter was inserted into the great cardiac vein draining the LAD perfused bed to obtain coronary venous blood samples during the treatments for the determination of myocardial oxygen consumption. The venous samples were analyzed by a Ciba-Corning 288 blood gas system (Medfield, Mass.) for oxygen tension, carbon dioxide tension, HCO_3^- , and oxygen content.

Series 2. Biopsy samples were obtained in a separate series of experiments ($n = 7/\text{group}$) to avoid biopsy drill-induced alterations in function and interstitial metabolites. Biopsy samples were taken from the LAD perfused area after 30 minutes of reperfusion before either dobutamine or pyruvate infusion. Twenty minutes were allowed to let the animal recover from the transient regional trauma associated with the biopsy drill. Intravenous dobutamine (10 $\mu\text{g/kg}$ per minute) or intracoronary pyruvate (1 ml/min, 150 mmol/L, pH 7.4) was then infused and biopsy samples were taken after 10 minutes of infusion.

At the conclusion of both series of experiments, the animals were killed with an intravenous injection of sodium pentobarbital and saturated KCl. The hearts were removed, and the placement of the crystals and dialysis fibers was carefully examined. The ischemic area was

Table 1. Effects of dobutamine and pyruvate on systemic hemodynamics and LAD blood flow

	HR (beats/min)	MAP (mm Hg)	LVEDP (mm Hg)	CBF (ml/min)
Dobutamine				
Baseline	95 ± 4	83.4 ± 2.8	4.6 ± 1.1	17.3 ± 1.5
30' RP	95 ± 4	79.8 ± 3.8	5.3 ± 1.8	18.4 ± 1.4
10' DOB	149 ± 14*	79.8 ± 4.0	4.7 ± 1.4	28.7 ± 1.7*
20' Recovery	94 ± 3	76.5 ± 3.1	5.1 ± 1.5	16.7 ± 1.3
Pyruvate				
Baseline	102 ± 10	83.4 ± 2.8	4.0 ± 1.4	17.6 ± 1.2
30' RP	105 ± 9	79.8 ± 3.8	4.1 ± 1.4	14.4 ± 1.2
10' PYR	105 ± 9	79.7 ± 4.0	3.4 ± 1.3	15.7 ± 1.2
20' Recovery	110 ± 10	76.5 ± 3.1	3.3 ± 1.6	15.1 ± 1.2

HR, Heart rate; MAP, mean arterial blood pressure; LVEDP, left ventricular end-diastolic pressure; CBF, coronary blood flow; Baseline, before ischemia; 30' RP, 30 minutes of reperfusion; 10' DOB, 10 minutes of intravenous dobutamine infusion; 10' PYR, 10 minutes of intracoronary pyruvate infusion; 20' Recovery, 20 minutes after cessation of drug administration. Values are expressed as mean plus or minus standard error of the mean.

* $p < 0.05$ versus 30 minutes of reperfusion.

confirmed by injection of India ink into the LAD at the site of occlusion.

Data analysis. Hemodynamic data were recorded on an 8-channel recorder. For the computation of SWT, end diastole was defined as the onset of positive dP/dt and end systole was taken as 20 msec before peak negative dP/dt . From the measurements of end-systolic wall thickness (ESWT) and end-diastolic wall thickness (EDWT), wall thickness changes were calculated as $\Delta WT = ESWT - EDWT$ and expressed as relative wall thickening by the formula $\%WT = \Delta WT/EDWT \cdot 100\%$. Values of post-ischemic relative wall thickening were then normalized to percent of preischemia levels. Myocardial oxygen consumption was calculated as (arterial O_2 content - venous O_2 content) $\times$ (LAD blood flow).

Statistical analysis. Values are expressed as mean plus or minus the standard error of the mean. The data were first analyzed by multivariate repeated analysis of variance. Differences between the time points were compared with linear contrast. All calculations were done with the SAS procedure PROC GLM (SAS Institute, Inc., Cary, N.C.). A p value of less than 0.05 was considered as significant.

Results

In series 1 experiments, heart rate, mean arterial blood pressure, left ventricular end-diastolic pressure, and LAD blood flow were similar in both groups before ischemia and after 30 minutes of reperfusion (Table I). After 10 minutes of intravenous infusion, dobutamine significantly increased heart rate and LAD blood flow, but had no effect on mean arterial blood pressure or left ventricular end-diastolic pressure. Heart rate and coronary blood flow returned to preinfusion levels after a 20-minute washout of dobutamine. In contrast, 10 minutes of intracoronary pyruvate infusion had no significant effects on systemic hemodynamics.

Regional ventricular function. The effects of ischemia, dobutamine, and pyruvate on SWT are

illustrated in Fig. 1. Before ischemia there were no differences between the two groups with respect to absolute LAD SWT ($32.3\% \pm 3.6\%$ versus $27.7\% \pm 2.4\%$). Ten minutes of LAD occlusion and 30 minutes of reperfusion resulted in similar decreases in SWT: $31.2\% \pm 1.0\%$ and $30.0\% \pm 1.2\%$ of pre-ischemic values, respectively. Regional function in the nonischemic left circumflex artery perfused bed remained stable throughout the ischemia-reperfusion period. After 30 minutes of reperfusion left circumflex artery SWT was $91.3\% \pm 3.8\%$ and $94.2\% \pm 4.5\%$ of baseline values in the dobutamine- and pyruvate-treated animals, respectively.

Postischemic intravenous dobutamine infusion increased SWT in the stunned myocardium from $31.2\% \pm 1.0\%$ to $57.3\% \pm 5.4\%$ of baseline. Twenty minutes after cessation of the dobutamine infusion, the SWT value was lower than preinfusion values: $21.7\% \pm 2.3\%$ versus $31.2\% \pm 1.0\%$ ($p < 0.05$). Intravenous dobutamine also increased SWT in the left circumflex artery nonischemic control region; however, 20 minutes after the dobutamine treatment was discontinued, the SWT value in the left circumflex artery bed was similar to preinfusion values ($94.8\% \pm 6.3\%$ versus $91.3\% \pm 3.8\%$ of baseline SWT).

Intracoronary pyruvate infusion resulted in a more than twofold increase in SWT ($31.0\% \pm 4.5\%$ to $75.2\% \pm 10.8\%$ of preischemic values, $p < 0.01$). During pyruvate washout, SWT levels gradually returned to, but did not fall below, preinfusion levels. Intracoronary pyruvate infusion did not alter SWT values in the left circumflex artery control bed during and after the LAD coronary infusion.

Myocardial oxygen consumption. The effects of dobutamine and pyruvate on myocardial oxygen

Fig. 1. Effects of ischemia (ISCH), reperfusion (30' RP), and intravenous dobutamine (DOB) and intracoronary pyruvate (PYR) infusions on SWT. SWT is expressed as percent of baseline (Base) values before ischemia. Values are expressed as mean plus or minus standard error of mean. * $p < 0.05$ versus reperfusion.

consumption are illustrated in Fig. 2. The myocardial oxygen consumption was the same for both groups before ischemia (7.3 ± 0.2 versus 7.1 ± 0.2 ml/min per 100 gm). Ischemia and reperfusion did not significantly decrease myocardial oxygen consumption in either group although regional contractile function was reduced by approximately 70%. The inotropic effect of dobutamine was associated with an increase in myocardial oxygen consumption from 7.2 ± 0.2 to 12.0 ± 0.4 ml/min per 100 gm ($p < 0.05$). On cessation of the dobutamine infusion, the myocardial oxygen consumption returned to preinfusion levels within 20 minutes. In contrast to that with dobutamine, the inotropic effect of pyruvate was associated with little change in myocardial oxygen consumption (from 6.7 ± 0.3 to 7.2 ± 0.2 ml/min per 100 gm).

Dialysate adenosine levels. Preischemic dialysate adenosine levels were similar in both groups. Dialysate adenosine concentration increased more than 20-fold during ischemia in both groups (14.91 ± 3.05 μ mol/L and 13.65 ± 3.53 μ mol/L). During 10 minutes of dobutamine infusion in the stunned heart, dialysate adenosine concentrations increased from 0.47 ± 0.05 to 0.75 ± 0.09 μ mol/L (Fig. 3, $p < 0.05$).

Dialysate inosine levels increased from 1.75 ± 0.28 to 2.09 ± 0.3 μ mol/L, and hypoxanthine levels increased from 11.46 ± 0.16 to 13.09 ± 0.19 μ mol/L. After a 20-minute washout, adenosine levels returned to preinfusion values. Intracoronary pyruvate infusion did not increase dialysate adenosine (0.58 ± 0.08 versus 0.57 ± 0.07 μ mol/L), inosine, or hypoxanthine levels.

Myocardial phosphorylation potential. The effects of dobutamine and pyruvate on the CrP/Cr · P_i ratio, which was used as an index of the myocardial phosphorylation potential, are illustrated in Fig. 4. Both groups had comparable CrP/Cr · P_i ratios and total tissue ATP content after 30 minutes of reperfusion. In the stunned heart, intravenous dobutamine infusion decreased the CrP/Cr · P_i ratio from 0.17 ± 0.02 to 0.09 ± 0.02 . Dobutamine did not alter venous pH (7.33 ± 0.01 versus 7.34 ± 0.02), carbon dioxide tension levels (56.22 ± 2.72 versus 54.8 ± 4.32 mm Hg), HCO₃⁻ levels (33.2 ± 0.6 versus 31.8 ± 1.2 mmol/L), or tissue ATP content (3.84 ± 0.27 μ mol/gm wet weight before infusion versus 3.79 ± 0.26 μ mol/gm wet weight after infusion).

In contrast to dobutamine, intracoronary pyruvate infusion resulted in more than a twofold in-

Fig. 2. Myocardial oxygen consumption (MVO_2) at baseline (*Base*), reperfusion (*30' RP*), intravenous dobutamine (*DOB*) administration, intracoronary pyruvate (*PYR*) infusion, and 20 minutes of recovery. Myocardial oxygen consumption is expressed as milliliters per minute per 100 gm left ventricular tissue. Values are expressed as mean plus or minus standard error of mean. * $p < 0.05$ versus reperfusion.

crease in the $CrP/Cr \cdot P_i$ ratio from 0.20 ± 0.03 to 0.55 ± 0.08 . The increase in the $CrP/Cr \cdot P_i$ ratio was not associated with measurable changes in venous pH (7.35 ± 0.04 versus 7.34 ± 0.01), HCO_3^- level (32.1 ± 0.7 versus 34.1 ± 1.06 mmol/L), carbon dioxide tension level (59.9 ± 1.1 versus 62.0 ± 1.5 mm Hg), or tissue ATP content (3.56 ± 0.22 versus 3.69 ± 0.36 μ mol/gm wet weight).

Discussion

The results of this study indicate that the positive inotropic effect of dobutamine in the stunned heart is associated with increased myocardial adenosine release and decreased phosphorylation potential. In contrast, the positive inotropic effect of pyruvate had no effect on interstitial adenosine levels and significantly increased the phosphorylation potential. These findings suggest that dobutamine, in contrast to pyruvate, increases function in stunned myocardium at the expense of myocardial energetics.

The rationale for treating the stunned heart with positive inotropic agents such as dobutamine is based on the fact that postischemic dysfunction may be related to decreased myofibrillar Ca^{2+} sensitivity.⁴ The inotropic effect of dobutamine is mediated by the β -adrenergic receptor-induced increase in adenyl cyclase activity and a concomitant increase

in cytosolic calcium level¹⁶ and increased phosphorylation of regulatory and contractile proteins.¹⁷ However, the metabolic effects associated with such mechanisms may not be beneficial to the stunned heart. The inotropic effect of dobutamine is associated with a considerable increase in oxygen consumption and markedly elevated interstitial adenosine levels, which indicate enhanced metabolic stress on the cardiomyocytes.^{7,18} These effects may de-energize already jeopardized stunned myocardium, which exhibits elevated oxygen consumption relative to its depressed contractile state.¹⁹ Dobutamine also increases intracellular Ca^{2+} at a time when the stunned heart may have a reduced ability to regulate intracellular Ca^{2+} , that is, when it has reduced sarcoplasmic reticulum Ca^{2+} -ATPase activity.³ Moreover, the potential effects of dobutamine on reducing calcium sensitivity may be another undesirable side effect associated with its inotropic effect, because stunning itself is associated with reduced myofibrillar Ca^{2+} sensitivity.⁴

Myocardial phosphorylation potential and contractile function. The energy that is available to intracellular ion pumps, contractile proteins, and other ATP hydrolytic reactions is determined by the myocardial phosphorylation potential. This parameter monitors the Gibbs free energy change of ATP hydrolysis as shown in the equation

Fig. 3. Effects of intravenous dobutamine (DOB) and intracoronary pyruvate (PYR) on dialysate adenosine concentration ([ADO]). Ten-minute dialysate samples were collected before ischemia (Base), at 20 to 30 minutes of reperfusion (30' RP), during 10-minute dobutamine or pyruvate infusions, and during 10 to 20 minutes of recovery from infusions. Values (micromoles) are expressed as mean plus or minus standard error of mean. * $p < 0.05$ versus reperfusion.

$$G_{\text{ATP}} = G_{\text{ATP}}^0 + RT \cdot \ln \frac{[\text{ADP}] [\text{P}_i]}{[\text{ATP}]}$$

where ΔG^0 is the standard free energy change of ATP hydrolysis, R is gas constant, and T is absolute temperature.

Under many conditions, because ΔG^0 is relatively constant, the main determinant of the equation is the $[\text{ATP}]/[\text{ADP}] \cdot [\text{P}_i]$ ratio. Direct measurement of cellular myocardial phosphorylation potential is complicated by the fact that intracellular ADP is primarily bound to myofibrils and compartmented between cytosol and mitochondria.^{20, 21} However, the $\text{CrP}/\text{Cr} \cdot \text{P}_i$ ratio can be accepted as an index for cytosolic phosphorylation potential under various conditions as shown in the equation

$$\frac{[\text{ATP}]}{[\text{ADP}] \cdot [\text{P}_i]} = \frac{[\text{CrP}]}{[\text{Cr}] \cdot [\text{P}_i]} \cdot \frac{[\text{H}^+]}{K_{\text{eq}}}$$

where K_{eq} is the pH and Mg^{2+} dependent equilibrium constant of creatine kinase. This equation is based on the creatine kinase reaction at equilibrium.²⁰ Measurement of levels of creatine phosphate, creatine, and inorganic phosphate can be accom-

plished by measuring the respective total tissue contents because creatine phosphate, creatine, and the bulk of inorganic phosphate are located primarily in the cytosol.²¹

Although it has been reported that isolated perfused hearts exhibit lower phosphorylation potential after ischemia,¹⁰ it is not known what role myocardial phosphorylation potential plays in in vivo myocardial stunning or what effects inotropic agents have on phosphorylation potential under such conditions. The potential side effects of β -adrenergic receptor agonists used in the management of post-ischemic dysfunction have been studied by several investigators; however, their effects on myocardial phosphorylation potential have not been fully addressed. Bolli and associates²² tested isoproterenol (0.1 $\mu\text{g}/\text{kg}$ per minute) for 30 minutes in stunned myocardium in regard to regional function; however, they did not measure high-energy phosphates. Arnold and associates²³ reported that infusion of dopamine for 3 hours did not increase infarct size after 2 hours of ischemia and 45 minutes of reperfusion. Although they reported no change in ATP and creatine phosphate content as a result of dobut-

Fig. 4. CrP/Cr·Pi ratio at 30 minutes of reperfusion (30' RP) and during 10 minutes of intravenous dobutamine (DOB) or intracoronary pyruvate (PYR) infusion. Values are expressed as mean plus or minus standard error of mean. * $p < 0.05$ versus reperfusion.

amine infusion, they did not measure creatine and inorganic phosphate contents, which are crucial to the assessment of myocardial phosphorylation potential. In accord with our data, Buser and colleagues,²⁴ with the use of noninvasive ³¹P nuclear magnetic resonance techniques, reported that dobutamine administration decreased the CrP/Cr·Pi ratio. Thus it is unlikely that the observed CrP/Cr·Pi decrease observed in this study was an artifact of the multiple biopsy protocol; indeed biopsy samples were first taken from the apical region such that the blood supply to the proximal regions, from which subsequent biopsy samples were taken, was not compromised.

Although Bolli and associates²² reported that regional function in stunned canine myocardium did not deteriorate after cessation of isoproterenol infusion, in this study regional function in stunned porcine myocardium decreased to less than preinfusion levels after cessation of dobutamine treatment. It is likely that regional function would have returned to levels similar to those before dobutamine infusion, but we only monitored the animals for 20 minutes after dobutamine administration. Because SWT is preload and afterload dependent, it is possible that this effect was not a true decrease in contractility. However, the observations that dobutamine increased interstitial fluid adenosine levels

and decreased phosphorylation potential suggest that this effect may have been related to the additional energetic stress caused by dobutamine in the stunned heart. The reduction after dobutamine infusion in SWT occurred only in the stunned LAD bed, inasmuch as SWT in the nonischemic left circumflex artery bed returned to preinfusion levels after intravenous dobutamine infusion. Pyruvate infusion increased contractile function comparable to that with dobutamine, but SWT did not deteriorate after cessation of the pyruvate infusion, most likely because of the salutary effect of this agent on myocardial phosphorylation potential.

The exact link between myocardial phosphorylation potential and contractile function remains unknown. Enhanced myocardial energetics benefit all intracellular energy-dependent reactions such as ion pumps, contractile proteins, and metabolic synthetic proteins. The calculated minimum energy required to maintain intracellular calcium homeostasis is about 52 kJ/mol, and normal physiologic ΔG_{ATP} is about 60 kJ/mol. Mallet and Bünger²⁵ recently reported that the inotropic effect of pyruvate in isolated perfused guinea pig hearts was associated with improved sarcoplasmic reticulum calcium handling. Therefore, considering the decreased sarcoplasmic reticulum function and impaired energy metabolism in stunned myocardium, pyruvate may

be of value in the treatment of myocardial stunning on the basis of its beneficial effects on both function and energy metabolism.

Limitations. One potential limitation in using the $\text{CrP/Cr} \cdot \text{P}_i$ ratio to assess myocardial phosphorylation potential is that creatine kinase depends on intracellular pH and Mg^{2+} .^{10, 20} The stability of the regional venous pH, HCO_3^- , and carbon dioxide tension levels during our protocols implies relatively stable intracellular pH level. Moreover, that ATP, the main chelator of $[\text{Mg}^{2+}]_i$, did not change during the dobutamine and pyruvate infusions suggests that there were no major alterations in $[\text{Mg}^{2+}]_i$.

Another limitation in this study was the assessment of regional ventricular function by SWT, which is dependent on preload, afterload, and heart rate. Because intracoronary pyruvate had no effect on any of these parameters (Table I) it is likely that the increase in LAD SWT with pyruvate was indicative of increased contractility. Dobutamine initially increased arterial pressure, but after 10 minutes of infusion the only systemic effect of dobutamine was an increase in heart rate, an effect that would decrease SWT. The decrease in SWT after termination of the dobutamine infusion was most likely because of an imbalance in oxygen supply and demand as evidenced by the increase in interstitial adenosine and the decrease in phosphorylation potential. Although it is likely that this was only a transient effect and may have been caused by the dobutamine-induced increase in heart rate, we have observed in similar studies that lower doses of dobutamine that have little effect on heart rate also decrease myocardial phosphorylation potential.

Finally, it must be pointed out that the results in this study provide no insight into the relative potency of pyruvate versus dobutamine, because these agents were infused via different routes; that is, dobutamine intravenously versus pyruvate by intracoronary infusion. Intracoronary infusion of dobutamine caused asynchronous wall motion in the area of interest, which has been observed in other in vivo regional ischemic models.²⁶ Pyruvate was infused locally to minimize potential confounding effects of high systemic levels of pyruvate, which is an area not well explored at the present.

Conclusion. The results from this study highlight the dilemma in the treatment of myocardial stunning with dobutamine. Dobutamine is one of the most potent inotropes clinically available, but its possible deleterious effects on myocardial energetics

are of major concern. The findings in this study emphasize the need to elucidate the optimal inotropic therapy for the stunned heart.

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Discussion

Dr. Sidney Levitsky (*Boston, Mass.*). I am particularly pleased with this report because it substantiates a personal, long-held hypothesis that all inotropic agents presently approved by the Food and Drug Administration administered after cardiac operation are potentially deleterious to myocardial function because they increase myocardial oxygen consumption during the period of cell membrane instability associated with reperfusion-induced myocardial stunning. These adverse effects are potentiated in the patient undergoing coronary revascularization who, despite our attempts at total revascularization, has regional areas that are incompletely revascularized and energetically deficient.

I have several questions. Are you able to discriminate whether the increase in myocardial oxygen consumption noted in the dobutamine group was associated with an increase in the contractile state of the heart or was related to an increase in the heart rate that was not noted with the pyruvate group? This question could have been clarified if the authors had blocked the sinoatrial or the atrioventricular node in the experimental preparation and controlled the heart rate.

Dr. Pedro del Nido and his associates in 1992 performed a similar experiment with use of a Langendorff-perfused model and did not find depression of myocardial function after dobutamine infusion. Was this related to differing function measurements because they used a rate-pressure product and you used systolic wall thickening, or was this related to a change in the geometric configuration of the left ventricular cavity particularly in the left circumflex artery area, which was the control group? Similar to the present report, del Nido found that pyruvate improved the energetic state of the heart compared with results with dobutamine.

Finally, there appears to be increasing experimental evidence that pyruvate may be the inotropic drug of choice to treat myocardial stunning after operation. Do you know of any potential adverse effects that would prevent its clinical use?

Dr. Zhou. In response to the first question, it is probable that the increase in myocardial oxygen consumption during dobutamine infusion was caused in part by the approximately 50% increase in heart rate. However, in additional studies in the stunned heart we have observed that lower doses of dobutamine, which have little effect on heart rate, decrease myocardial phosphorylation potential, suggesting that myocardial oxygen consumption increased. With respect to the second question regarding functional depression after cessation of dobutamine infusion, we agree that this observation may be a result of the conditions used in this study. Ventricular function was assessed by SWT and the hearts were monitored for only 20 minutes after dobutamine administration was discontinued. More recent studies in our laboratory indicate that 30 minutes after cessation of dobutamine administration, regional contractility, measured by the slope of the end-systolic pressure thickness relationship, is similar to that before dobutamine administration.

Regarding the clinical utility of pyruvate, it is not known to what extent plasma pyruvate levels need to be elevated to exert a positive inotropic effect nor what effects pro-

longed pyruvate infusion has on systemic hemodynamics or metabolism. One potential adverse effect may be alkalosis because pyruvate is cotransported across the sarcolemma with hydrogen ions.

Dr. Dimitri Novitzky (*Tampa, Fla.*). We have studied the stunned myocardium in dogs with use of pressure-volume relationships looking at the area at risk and the nonischemic myocardium with sonomicrometers. We have observed after 30 minutes of reperfusion of the heart that the dyskinetic area at risk (stunned myocardium) after triiodothyronine administration recovers to control status (before stunning). We notice also no impact of T_3 on the nonischemic myocardium. This study with the use of pressure-volume relationships shows clearly an excellent recovery of the left ventricular arterial coupling; therefore T_3 has also a significant peripheral effect.

T_3 activates the pyruvate dehydrogenase system, allowing more pyruvate to get into the mitochondria, and it also activates the adenylate nucleotide transferase, allowing ATP transfer out of the mitochondria and ADP back into it. Do you think that by increasing pyruvate availability to the mitochondria that the observed inotropic effect is an indirect one increasing substrate for the aerobic metabolism resulting in high-energy phosphate production, rather than a direct inotropic effect on the sarcomere? With administration of T_3 we have observed similar effects on the myocardium and also a synergistic effect of T_3 and the catecholamines on the beta receptors.

Dr. Zhou. We do not know at the present the exact mechanism of the positive inotropic effect of pyruvate. With respect to pyruvate dehydrogenase, it has been reported that in isolated perfused hearts, pyruvate is more

effective in attenuating stunning than activating pyruvate dehydrogenase with sodium dichloroacetate. One of our working hypotheses is that the pyruvate-induced increase in phosphorylation potential provides more energy to drive the highly energy-dependent sarcoplasmic reticulum Ca^{2+} -ATPase, which has been reported to be depressed in stunned myocardium. In fact, Mallet and Bünger recently reported that the positive inotropic effect of pyruvate in isolated guinea pig hearts was associated with increased sarcoplasmic reticulum calcium handling.

Dr. Gerald D. Buckberg (*Los Angeles, Calif.*). My question relates to whether the authors think that pyruvate is an inotropic stimulus or that it restores depleted substrate. Stated differently, are we whipping a tired horse in one situation and are we feeding one in another? I suppose that can be answered by determining whether pyruvate increased contractility of the nonischemic segment. These observations would help determine whether these results reflect a restoration of a depleted substrate from ischemia or direct inotropism by an agent that effects calcium handling in a different way.

Dr. Zhou. As stated in response to the previous question, we do not know the exact mechanism of the positive inotropic effect of pyruvate. However, it is unlikely that pyruvate is acting by merely restoring depleted substrates, because we have previously reported that pyruvate exerts similar inotropic effects in nonstunned canine myocardium. In addition, in both the normal and stunned heart, the onset of the positive inotropic effect occurs within 1 to 2 minutes after initiation of the pyruvate infusion and dissipates just as rapidly when the pyruvate infusion is terminated.

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