Effect of Pyruvate on Regional Ventricular Function in Normal and Stunned Myocardium

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The prolonged ventricular dysfunction following brief periods of coronary artery occlusion that does not produce irreversible damage has been termed the "stunned" myocardium. Although ventricular function returns to preischemic values by 1 to 7 days after reperfusion is established, inotropic therapy may be necessary to enhance contractility in the stunned heart. The purpose of this study was to determine the effect of pyruvate on ventricular function in normal and stunned myocardium. Eight chloralose/urethane anesthetized dogs were instrumented with ultrasonic crystals to measure systolic wall thickening in the left anterior descending artery (LAD) and left circumflex artery perfused regions of the left ventricle. Pyruvate (1 ml/min of 150 mM sodium pyruvate, pH 7.4) was infused directly into the LAD prior to and 30 minutes after a 10 minute LAD occlusion. Prior to LAD occlusion, LAD pyruvate infusion increased systolic wall thickening in the LAD-perfused region from 16.2% ± 4.3% to 23.4% ± 5.1% ($p < 0.05$). Thirty minutes after LAD occlusion, regional wall thickening was depressed (3.3% ± 2.6%; $p < 0.05$), which is indicative of stunned myocardium. Subsequent LAD pyruvate infusion increased wall thickening in the stunned myocardium to 12.7% ± 2.5%. The improvement of regional ventricular function was maintained only during the pyruvate infusion, as function returned to prepyruvate levels within 20 minutes after cessation of pyruvate infusion. These data indicate that pyruvate exerts a positive inotropic effect in normal and stunned myocardium. If pyruvate, a key intermediate in energy-producing pathways, exerts its inotropic effect through an enhancement of the energy state of the heart, it may have advantages over traditional inotropic agents in the treatment of postischemic contractile dysfunction.

WITH RECENT ADVANCES IN CARDIAC SURGERY, thrombolytic therapy, and angioplasty, the salvage of viable myocardium on reperfusion has been greatly enhanced. However, even when reperfusion occurs early enough to prevent irreversible damage and necrosis, postischemic function may remain depressed. This prolonged but reversible postischemic dysfunction has been termed the "stunned" myocardium.[1-3] Because a patient's ability to survive an ischemic episode may necessitate attempts to improve ventricular function in the stunned heart, several recent studies have evaluated the response of stunned myocardium to inotropic agents such as dopamine, dobutamine, isoproterenol, epinephrine, and norepinephrine.[4-10] These agents markedly improve ventricular function in stunned myocardium, demonstrating that stunned myocardium retains substantial functional reserve. However, because these agents primarily elevate oxygen requirements, prolonged treatment may be deleterious in a diseased heart. Therefore, an intervention that increases the inotropic state of the heart secondary to improvements in energy production and the myocardial energy state may be preferable to traditional inotropic therapy. Treatment with pyruvate is one such metabolic intervention that may enhance both the energy state and contractile function. Although pyruvate treatment has produced conflicting results in its ability to reduce myocardial infarct size,[11,12] it has been shown to improve function in the normoxic, hypoxic, and postischemic isolated perfused guinea pig heart,[13,14] and in the normal and moderately ischemic swine heart.[15] While the mechanism by which pyruvate enhances contractility has not been completely elucidated, its importance as a key intermediate in glycolytic and pyruvate dehydrogenase pathways suggests that it may improve function by an optimization of energy production with subsequent enhancement of the myocardial energy state.

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Because the effect of pyruvate on regional ventricular function in the stunned blood-perfused heart is unknown, the purpose of this study was to determine whether pyruvate enhances regional ventricular function in the postischemic stunned myocardium in an open-chest canine heart preparation.

Materials and Methods

Animal Preparation

Adult mongrel dogs (n = 8) of either sex weighing 20 to 30 kg were premedicated with 3 mg/kg morphine and then anesthetized with 0.5 mg/kg thiamylol i.v., followed by 70 mg/kg α-D-chloralose and 200 mg/kg urethane i.v. The animals were intubated and ventilated (Model 613 Dog Respirator; Harvard Apparatus, South Natick, MA) with a mixture of 100% oxygen and air. Tidal volume, respiratory rate, and percentage of oxygen in the inspired air were adjusted to maintain normal blood gas values (Model 170 pH/Blood Gas Analyzer; Ciba Corning Diagnostics Corp., Medfield, MA). Core body temperature was monitored with an esophageal temperature probe and maintained with a heating pad at approximately 38°C. The right femoral artery was cannulated for the measurement of arterial blood pressure and the determination of arterial blood gases. The left femoral vein was cannulated for infusion of fluids and anesthetic supplement when necessary.

A thoracotomy was performed through the fifth intercostal space and the pericardium was incised to expose the left anterior descending artery (LAD) and left circumflex artery (LC) perfused regions of the left ventricle. A Konigsberg micromanometer (Konigsberg Instruments Inc., Pasadena, CA) was inserted through the apex of the left ventricle for the measurement of left ventricular pressure and was subsequently differentiated to yield measurements of left ventricular dP/dt.

Regional ventricular function was measured sonomicrometrically from the LAD and LC perfused regions of the heart using wall thickening during systole as an index of regional ventricular function. Sonomicrometric measurements of left ventricular wall thickness were made using pairs of piezoelectric crystals and a sonomicrometer (Model 120; Triton Technology Inc., San Diego, CA). One of the crystals was placed in the subendocardium and the second crystal, incorporated into a dacron patch, was sewn onto the epicardium at the location that indicated minimum distance between the two crystals. Pairs of crystals were placed in both the LAD perfused region and the LC perfused region. The position, orientation, and depth of the crystals were assessed at the conclusion of the experiment.

The regional ischemia protocol consisted of a brief period of LAD occlusion followed by reperfusion. In preparation for the LAD occlusion, a short length of the LAD was dissected free from the surrounding tissue and a snare was passed around the vessel. A 24-gauge angiocatheter was inserted into the LAD for regional pyruvate infusion.

At the completion of the surgical preparation, a minimum of 30 minutes was allowed for stabilization prior to initiation of the protocol. Arterial blood pressure, left ventricular pressure, left ventricular dP/dt, heart rate, and wall-thickening data were recorded on an eight-channel recorder (Model 3800; Gould Inc., Cleveland, OH).

Protocol

The protocol was designed to determine the effect of LAD pyruvate infusion in normal and stunned myocardium. In these animals (n = 8), preischemia wall-thickening was determined both during control conditions and at the end of a 10-minute LAD pyruvate infusion (1 ml/min of 150 mM sodium pyruvate, pH 7.4). After the pyruvate infusion, a 20-minute recovery period was allowed prior to LAD occlusion. The LAD was occluded for 10 minutes in order to create stunned myocardium in the LAD-perfused region. After release of the LAD occlusion, 30 minutes of reperfusion in the absence of pyruvate was allowed, during which measurements were made every 10 minutes. The effect of pyruvate on regional ventricular function in stunned myocardium was then determined by a 10-minute LAD pyruvate infusion (1 ml/min of 150 mM sodium pyruvate, pH 7.4). This was followed by 20 minutes without pyruvate. The 10 minutes of LAD pyruvate infusion followed by 20 minutes without pyruvate was then repeated. The dose of LAD pyruvate was chosen based on preliminary studies indicating that 1 ml/min of 150 mM sodium pyruvate consistently improved regional ventricular function.

Calculations

For the computation of systolic wall thickening, the dP/dt recording was used to define systole, with end-diastole defined as the onset of positive dP/dt and end-systole defined as 20 msec prior to peak negative dP/dt. From the measurements of end-systolic wall thickness (ESWT) and end-diastolic wall thickness (EDWT), wall-thickness excursion was calculated as $\Delta WT = ESWT—EDWT$ and percentage wall thickening by the formula $\%WT = \Delta WT/EDWT \times 100$.

Statistical Analysis

Mean values and standard errors of the means were calculated for all the data. Differences between mean values were determined by ANOVA followed by Duncan's multiple range test for repeated measures. A $p < 0.05$ was accepted as indication of a statistically significant difference.

TABLE 1. *Hemodynamic Parameters**

Condition	MAP (mmHg)	HR (b/min)	dP/dt (% Control)
Control	103.8 ± 4.7	116.9 ± 10.9	100
10′ LAD Pyruvate Infusion	105.6 ± 4.6	116.6 ± 11.0	100.0 ± 0.0
20′ Post LAD Pyruvate Infusion	107.1 ± 4.1	121.4 ± 10.9	96.0 ± 1.6
LAD Occlusion	103.5 ± 3.3	136.3 ± 8.3	89.0 ± 5.6
10′ Reperfusion	104.2 ± 4.4	139.7 ± 8.4	84.9 ± 6.0
20′ Reperfusion	100.8 ± 4.4	139.7 ± 10.4	85.6 ± 5.1
30′ Reperfusion	96.5 ± 4.8	136.0 ± 13.3	81.9 ± 5.4
10′ LAD Pyruvate Infusion	99.4 ± 5.7	135.7 ± 13.6	81.8 ± 5.2
20′ Post LAD Pyruvate Infusion	97.8 ± 3.8	146.3 ± 10.8	84.5 ± 6.0
10′ LAD Pyruvate Infusion	87.8 ± 7.2	153.0 ± 6.7	73.7 ± 2.3
20′ Post LAD Pyruvate Infusion	86.9 ± 9.6	157.3 ± 4.9	71.0 ± 4.2

* Values expressed as mean ± SEM.

Results

The hemodynamic data is shown in Table 1. Mean arterial pressure was relatively stable throughout the protocol, although heart rate increased and dP/dt decreased gradually throughout the protocol. This is likely due to the length of time necessary to complete the experimental protocol.

An example of the sonomicrometric measurements from an individual animal is shown in Figure 1. In this example, LAD wall thickening increased during LAD pyruvate infusion in the preischemic heart from 20% to 30.6%. In the postischemic stunned heart, wall thickening was 14.6% and increased to 24.3% with pyruvate. Ventricular function in the nonischemic LC region did not change during LAD pyruvate infusion and was not different after LAD occlusion.

The average data from eight dogs is shown in Figure 2. Although average wall thickening was greater in the LAD region than in the LC region during preischemic conditions, the nonischemic LC region remained relatively stable throughout the protocol. In the preischemic period, LAD pyruvate infusion resulted in a significant increase in LAD wall thickening that was reversible at 20 minutes after the pyruvate infusion was stopped. LAD wall thickening became dyskinetic during the LAD occlusion and recovered to approximately 20% of control after 30 minutes of reperfusion. In the stunned heart, LAD pyruvate infusion resulted in a marked improvement in function, increasing wall thickening to a level that was not statistically different from control without pyruvate, but less than that during the preischemic pyruvate infusion. This inotropic effect of pyruvate was reversible and

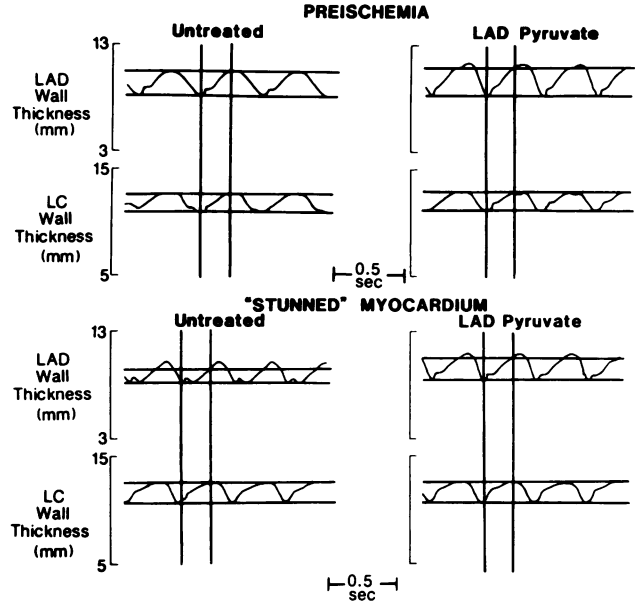

FIG. 1. Example of the effect of left anterior descending artery (LAD) pyruvate infusion on wall-thickness changes in the LAD and left circumflex artery (LC) perfused regions in an individual animal. Tracings are shown during both preischemic conditions and in the postischemic stunned myocardium. The area between the vertical lines indicates the period of systole, as defined from the left ventricular dP/dt tracing (not shown), and the area between the horizontal lines illustrates the systolic wall thickening during the cardiac cycles shown.

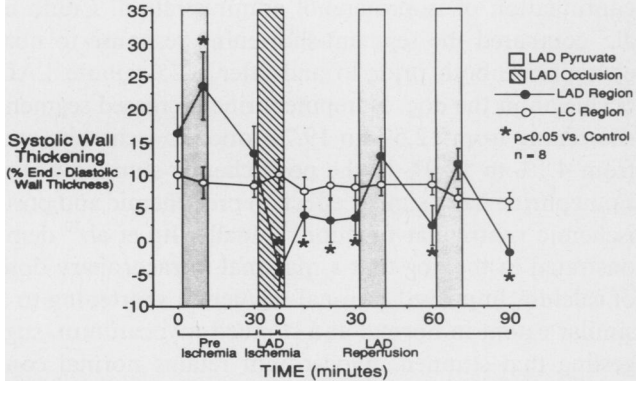

FIG. 2. Systolic wall thickening (mean ± SEM) in the left anterior descending artery (LAD) and left circumflex artery (LC) perfused regions during a 30-minute preischemic period, a 10-minute period of LAD occlusion (indicated by the bar filled with diagonal lines), and a 90-minute reperfusion period. The 10-minute periods of LAD pyruvate infusion are indicated by the open bars; * = $p < 0.05$ compared to control (preischemia, prepyruvate value).

reproducible, as cessation of pyruvate infusion returned LAD wall thickening to the stunned level and a second pyruvate infusion once again improved LAD function.

Discussion

The major finding of this study was the observation that LAD pyruvate infusion improves regional ventricular function in both normal and stunned myocardium. Although a positive inotropic effect of pyruvate has been described,[13-15] to our knowledge this is the first demonstration of enhanced regional ventricular function *in vivo* in the stunned myocardium.

Several investigators have documented the ability of non-necrotic postischemic myocardium to respond to inotropic stimulation. Thirty minutes after a 30-minute LAD occlusion in the dog, Mercier et al.[7] demonstrated that dopamine improved regional systolic segment shortening from 20% of control to levels similar to preischemic values. An improvement in postischemic function with dopamine was also observed in dogs subjected to three hours of LAD occlusion, provided that a portion of the myocardium remained non-necrotic. Arnold et al.[6] and Ellis et al.[8] reported that dopamine infusion resulted in a sustained improvement in regional systolic wall thickening in the dog following two hours of LAD occlusion. This improvement in function was observed when the drug was given as early as 45 minutes postischemia[6] or as late as 24 hours postischemia.[8] After cessation of dopamine treatment, ventricular function returned to the previously depressed stunned level.[6] Bolli et al.[4] observed that three hours following a 15-minute LAD occlusion in the dog, isoproterenol increased wall-thickening values from approximately 15% of control to values similar to preischemia levels. This response was reversible, as function returned to preisoproterenol values 30 minutes after discontinuation of isoproterenol administration. Ciuffo et al.[5] compared the segment-shortening response to norepinephrine both prior to and after a 25-minute LAD occlusion in the dog. Norepinephrine increased segment shortening from 12.5% to 19.7% prior to ischemia, and from 4.1% to 12.9% in the postischemic stunned heart. Epinephrine has a similar effect on preischemic and postischemic ventricular function.[9] Finally, Ito et al.[10] demonstrated in the dog that a maximal intracoronary dose of calcium improved regional segmental shortening to a similar extent in normal and stunned myocardium, suggesting that stunned myocardium retains normal contractile reserve. In summary, considering the variety of ischemia and reperfusion durations tested, there seems to be little doubt that postischemic stunned myocardium contains considerable functional reserve that can be tapped by well-known positive inotropic agents.

Nevertheless, concern exists as to the potentially deleterious effect that these inotropic agents may have on the stunned myocardium. Three lines of evidence argue against a major adverse effect of inotropic agents on stunned myocardium. First, regional ventricular function is not further depressed from the stunned level once inotropic stimulation is discontinued.[4,6,10] We obtained similar results with pyruvate, as regional ventricular function returned to prepyruvate levels once the pyruvate was discontinued. Second, inotropic therapy in the stunned heart does not appear to induce or increase infarct development.[6,16] Third, myocardial high-energy phosphates are not further depressed from the stunned level during[17] or after[6] inotropic therapy. However, in one study dopamine did appear to cause some depletion of endocardial high-energy phosphates while exerting its positive inotropic effect.[6]

Our present findings are similar to the results obtained with conventional inotropic agents, as intracoronary pyruvate improved postischemic function to a level similar to untreated preischemic levels but less than preischemic pyruvate-treated levels. In addition, these new data substantiate the previous studies of Bünger and colleagues[13,14] and Liedtke and Nellis[15] that pyruvate, in supraphysiologic doses, exerts a positive inotropic effect on normal and postischemic myocardium. Although further studies will be necessary to determine whether pyruvate improves function in the *in vivo* stunned heart by enhancing high-energy phosphate production and thus the adenine nucleotide pool, studies in isolated perfused hearts have shown that pyruvate improves postischemic function in a concentration-dependent manner parallel with an increase in the cytosolic phosphorylation potential (ATP/ADPxP$_i$), an index of myocardial energy state.[14] Of particular interest is the observation that only pyruvate, not lactate or acetate, maintained function and re-energized the postischemic isolated perfused guinea pig heart.

Although the biochemical mechanism of pyruvate action has not been fully delineated, there are three likely sites of action. First, pyruvate may stabilize the cytosolic phosphorylation potential due to a decrease in the cytosolic NADH/NAD$^+$ ratio. This effect, which is mediated by lactate dehydrogenase, would tend to increase the cytosolic phosphorylation potential *via* two other powerful glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase. Second, because pyruvate is both the immediate substrate for and activator of pyruvate dehydrogenase, improvements in the myocardial energy state with pyruvate may be mediated by increased mitochondrial pyruvate dehydrogenase flux and subsequent acetyl-CoA production. The acetyl-CoA is then available as an energy-producing substrate for the tricarboxylic acid cycle and oxidative phos-

phorylation, resulting in the rephosphorylation of ADP to ATP in accordance with the instantaneous myocardial energy requirements. Finally, although pyruvate decreases the NADH/NAD^+ ratio in the cytosol, it increases this ratio in the mitochondria. This increase in the mitochondrial NADH/NAD^+ ratio would tend to increase the thermodynamic driving force for mitochondrial electron transport and thus stimulate myocardial oxygen uptake.

While the relative importance of the cytosolic and mitochondrial effects of pyruvate remain to be elucidated, the studies of Bünger et al.[13,14] suggest that the overall result appears to be an enhancement of the cytosolic phosphorylation potential. Because the sarcoplasmic reticulum calcium pump establishes the concentration gradient for free calcium across the sarcoplasmic reticulum in accordance with the cytosolic phosphorylation potential, pyruvate may ultimately improve calcium handling by the sarcoplasmic reticulum, leading to improved contractile function. If this is in fact the mechanism of action of pyruvate, then pyruvate exerts a positive inotropic effect secondary to improvements in the myocardial energy state, an effect that distinguishes it from conventional inotropic agents.

In support of these proposed biochemical mechanisms, Bünger et al.[14] recently reported that the improvement in postischemic function and myocardial energy state in the isolated perfused guinea pig heart was associated with a concentration-dependent increase in myocardial oxygen consumption, reflecting increased mitochondrial respiration and oxidative phosphorylation. We feel that these observations, combined with the findings of the present study indicating that intracoronary pyruvate improves regional myocardial function in the *in vivo* stunned heart, warrant further consideration of the clinical use of pyruvate in the treatment of postischemic ventricular dysfunction.

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Discussion

DR. ROBERT B. WALLACE (Washington, D.C.): Thank you, Dr. Polk. Dr. Jones, Fellows, and Guests: The studies of Dr. Flint, Mentzer, and their colleagues exemplifies a continuing search for means of altering the effects of ischemia on the myocardium. The authors have attempted to reduce the effects of ischemia by metabolic substrate enhancement using pyruvate infusion prior to and following a five-minute period of myocardial ischemia.

Braunwald introduced the concept of myocardial stunning, which might be described as reversible postischemic myocardial injury. In addition to the adverse effects of ischemia, Jennings, Buckberg, and others have stressed the significance of reperfusion as an additional insult to the myocardium.

Dr. Dan Miller, one of our surgical residents, working with Dr. Marc Visner in our cardiovascular research laboratory, has studied the effects of myocardial ischemia and how they might be altered by modifying reperfusion.

The model used by Dr. Miller is similar to that used by Dr. Mentzer and his colleagues. Regional function was assessed by determining regional ejectional shortening before and after a 15-minute period of occlusion of the left anterior descending artery. Following unmodified reperfusion, functional recovery was 60% of baseline at six hours. Animals in whom the first 15 minutes of reperfusion was with normothermic cardioplegic