MINIREVIEW

Pyruvate: Metabolic Protector of Cardiac Performance (44472)

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Abstract. **Pyruvate, a metabolic product of glycolysis and an oxidizable fuel in myocardium, increases cardiac mechanical performance and energy reserves, especially when supplied at supraphysiological concentrations. The inotropic effects of pyruvate are most impressive in hearts that have been reversibly injured (stunned) by ischemia/reperfusion stress. Glucose appears to be an essential co-substrate for pyruvate's salutary effects in stunned hearts, but other fuels including lactate, acetate, fatty acids, and ketone bodies produce little or no improvement in postischemic function over glucose alone. In contrast to pharmacological inotropism by catecholamines, metabolic inotropism by pyruvate increases cardiac energy reserves and bolsters the endogenous glutathione antioxidant system. Pyruvate enhancement of cardiac function may result from one or more of the following mechanisms: increased cytosolic ATP phosphorylation potential and Gibbs free energy of ATP hydrolysis, enhanced sarcoplasmic reticular calcium ion uptake and release, decreased cytosolic inorganic phosphate concentration, oxyradical scavenging *via* direct neutralization of peroxides and/or enhancement of the intracellular glutathione/NADPH antioxidant system, and/or closure of mitochondrial permeability transition pores. This review aims to summarize evidence for each of these mechanisms and to consider the potential utility of pyruvate as a therapeutic intervention for clinical management of cardiac insufficiency.**

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Catecholamines are used extensively to provide inotropic support for clinical management of inadequate cardiac function in a variety of scenarios, including acute cardiac failure, postoperative recovery following heart surgery or coronary revascularization, and treatment of low cardiac output associated with cardiogenic, hypovolemic, or septic shock (1). Reversibly injured, stunned myocardium retains significant inotropic reserve (2) and is highly responsive to β-adrenergic inotropes (3, 4). However, β-adrenergic stimulation exacts a cost: catecholamines produce disproportionate increases in myocardial oxygen requirements relative to increases in mechanical function, thereby lowering myocardial O_2 utilization efficiency (5, 6), and depleting myocardial energy reserves (6–8). Thus β-adrenergic stimulation jeopardizes stunned or failing myocardium by increasing its energy demand at a time when its energy reserves are low.

Over the last two decades, considerable effort has been directed to finding alternatives to catecholamines for treatment of cardiac stunning and failure. Studies in isolated and *in situ* heart preparations have demonstrated that pyruvate, a natural aliphatic monocarboxylate and a central metabolic intermediate in mammalian cells, is capable of increasing contractile function of ischemically injured, stunned myocardium. Unlike catecholamines, pyruvate does not deplete

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[2]Enzymes discussed in this article include: Adenylate cyclase (EC 4.6.1.1); aldolase (EC 4.1.2.13), Ca^{2+} ATPase (EC 3.6.1.38), citrate synthase (EC 4.1.3.7), creatine kinase (EC 2.7.3.2), glutamate-oxaloacetate transaminase (EC 2.6.1.1), glutamate-pyruvate transaminase (EC 2.6.1.2), glutathione peroxidase (EC 1.11.1.9), glutathione reductase (EC 1.8.1.4), glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12), lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.39/40), Na^+,K^+ ATPase (EC 3.6.1.37), phosphofructokinase (EC 2.7.1.11), phosphoglycerate kinase (EC 2.7.2.3), pyruvate carboxylase (EC 6.4.1.1), pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.6.4.3), pyruvate dehydrogenase kinase (EC 2.7.1.99), pyruvate dehydrogenase phosphatase (EC 3.1.3.43), and pyruvate kinase (EC 2.7.1.40)

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but instead substantially increases cardiac energy reserves in parallel with cardiac function. Moreover, pyruvate is a potent antioxidant, whereas catecholamines stimulate formation of harmful reactive oxygen species (7, 9, 10). These favorable metabolic properties, detailed below, make pyruvate a promising alternative or adjunct to catecholamines for treatment of inadequate cardiac performance.

After surveying the pathways of myocardial pyruvate metabolism, this article reviews pyruvate's salutary effects on cardiac function, with an emphasis on the ischemically stunned heart. Potential mechanisms for these beneficial effects are then presented. The article concludes by considering pyruvate's therapeutic potential for providing metabolic inotropic support to injured and failing human heart.

Pyruvate Metabolism in Cardiomyocytes

The distinctive patterns of pyruvate metabolism in myocardium, which differ in many respects from the oxidative metabolism of other cardiac fuels, provide the foundation for pyruvate's cardioprotective properties.

Sarcolemmal Transport and Cytosolic Metabolism of Pyruvate. Plasma pyruvate concentration in resting, fasted humans is normally about 80–100 μM, well below that of glucose and lactate (11, 12). At these low concentrations, pyruvate is not an important blood-borne fuel for the myocardium. Circulating pyruvate concentration transiently increases two- to three-fold following physical exertion (12, 13) and ingestion of mixed meals (11, 14), where plasma pyruvate parallels a much larger increase in plasma lactate concentration.

Sarcolemmal pyruvate uptake is mediated by a H^+-monocarboxylate symporter. Although this carrier transports other aliphatic monocarboxylates, including lactate and ketone bodies (15, 16), its pyruvate affinity is much higher. The K_m for pyruvate is 0.07 mM in guinea-pig and 0.2 mM in rat myocardium (i.e., near the plasma pyruvate concentration). Sarcolemmal pyruvate uptake varies exponentially with extracellular pyruvate concentration (17), so pyruvate metabolism could increase in postprandial and postexercise states although it may be constrained by concomitantly increased extracellular lactate. At a pK_a of 2.49, pyruvic acid is almost completely dissociated to its anionic conjugate base at physiological plasma pH, so simple diffusion of the neutral acid through the lipid bilayer is negligible.

Under most physiological conditions, the bulk of intracellular pyruvate is generated endogenously by glycolysis or lactate oxidation (Fig. 1). An abundant cytosolic enzyme, lactate dehydrogenase (LDH) catalyzes a reversible near-equilibrium redox reaction, in which the electron acceptor NAD^+ is stoichiometrically reduced to NADH as lactate is oxidized. When myocardial O_2 delivery is compromised by hypoxemia or ischemia, cytosolic NADH accumulates and shifts the LDH equilibrium in favor of net lactate formation.

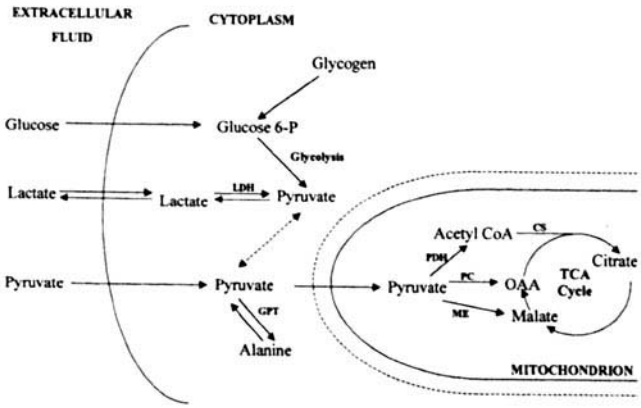

Figure 1. Pyruvate metabolism and compartmentalization in cardiomyocytes. Cytosolic pyruvate compartmentalization into glycolytic and exogenous pools and mitochondrial pathways of pyruvate carboxylation are depicted. Near-equilibrium processes are indicated by double arrows. LDH: lactate dehydrogenase; GPT: glutamate-pyruvate transaminase; PDH: pyruvate dehydrogenase; ME: malic enzyme; PC: pyruvate carboxylase; CS: citrate synthase; OAA: oxaloacetate.

Cardiac LDH is a tetramer consisting of different combinations of heart (H) and muscle (M) subunits. LDH-1, which contains four H subunits, is the most abundant LDH isoenzyme in myocardium (18). LDH isoenzymes with M subunits are less abundant than LDH-1; LDH-5, which contains four M subunits, comprises only 1% of total myocardial LDH (18). The Michaelis-Menten kinetics of these isoenzymes differ; K_m values for pyruvate were 0.1 mM in rabbit myocardium, where LDH-1 is the major isoform, versus 0.35 mM in skeletal muscle, where LDH-5 predominates (19). Similar pyruvate K_ms were reported in rat (20) and bovine (21) myocardium. Cytosolic pyruvate concentrations are usually at or below 0.1 mM, so increases in cytosolic pyruvate concentration favor increased lactate formation.

At supraphysiological concentrations, pyruvate inhibited LDH (19, 20). The LDH-1 isoenzyme was much more susceptible to pyruvate inhibition than LDH-5 (18, 19); indeed, 5 mM pyruvate inhibited rabbit heart LDH-1 by 70%, but did not inhibit LDH-5. However, it should be noted that pyruvate inhibition only occurred when LDH was heavily diluted; at LDH-1 concentrations near that of the cardiocyte cytosol, even 20 mM pyruvate did not inhibit the enzyme (18).

Transamination of pyruvate to generate alanine occurs mainly in the cytosol where the bulk of glutamate-pyruvate transaminase is located (22, 23). Alanine formation from pyruvate is stoichiometrically linked to equimolar disappearance of glutamate and aspartate (24, 25); glutamate is the immediate amino donor, and aspartate's amino group is incorporated into alanine *via* the combined glutamate-oxaloacetate and glutamate-pyruvate transaminases. Glutamate-pyruvate transaminase is readily reversible, so alanine can serve as a precursor of cytosolic pyruvate.

Cytosolic Pyruvate Compartmentalization. Glycolytically generated and exogenous pyruvate appear to exist as separate, slowly exchanging cytosolic pools (Fig.

1). In isolated rat hearts perfused with [1–^{14}C]pyruvate, specific activities of tissue alanine, mitochondrial pyruvate, and coronary venous effluent lactate were higher than that of intracellular lactate (26). When [1–^{14}C]lactate was applied, specific activities of tissue and effluent pyruvate and tissue alanine were similar, but less than that of effluent lactate (27). In isolated working hearts perfused with [U-^{14}C]glucose, the specific activity of coronary effluent lactate plateaued within 8 min, but $^{14}CO_2$ release rose more gradually and stabilized only after 20 min (28). When 1–5 m*M* [1–^{14}C]pyruvate was applied, the specific activity of effluent lactate was roughly half that of tracer pyruvate. In isolated rat hearts perfused with [3–^{13}C]pyruvate, ^{13}C fractional enrichment of C3 of alanine was similar to that of acetate C2, but well above lactate C3 enrichment (29). Thus, alanine appeared to be in equilibrium with the cytosolic pyruvate pool ultimately oxidized in the mitochondria, whereas a large lactate fraction did not arise from extracellular pyruvate.

Damico *et al.* (30) perfused isolated rabbit hearts with 5 m*M* [2–^{13}C]glucose + 2.5 m*M* [3–^{13}C]pyruvate. In these hearts, ^{13}C labeling patterns revealed that glycolysis from exogenous glucose accounted for 22% and 10% of lactate and alanine formation, respectively, whereas 36% of lactate and 86% of alanine were generated from exogenous pyruvate. Exogenous pyruvate was the predominant fuel for oxidative metabolism, and very little exogenous glucose was oxidized; thus, at supraphysiological concentrations, pyruvate suppressed glucose oxidation. Taken together, these studies indicate the presence of two slowly exchanging cytosolic pyruvate pools (Fig. 1), one generated by glycolysis and preferentially reduced to lactate, and the other, derived from exogenous pyruvate, preferentially oxidized or transaminated to generate alanine. The histologic basis of this pyruvate compartmentalization has not been delineated, but these studies suggest a subcellular distribution of the glycolytic enzymes and LDH distinct from that of glutamate-pyruvate transaminase and the mitochondria.

Mitochondrial Pyruvate Transport. Pyruvate oxidation occurs exclusively in the mitochondria. The inner mitochondrial membrane does not permit simple diffusion of hydrophilic substances, and a carrier mechanism is required to transfer pyruvate from the cytosol to the mitochondrial matrix (31). The pyruvate transporter has been characterized by Halestrap *et al.* (32), who employed cyanocinnamate derivatives to inhibit the transporter and study its kinetics. Like the sarcolemmal transporter, the mitochondrial carrier symports aliphatic monocarboxylates with a proton. This reversible carrier can exchange cytosolic pyruvate for mitochondrial monocarboxylates including acetoacetate and β-hydroxybutyrate (33), but not lactate, which has a much lower affinity for the carrier (33, 34).

In hearts perfused with supraphysiological pyruvate, cytosolic pyruvate concentration remains below that of the extracellular fluid due to simultaneous mitochondrial pyruvate combustion. When the mitochondrial transporter is pharmacologically disabled with α-cyano-3-hydroxycinnamate, cytosolic pyruvate concentration increases (25), and a Donnan equilibrium is established in accordance with the *trans*-sarcolemmal pH gradient (35). Under these conditions, the cytosolic pyruvate-consuming pathways are enhanced: myocardial alanine content increases at the expense of glutamate and aspartate (25), and cardiac lactate excretion increases several-fold (35).

Pyruvate Dehydrogenase. Pyruvate dehydrogenase (PDH) catalyzes irreversible oxidative decarboxylation of pyruvate to acetyl CoA in the mitochondrial matrix. This multienzyme complex occupies a central crossroads of intermediary metabolism, the point at which carbon derived from glycolysis and lactate is committed to oxidation in the TCA cycle. The PDH complex contains multiple subunits of three different enzymes that catalyze four distinct reactions. The first reaction, pyruvate decarboxylase, cleaves the pyruvate molecule into CO_2 and a two-carbon hydroxyethyl fragment covalently bound to a thiamine pyrophosphate prosthetic group of the decarboxylase. This reaction is irreversible and controls the rate of the overall PDH reaction. Next, the hydroxyethyl fragment is transferred to the lipoamide moiety of dihydrolipoyl transacetylase, yielding acetyl hydrolipoamide and regenerating thiamine pyrophosphate. In the third reaction, also catalyzed by dihydrolipoyl transacetylase, coenzyme A is added to the acetyl moiety of acetyl hydrolipoamide; acetyl CoA is produced, and reducing equivalents are conserved as dihydrolipoamide. Lastly, dihydrolipoamide dehydrogenase transfers reducing equivalents from dihydrolipoate to NAD^+, yielding NADH and regenerating the oxidized lipoamide moiety of dihydrolipoyl transacetylase. The net PDH reaction is pyruvate + $NAD^+ \rightarrow$ acetyl CoA + CO_2 + NADH; the acetyl CoA product enters the TCA cycle *via* citrate synthase and is ultimately oxidized.

Pyruvate dehydrogenase activity and flux are exquisitely controlled by two distinct mechanisms (Fig. 2) (36, 37). Firstly, PDH flux is modulated by the intramitochon-

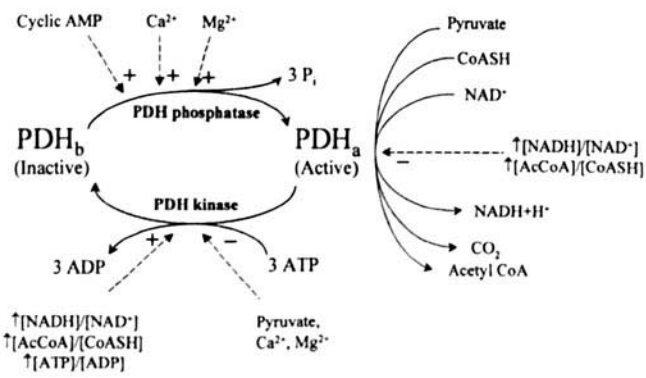

Figure 2. Pyruvate dehydrogenase regulation. Pyruvate dehydrogenase (PDH) is interconverted between its active (PDH_a) and inactive (PDH_b) forms by the PDH kinase/phosphatase system. Metabolic factors that regulate the kinase and phosphatase are indicated. In addition, active PDH_a is inhibited by increased concentrations of its products, acetyl CoA and NADH, relative to its substrates free coenzyme A (CoASH) and NAD^+.

drial molar concentration ratios of acetyl CoA to free CoA and NADH to NAD^+ (38–41); increases in these ratios impose end-product inhibition of the reversible dihydrolipoyl transacetylase and dihydrolipoamide dehydrogenase reactions, respectively. Secondly, PDH is covalently regulated by a kinase/phosphatase system, which converts PDH between its dephosphorylated, active form (PDH_a) and phosphorylated, inactive PDH_b (36, 42). Pyruvate dehydrogenase kinase inactivates PDH by phosphorylating an α subunit of pyruvate decarboxylase; pyruvate dehydrogenase phosphatase reverses this inhibition by dephosphorylating the decarboxylase. Kinase and phosphatase activities are regulated by acetyl CoA/free CoA, NADH/NAD^+ and ATP/ADP concentration ratios in the mitochondrial matrix (39, 40, 43, 44). Increased ratios activate PDH kinase and, thus, promote PDH inactivation, whereas decreased ratios activate PDH by inhibiting the kinase. These mechanisms adjust myocardial fuel selection between carbohydrate and fatty acid/ketones in response to changes in plasma fuel composition and neuroendocrine factors. β-Oxidation of fatty acids and ketone bodies produces acetyl CoA and NADH that suppress pyruvate oxidation, enabling fatty acids and ketones to compete with carbohydrates to supply acetyl CoA to the TCA cycle (40, 45). Pyruvate promotes its own oxidation by activating the PDH complex directly (46) and inhibiting PDH kinase, thus increasing the PDH_a fraction (40, 43, 44, 47). Indeed, high concentrations of pyruvate (≈ 5 m*M*) can at least partially overcome PDH inhibition by high fatty acid concentrations (45).

Pyruvate dehydrogenase is also activated by physiological concentrations of Mg^{2+} and Ca^{2+}, which inhibit PDH kinase (44) and activate PDH phosphatase (48). However, in hearts perfused with high concentrations (1–5 m*M*) of pyruvate, Ca^{2+} appears to stimulate PDH by increasing cardiac workload without altering kinase or phosphatase activities (49). Weiland and Siess (50) reported cAMP activation of PDH phosphatase, and proposed a separate protein kinase/phosphatase system to modulate activity of the phosphatase, analogous to mechanisms regulating cardiac glycogen turnover.

Pyruvate Carboxylation. The mitochondrial matrix of cardiomyocytes contains enzymes capable of condensing pyruvate and CO_2 to generate four-carbon TCA cycle intermediates. Pyruvate carboxylation increases steady-state contents of citrate and other TCA intermediates (51). This anaplerotic process can be substantial: ^{13}C-NMR revealed pyruvate carboxylation to be 13%–18% of overall citrate synthase flux in hearts perfused with 2.5 m*M* pyruvate (30, 52). Cardiac mitochondria contain appreciable activities of pyruvate carboxylase (26, 51–53), which generates oxaloacetate at the expense of one molecule of ATP, and $NADP^+$-dependent malic enzyme (54–56), which yields malate and consumes one NADPH. The relative contributions of these two enzymes to the overall rate of pyruvate carboxylation have not been defined. However, studies in isolated rat hearts implicate malic enzyme as the major pyruvate carboxylating enzyme; carboxylation was unaffected by severe biotin deficiency that lowered pyruvate carboxylase activity by 90% (55), whereas hydroxymalonate, an inhibitor of malic enzyme and malate dehydrogenase, blunted (but did not completely eliminate) acetoacetate-enhanced pyruvate carboxylation (56).

Pyruvate Enhancement of Cardiac Mechanical Function

Although the myocardium is capable of oxidizing a variety of fuels to generate energy for contractile function, pyruvate appears uniquely able to augment cardiac performance. Pyruvate's effects on mechanical function have been studied in isolated (25, 57–60) and *in situ* hearts (61–63). At supraphysiological concentrations, pyruvate increases contractile function of hearts metabolizing glucose (59, 60) or fatty acid (25). Pyruvate's inotropic effect is especially striking in hearts reversibly injured (stunned) by ischemia/reperfusion stress. In isolated working guinea-pig hearts consuming glucose + lactate as basal fuels, pyruvate concentration-dependently improved postischemic mechanical function (64). Near-physiological 0.2 m*M* pyruvate improved function only slightly, but higher pyruvate concentrations increased left ventricular developed pressure and power several-fold. These effects plateaued at 5–10 m*M* pyruvate. Similarly, van Bilsen *et al.* (58) demonstrated near-complete restoration of cardiac output by 5 m*M* pyruvate in ischemically stunned, glucose-perfused rat hearts. Pyruvate was also effective in regionally stunned, *in vivo* canine (63) and porcine (6, 61, 62) myocardium. In these studies, intracoronary pyruvate increased postischemic systolic wall motion and contractile force several-fold, to near the respective preischemic baselines, without altering function of remote, nonischemic myocardium.

Figure 3 presents a typical experiment demonstrating the salutary effects of pyruvate on mechanical function of ischemically stunned guinea-pig heart. Glucose (5 m*M*) was provided as basal metabolic fuel. Left ventricular pressure and dP/dt were monitored with a pressure transducer, and stroke work was computed from aortic and coronary flows. After measurement of preischemic function, the heart was stunned by 45 min hypoperfusion with concomitant *L*-norepinephrine stimulation (64, 65). At 15 min reperfusion, cardiac function was markedly depressed relative to preischemia. Left ventricular pressure development, dP/dt, and stroke work were sharply lowered, and the heart could not generate aortic flow. Pyruvate was then infused to an intracoronary concentration of 5 m*M*. By 15 min pyruvate infusion, cardiac function recovered nearly to the preischemic level, although heart rate was only slightly increased versus pretreatment. However, pyruvate's effects did not persist after infusion was discontinued. When pyruvate was withdrawn, function quickly fell and stabilized at a level which, although somewhat higher than prepyruvate, was commensurate with spontaneous, partial contractile

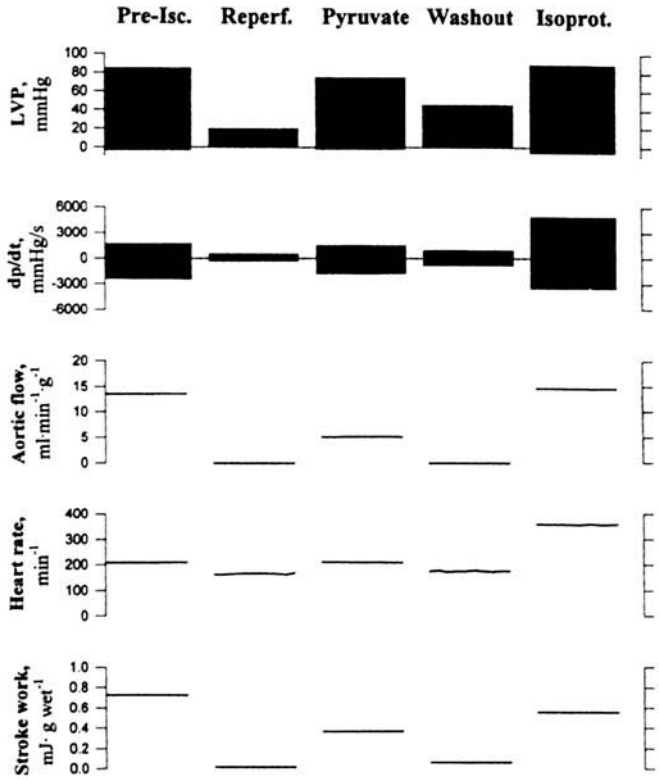

Figure 3. Effects of pyruvate versus isoproterenol on contractile function of stunned working heart. A Millar high-fidelity pressure transducer was placed in the left ventricular lumen *via* the left atrium for measurement of left ventricular pressure (LVP) and its first derivative (dP/dt). Tracings were obtained in the last minute of five phases of a typical experiment in which the heart was stunned by 45 min low-flow ischemia (65). Pre-Isc.: preischemic baseline; Reperf.: 15 min postischemic reperfusion; Pyruvate: pyruvate infusion to 5 m*M* left atrial concentration at 15–30 min reperfusion; Washout: pyruvate-free perfusion at 30–45 min reperfusion; Isoprot.: 10 n*M* isoproterenol infusion at 45–60 min reperfusion. 10 m*M* glucose was provided throughout the experiment.

recovery during prolonged reperfusion of these stunned hearts (66). Lastly, the heart was stimulated with the β-adrenergic agent isoproterenol, which increased left ventricular inotropism to levels above preischemic baseline. Significant inotropic reserve is a hallmark of cardiac stunning (2–4, 6, 67), and distinguishes stunned from irreversibly injured myocardium.

Not shown in Figure 3 is a transient, moderate decline in cardiac function that occurs during the first few minutes of pyruvate treatment. This phenomenon is routinely observed both in preischemic and stunned hearts in the author's laboratory, and has been noted by other investigators (68). The mechanism of pyruvate's temporary cardiodepressant effect is unknown, but may result from sarcolemmal H^+ symport. Cytosolic acidification dampens contractile force at the myofilament level (69–72). This H^+ effect would then be overcome by subsequent enhancement by pyruvate metabolism of cytosolic energetics and Ca^{2+} transport; also, pyruvate:H^+ symport into the mitochondria would effectively remove from the cytosol the H^+ that entered the cell with pyruvate.

Other fuels including lactate, acetate, and octanoate failed to improve and in some cases even worsened contractile failure of glucose-perfused stunned hearts (8, 64). Like pyruvate, each of these compounds is readily oxidized in myocardium, and the basis of pyruvate's superiority for restoring cardiac function is not immediately obvious. It seems likely that the peculiarities of pyruvate metabolism may be responsible for its salutary effects. Possible mechanisms for pyruvate's metabolic inotropism are considered in the following sections.

Enhancement of Cardiac Energetics

Cardiac mechanical performance requires a continuous supply of chemical energy provided by ATP hydrolysis. Therefore, it is not surprising that cardiac function responds to changes in myocytic energy reserves (8, 57, 59). A readily oxidized substrate, pyruvate has proven more effective than other fuels at augmenting cardiac energy reserves, especially in energy-depleted postischemic myocardium. Pyruvate (5 m*M*) doubled cytosolic ATP phosphorylation potential (i.e., [ATP]/([ADP][P_i]), where [ ] denotes free cytosolic metabolite concentration and P_i is inorganic phosphate) in preischemic and stunned guinea-pig hearts metabolizing glucose as basal fuel (8, 64). This doubling of ATP phosphorylation potential increased by ≈ 2 kJ/mol the cytosolic Gibbs free energy of ATP hydrolysis (ΔG_{ATP}), the thermodynamic driving force for acto-myosin crossbridge cycling, ion transport, and other energy-consuming processes of working heart muscle. Lasley's group (6, 73) has demonstrated marked pyruvate enhancement of cytosolic energetics in ischemically stunned, *in situ* pig myocardium. Thus, supraphysiological concentrations of intracoronary pyruvate increase cytosolic energy reserves in tandem with contractile function both in isolated and *in vivo* myocardium. Possible mechanisms to produce this pivotal energetic effect are described next.

Oxidation of Cytosolic Pyridine Nucleotides. Although the biochemical mechanisms for pyruvate enhancement of cytosolic phosphorylation potential have not been established conclusively, the pattern of pyruvate metabolism could make it superior to other fuels. Pyruvate oxidation generates large amounts of ATP, yet other readily oxidized, ATP-generating fuels including fatty acid, acetate, ketone bodies, and lactate do not increase phosphorylation potential above the baseline established by glucose alone. Mitochondrial oxidation of each of these fuels generates reducing equivalents to power electron transport and oxidative phosphorylation. Unlike the other substrates, pyruvate is a powerful cytosolic oxidant, where it increases the NAD^+/NADH concentration ratio *via* the LDH equilibrium. Veech *et al.* (74, 75) proposed that the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase could catalyze near-equilibrium between cytosolic NAD^+/NADH and ATP phosphorylation potential. By this mechanism, oxidation of the cytosolic pyridine

nucleotide redox state by pyruvate would further increase ATP phosphorylation potential above the level sustained by oxidative phosphorylation. Indeed, near-equilibrium between cytosolic redox and adenylate systems was demonstrated in retrogradely perfused, nonworking guinea-pig hearts (76). However, this enzyme system appeared to be displaced from equilibrium at the higher metabolic rates of hearts performing physiological levels of external work (77, 78). In these working hearts, pyruvate increased phosphorylation potential, but not as much as predicted from the cytosolic $NAD^+/NADH$ ratio. Moreover, when mitochondrial pyruvate metabolism was blocked selectively by α-cyano-3-hydroxycinnamate, cytosolic redox and phosphorylation states were dissociated; cytosolic $NAD^+/NADH$ increased further, yet cytosolic ATP phosphorylation potential fell to the pyruvate-free control level (25). Taken together, these studies leave little doubt that near-equilibrium of the cytosolic redox and phosphorylation potentials does not prevail in physiologically working myocardium. However, it is still possible that pyruvate's redox effects could incrementally increase cytosolic energetic state, provided mitochondrial pyruvate metabolism is intact.

Pyruvate Dehydrogenase Activation. Reductive decarboxylation of pyruvate by PDH generates reducing equivalents within the mitochondrial matrix, and commits the pyruvate carbon skeleton to combustion in the TCA cycle. Pyruvate activates PDH and promotes its own oxidation by inhibiting PDH kinase, and it has been proposed that this mechanism could be responsible for pyruvate enhancement of cardiac function and energetics (47). Pyruvate dehydrogenase is inactivated in ischemic myocardium by its accumulated products acetyl CoA and NADH (79–81). Dichloroacetate, a nonmetabolizable, pharmacological PDH kinase inhibitor, has been shown to increase contractile function (82–84), improve oxygen utilization efficiency (82, 83) and increase lactate consumption (85) in several experimental models of cardiac insufficiency (86). The author examined dichloroacetate's effects in stunned, glucose-perfused guinea-pig hearts (87). Dichloroacetate was applied at 2 m*M*, a concentration that maximally activates cardiac PDH (88, 89). Dichloroacetate moderately increased postischemic function in these hearts, but 5 m*M* pyruvate was much more effective. Furthermore, cytosolic phosphorylation potential was doubled by pyruvate but unaltered by dichloroacetate. Dichloroacetate also failed to increase cardiac function and energetics in several other investigations (85, 89, 90). Thus, PDH activation likely contributes to, but is probably not solely responsible for pyruvate enhancement of postischemic function and energetics.

Cytosolic Inorganic Phosphate Concentration. The concentration of P_i in the cytosolic compartment modulates myocardial function (91–94). This effect stems from two properties of P_i. Inorganic phosphate is liberated by ATP hydrolysis and is a component of the denominator of the ATP phosphorylation potential, so P_i accumulation lowers ΔG_{ATP}, the thermodynamic energy source that powers cardiac function. Secondly, P_i directly interferes with actomyosin crossbridge kinetics, which lowers the amount of active force developed per ATP hydrolyzed (91, 93, 95).

Reduction of cytosolic P_i concentration by pyruvate has been demonstrated in isolated and *in vivo* hearts by metabolite extraction (6, 25, 59) and by ^{31}P NMR spectroscopy (57, 60, 96). Pyruvate lowers cytosolic P_i by at least two mechanisms. Pyruvate metabolism increases phosphocreatine concentration (25, 57, 96), which effectively sequesters P_i and lowers its soluble concentration. Mitochondrial pyruvate carboxylation increases steady-state citrate concentration. By inhibiting phosphofructokinase (97), citrate restricts glycolytic flux, causing the hexose monophosphates glucose 6-phosphate and fructose 6-phosphate to accumulate (66, 87). These compounds, too, sequester cytosolic P_i. By lowering P_i, pyruvate increases cytosolic phosphorylation potential and ΔG_{ATP}. These mechanisms would also relieve P_i dampening of crossbridge force development. In effect, by lowering P_i, pyruvate may act as a myofilament Ca^{2+} sensitizer to increase Ca^{2+}-activated force and lower the energy cost of cardiac work. Thus, by lowering cytosolic P_i, pyruvate could increase myocardial function both by thermodynamic and crossbridge kinetic mechanisms.

Sarcoplasmic Reticular Ca^{2+} Transport

Sequential release and sequestration of calcium ion by the sarcoplasmic reticulum (SR) produces the phasic activation of the contractile machinery fundamental to the cardiac cycle. A Ca^{2+} ATPase in the longitudinal tubular network pumps cytosolic Ca^{2+} into the SR. This ATP-consuming ion pump facilitates diastolic relaxation by lowering the free cytosolic Ca^{2+} concentration, and, by Ca^{2+}-loading the SR, primes the terminal cisternae to release sufficient Ca^{2+} to trigger a forceful systolic contraction. Thus, SR Ca^{2+} pumping is crucial for normal cardiac function. The rate of Ca^{2+} accumulation by the ATPase modulates contractile force: increased SR Ca^{2+} uptake allows more complete diastolic relaxation and increased filling of the more compliant ventricle, and a more Ca^{2+}-loaded SR releases more Ca^{2+} to activate a more forceful systolic contraction (69, 98).

Pyruvate increases cytosolic ΔG_{ATP}, the immediate energy source for the Ca^{2+} pump. The author conducted studies to test the hypothesis that ΔG_{ATP} can modulate SR Ca^{2+} transport and hence cardiac function (25, 59). A special ^{45}Ca labeling/washout procedure was developed to examine the effects of cytosolic energetics on SR Ca^{2+} turnover. Hearts were perfused with a standard fuel supply and ^{45}Ca for 6000 heart beats, followed by 40 min ^{45}Ca washout in the presence of different exogenous fuels to modulate cytosolic energetics, and then treated with 10 m*M* caffeine to release residual SR ^{45}Ca. An inverse relationship between cytosolic phosphorylation potential and the caffeine-mobilized ^{45}Ca pool size was observed: pyruvate-energized

hearts released 50%–60% less ^{45}Ca than control hearts perfused with glucose or octanoate alone, whereas hearts perfused without substrate to deplete energy reserves released 2.5-fold more ^{45}Ca during caffeine infusion than controls. These results were interpreted to indicate that pyruvate increased SR Ca^{2+} uptake and release, which increased the extent of precaffeine ^{45}Ca washout. When mitochondrial pyruvate metabolism was pharmacologically inhibited, pyruvate enhancement of cardiac function, cytosolic ΔG_{ATP}, and SR Ca^{2+} transport were abrogated despite a compensatory increase in octanoate oxidation (25).

Two recent reports independently support the concept of thermodynamic enhancement of SR Ca^{2+} turnover by pyruvate. In isolated rat ventricular myocytes, Martin *et al.* (99) demonstrated pyruvate enhancement of systolic Ca^{2+} transients that paralleled increased cell shortening; these effects were blunted by blocking mitochondrial pyruvate uptake. In isolated, perfused rabbit hearts, Chen *et al.* (60) reported that pyruvate increased SR Ca^{2+} concentration as well as left ventricular developed pressure and ΔG_{ATP}. Thus, pyruvate enhancement of cytosolic energetics promotes SR Ca^{2+} uptake and release, which likely contributes to increased cardiac mechanical performance.

Glucose: Essential Co-Substrate for Pyruvate Enhancement of Postischemic Function and Energetics

The salutary effects of pyruvate on postischemic contractile performance and cytosolic phosphorylation potential were only observed when glucose was also provided (87). In hearts supplied with pyruvate alone, preischemic function was normal, yet irreversible contracture ensued within 10 min of ischemic onset. When glucose was withdrawn immediately before reperfusion, the postischemic hearts fell into profound contractile failure by 5 min reperfusion. Glucose removal lowered cytosolic phosphorylation potential of these failing hearts by 91%. Thus, pyruvate's salutary effects required glucose, although pyruvate was readily oxidized and glycolysis provided only a small fraction of the heart's ATP requirements.

Studies by Becker *et al.* (100) confirmed that an intact glycolytic pathway is essential for pyruvate to restore postischemic cardiac performance. In isolated rabbit hearts metabolizing 5 m*M* pyruvate, these investigators demonstrated that pharmacological inhibition of glycolysis during reperfusion blunted contractile recovery. When glycolytic inhibition was delayed until 30 min after reperfusion, cardiac function was not compromised, indicating that glycolysis was most essential in the early stages of reperfusion (101). More recently, these workers delineated a subcellular structural basis for the glycolytic requirement (102). The entire sequence of glycolytic enzymes from aldolase to pyruvate kinase was found to be intimately associated or even bound to the outer leaflet of the SR membrane. Glycolytic ATP generated by phosphoglycerate kinase and pyruvate kinase in the presence of ADP was sufficient to power Ca^{2+} uptake by SR vesicles endowed with these enzymes. Interestingly, glycolytic ATP had a distinct kinetic advantage: it supported 15-fold higher rates of Ca^{2+} uptake than exogenous ATP. These findings strongly suggested that the glycolytic machinery was functionally coupled to the SR Ca^{2+} ATPase in a microcompartment (102).

The author's findings and those of Becker *et al.* (100) suggest the following scenario. At the onset of reperfusion, SR Ca^{2+} uptake is crucial to sustain renewed contractile activity and to manage the increased cellular Ca^{2+} load that accompanies reperfusion. Oxidative phosphorylation normally provides ample ATP for Ca^{2+} transport. As discussed below, oxyradicals generated upon reperfusion could impair mitochondrial function by opening permeability transition pores in the inner mitochondrial membrane and inactivating creatine kinase; such oxyradical effects would compromise mitochondrial energy supply to the Ca^{2+} ATPase. Under these circumstances, glycolysis could provide energy to sustain Ca^{2+} transport while the mitochondria and creatine kinase recover.

It should be recognized that other mechanisms may contribute to or even be primarily responsible for the glucose co-substrate requirement. For example, glucose, *via* its conversion to glucose 6-phosphate, provides substrate for the hexose monophosphate shunt, the major source of reducing equivalents to sustain the glutathione antioxidant system (see below). Removal of glucose could compromise these antioxidant defenses and expose cardiomyocytes to oxyradical attack. As described above, pyruvate metabolism suppresses glycolysis by generating citrate, an inhibitor of phosphofructokinase (97). Presumably, inhibition of this key enzyme would lessen rather than enhance glycolytic ATP supply to the SR Ca^{2+} pump. Thus, the mechanisms subtending the glucose co-substrate requirement for pyruvate's favorable effects have not been established conclusively.

Antioxidant Actions and Mechanisms

Reactive oxygen species generated in a burst at the onset of reperfusion play a central role in the pathogenesis of cardiac stunning (103–106) by modifying the chemical structures of cellular components including enzymes and membrane phospholipids. For example, critical enzymes including glyceraldehyde 3-phosphate dehydrogenase (107, 108), creatine kinase (109, 110), and sarcolemmal Na^+, K^+ ATPase (111–113) are inactivated when oxyradicals convert key sulfhydryl moieties in the catalytic domain to mixed disulfides (Fig. 4). Cells including cardiomyocytes are endowed with endogenous antioxidant systems, most importantly reduced glutathione (GSH), but these cellular defenses are overwhelmed and depleted by the large reperfusion prooxidant burst, leaving the cardiomyocytes vulnerable to oxyradical attack.

In addition to serving as a natural fuel for myocardium, pyruvate is a potent antioxidant capable of bolstering intracellular antioxidant defenses and neutralizing prooxidants

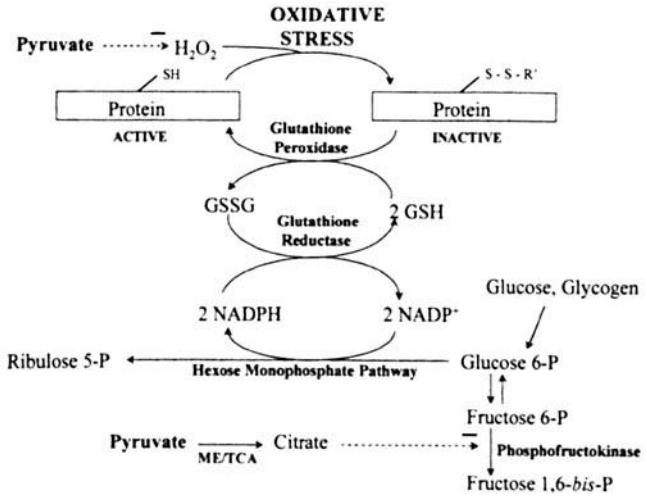

Figure 4. Antioxidant mechanisms of pyruvate. Oxidative stress inactivates proteins by oxidizing sulfhydryl moieties. Pyruvate prevents this oxidation by neutralizing hydrogen peroxide. Pyruvate could also indirectly reverse sulhydryl oxidation by increasing NADPH production by the hexose monophosphate shunt. Flux through this pathway is increased when glycolytic flux is restrained by citrate formed by pyruvate carboxylation. NADPH supplies reducing equivalents to regenerate reduced glutathione (GSH) from glutathione disulfide (GSSG); GSH restores protein sulfhydryls.

(114). Two different mechanisms mediate pyruvate's antioxidant effects. Pyruvate and other α-keto acids directly neutralize hydrogen peroxide and lipid peroxides in a nonenzymatic reaction (110, 115, 116):

$$\text{Pyruvate} + H_2O_2 \rightarrow \text{Acetate} + CO_2 + H_2O$$

A second, indirect antioxidant mechanism could operate in myocardium too (Fig. 4). Pyruvate carboxylation increases myocardial citrate content (25, 55, 56). As described above, citrate inhibits phosphofructokinase (97) and diverts glycolytic flux into the hexose monophosphate shunt, the principal source of NADPH to regenerate GSH from oxidized glutathione disulfide (GSSG). By these two mechanisms, supraphysiological 5 m*M* pyruvate restored the GSH/GSSG ratio of stunned myocardium, which had been lowered by ischemia/reperfusion, to the preischemic level (Fig. 5D).

Potentiation of β-Adrenergic Inotropism in Stunned Myocardium

Pyruvate's metabolic inotropism differs from pharmacological inotropism by catecholamines. Catecholamines stimulate function within a few seconds, but pyruvate enhancement of cardiac performance develops gradually over 5–10 min. Although the cardiac response to pyruvate is impressive, it is less striking than the β-adrenergic response. As described above, catecholamines and pyruvate produce opposite effects on myocardial energy reserves. Therefore, it seemed possible that pyruvate and catecholamines might exert additive inotropic effects when combined. To test this proposal, studies were conducted in the author's laboratory to define the effective concentration range of the β-adrenergic agent isoproterenol in stunned guinea-pig hearts (65). Maximum cardiac power elicited by high isoproterenol concentrations (30–100 n*M*) did not differ in nonischemic time control and postischemic stunned hearts, consistent with the notion that stunned hearts have normal inotropic reserves. However, the stunned hearts were far less responsive than time controls to lower isoproterenol doses: EC_{50} was 0.3 n*M* in controls but 5.2 n*M* in stunned hearts. A third group of hearts was treated with 5 m*M* pyruvate following reperfusion. Pyruvate did not increase the maximum isoproterenol effect, but partially restored cardiac sensitivity to isoproterenol: EC_{50} was lowered to 1.1 n*M* following pyruvate treatment.

To delineate the mechanisms of the isoproterenol:pyruvate interaction, cytosolic energetics, cyclic AMP, and glutathione antioxidant potential were determined in time con-

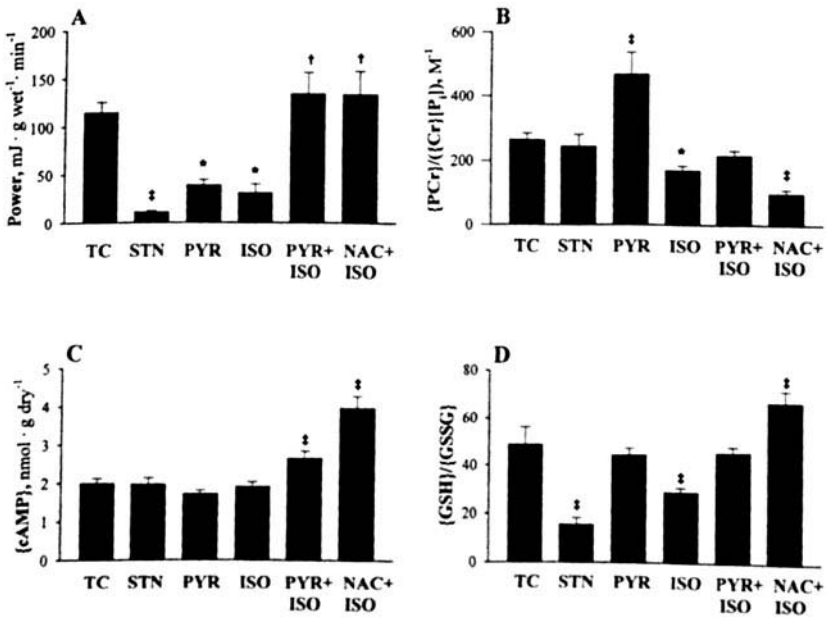

Figure 5. Pyruvate potentiation of β-adrenergic signal transduction and mechanical function in isoproterenol-stimulated stunned hearts. Time control hearts (TC) were perfused 90 min without ischemia; other groups were subjected to 45 min ischemia and 30 min reperfusion. Interventions (PYR: 5 m*M* pyruvate; ISO: 2 n*M* isoproterenol; NAC: 5 m*M* *N*-acetylcysteine) were administered at 15–30 min reperfusion. STN: untreated stunned hearts. (A) Cardiac function was assessed from power; (B) cytosolic energetics from phosphocreatine (PCr) potential (Cr: creatine; P_i: inorganic phosphate); and (D) cellular antioxidant potential from the ratio of reduced glutathione (GSH) to oxidized glutathione disulfide (GSSG). (C) Cyclic AMP (cAMP) content was also measured in these hearts. *$P < 0.05$ *vs.* TC; †$P < 0.05$ *vs.* ISO; ‡$P < 0.05$ *vs.* all other groups. (Data are from Refs. 65 and 66 and are presented with the permission of Academic Press, Ltd.)

trol and untreated stunned hearts, as well as in stunned hearts treated with pyruvate, isoproterenol, or pyruvate + isoproterenol (Fig. 5). An isoproterenol concentration (2 n*M*) near the mean of the three EC_{50}s, where cardiac function differed most markedly between groups, was used. Contractile function was severely impaired in untreated stunned hearts (Fig. 5A). Pyruvate and isoproterenol moderately increased function when applied separately, but isoproterenol was only 20% as effective as in preischemic control hearts (65). In combination, the two interventions dramatically increased cardiac power, indicating that pyruvate potentiated isoproterenol's inotropic effect. As expected, pyruvate and isoproterenol had opposite effects on cytosolic phosphorylation potential indexed by creatine kinase reactants (74–76). Pyruvate doubled but isoproterenol slightly lowered phosphorylation potential (Fig. 5B). Importantly, pyruvate maintained phosphorylation potential at time control levels when combined with isoproterenol, despite potentiating cardiac function and, thus, energy demand.

The β-adrenergic second messenger cyclic AMP was measured in these hearts to determine the effects of the different interventions on β-adrenergic signaling (Fig. 5C). Cyclic AMP was not altered by cardiac stunning, nor by pyruvate or isoproterenol alone; the lack of a measurable cyclic AMP response to isoproterenol was consistent with its modest inotropic effect. In contrast, cyclic AMP content increased significantly in the pyruvate + isoproterenol–treated hearts, which had much higher function. Thus, pyruvate restored the β-adrenergic signaling mechanism in these stunned hearts without compromising cytosolic energetics.

Pyruvate's antioxidant properties could mediate its β-adrenergic potentiation. Cardiac β-adrenergic signaling components including β-adrenoceptors and adenylate cyclase[2] are susceptible to oxyradical attack (117–119) and are inactivated in many cardiac stunning models (119–121). To examine the impact of pyruvate's antioxidant effects on β-adrenergic signaling, cardiac antioxidant reducing power was assessed from the GSH/GSSG ratio (Fig. 5D), and N-acetylcysteine (NAC), an antioxidant but not energy-yielding fuel, was substituted for pyruvate. Cardiac stunning sharply lowered the antioxidant GSH/GSSG ratio, indicating that ischemia/reperfusion inflicted oxidative stress in this model. Pyruvate restored GSH/GSSG in the absence and presence of isoproterenol. N-acetylcysteine alone did not increase postischemic function, but was as effective as pyruvate at potentiating the isoproterenol response (Fig. 5A). Treatment with NAC + isoproterenol depleted cytosolic energy reserves because NAC did not provide energy to meet the increased demand, but cyclic AMP content and GSH/GSSG increased even more than with pyruvate + isoproterenol. These results leave little doubt that antioxidant mechanisms are at least partially responsible for pyruvate potentiation of β-adrenergic inotropism (66).

Closure of Mitochondrial Permeability Transition Pores

The formation of permeability transition pores (MPTs) in the inner mitochondrial membrane has become recognized as a key event in the progression of injury in ischemic and reperfused myocardium (122). The efflux of matrix constituents through these pores (123) and collapse of the electrochemical proton gradient compromise mitochondrial function and exacerbate cellular energy depletion. On the other hand, if ischemic injury is moderate, the mitochondrial membrane could reseal during reperfusion, thereby rescuing cardiomyocytes and allowing recovery of cardiac performance. Reactive oxygen species and increased cytosolic Ca^{2+} have been incriminated as initiators of MPT formation, possibly by oxidizing sulhydryls of inner mitochondrial membrane proteins including adenine nucleotide translocase, and/or by crosslinking membrane proteins (122, 124). Inorganic phosphate has also been implicated in MPT induction (123); P_i may stimulate oxyradical formation at the level of reduced coenzyme Q in the respiratory chain (125).

A recent study by Halestrap *et al.* (126) demonstrated pyruvate's ability to promote MPT closure in postischemic isolated rat hearts. Using 2-deoxyglucose entrapment to monitor mitochondrial permeability, these workers found that 10 m*M* pyruvate resealed mitochondrial membranes and restored left ventricular pressure development to preischemic baseline.

Pyruvate may induce MPT resealing by a combination of energetic and antioxidant mechanisms. Pyruvate neutralization of hydrogen peroxide and enhancement of glutathione reducing power could limit oxyradical MPT induction; other antioxidant measures including catalase, dithiothreitol, and ascorbate have been shown to close MPTs and restore function of isolated mitochondria challenged by anoxia/reoxygenation (126). Pyruvate increases cytosolic ΔG_{ATP} and, thus, increases SR Ca^{2+} sequestration, which would minimize cytosolic Ca^{2+} accumulation. Thirdly, pyruvate lowers intracellular P_i concentration. Thus, pyruvate could arrest formation and promote closure of MPTs by removing three of the principal factors implicated in MPT formation. Evidence from Rigobello *et al.* (127) supports a fourth mechanism: oxidation of mitochondrial pyridine nucleotides promotes MPT formation by oxidizing mitochondrial membrane protein sulfhydryls, but pyruvate, a powerful mitochondrial reductant, may prevent MPTs by increasing NADH and NADPH concentrations in the mitochondrial matrix (128).

The Future: Clinical Application of Pyruvate

Pyruvate appears to hold promise as a safe, effective inotropic agent for treatment of cardiac stunning and failure in patients. Pyruvate's ability to protect and even enhance cytosolic phosphorylation potential distinguishes it from pharmacological inotropes such as dobutamine that can de-

plete cardiac energy reserves (6). In view of its many favorable effects in myocardium, it is somewhat surprising that pyruvate's clinical application has thus far been extremely limited. Recently, Hasenfuss *et al.* (129) provided the first clinical report of intracoronary pyruvate treatment, in which pyruvate's effects in patients with NYHA class III heart failure were examined. Pyruvate infusions to intracoronary concentrations of 3–6 m*M* markedly improved stroke volume and cardiac index and lowered pulmonary wedge pressure in these patients. Although the mechanisms for these promising pyruvate effects in failing human heart are unknown, it seems likely, in light of the extensive research in animal models described above, that enhancement of myocardial energetics could be central to pyruvate's improvement of human cardiac performance.

Despite its therapeutic potential, the limitations of pyruvate treatment of cardiac insufficiency should be recognized. Pyruvate's inotropic effects require plasma concentrations (≈ 5 m*M*) well above the physiological range. To produce such concentrations in the coronary circulation, the infusion line must be placed near the coronary ostia or advanced into the coronary artery. A small molecule, pyruvate readily diffuses throughout the extracellular fluid and is avidly transported into most cells; thus, intravenous infusion to effective plasma concentrations would require large amounts of pyruvate. Such systemic infusions would impose potentially harmful sodium loads if the sodium salt of pyruvate were administered. The therapeutic range of intracoronary pyruvate, although currently undefined, may have an upper limit: in Laughlin *et al.*'s study in *in situ* canine hearts (96), the heart beat became erratic, and blood pressure fell when plasma pyruvate exceeded 9 m*M*, and, in isolated stunned guinea-pig hearts, Bünger *et al.* (64) found pyruvate to be less effective at concentrations above 10 m*M*. As noted above, pyruvate's salutary effects on cardiac function, although impressive, do not persist after pyruvate is withdrawn. Despite these limitations, pyruvate at supraphysiological concentrations could serve as an effective adjuvant to acute inotropic treatments to improve function and survival of ischemically injured myocardium. Pyruvate would be most effective for treatment of reversibly injured but still viable myocardium. Not surprisingly, pyruvate could not salvage irreversibly injured, infarcted myocardium following prolonged coronary occlusions (130).

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