Influence of Pyruvate on Contractile Performance and Ca\textsuperscript{2+} Cycling in Isolated Failing Human Myocardium

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Background—Application of pyruvate was shown to improve contractile function in isolated animal myocardium and hemodynamics in patients with congestive heart failure. We assessed the influence of pyruvate on systolic and diastolic myocardial function and its subcellular mode of action in isolated myocardium from end-stage failing human hearts.

Methods and Results—In muscle strip preparations, concentration-dependent effects of pyruvate on developed and diastolic force (n=6), aequorin light emission reflecting intracellular Ca\textsuperscript{2+} transients (n=6), and rapid cooling contractures reflecting sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} content (n=11) were measured. Pyruvate resulted in a concentration-dependent increase in developed force and a decrease in diastolic force, with a maximum effect of 155% and 21%, respectively, at 20 mmol/L pyruvate (P<0.05). This was associated with a dose-dependent prolongation of time to peak tension and relaxation time. Pyruvate increased rapid cooling contractures by 51% and aequorin light signals by 85% (at 15 and 20 mmol/L; P<0.05). This indicates increased SR Ca\textsuperscript{2+} content and increased intracellular Ca\textsuperscript{2+} transients. The inotropic effect of pyruvate was still present after elimination of SR Ca\textsuperscript{2+} storage function with 10 μmol/L cyclopiazonic acid and 1 μmol/L ryanodine (n=8). Pyruvate significantly increased intracellular pH from 7.31±0.03 to 7.40±0.04 by BCECF fluorescence (n=6).

Conclusions—The present findings indicate that pyruvate improves contractile performance of failing human myocardium by increasing intracellular Ca\textsuperscript{2+} transients as well as myofilament Ca\textsuperscript{2+} sensitivity. The former seem to result from increased SR Ca\textsuperscript{2+} accumulation and release, the latter from increased intracellular pH. (Circulation. 2002;105:194-199.)

Key Words: contractility • sarcoplasmic reticulum • calcium • hemodynamics • heart failure

During the last decade, considerable progress has been made in long-term therapy of chronic congestive heart failure, whereas advances in treatment of acute heart failure were poor. For inotropic stimulation, only catecholamines or phosphodiesterase inhibitors currently are available, both of which increase intracellular cAMP levels. Although this results in increased intracellular Ca\textsuperscript{2+} turnover, increased heart rate, and increased energy consumption, application of these agents is generally unavoidable in patients with acute heart failure and cardiogenic shock.

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The glycolytic substrate pyruvate was shown to improve myocardial function in normal and postischemic failing myocardium from various animal species.\textsuperscript{1-4} Furthermore, it was shown recently that intracoronary application of pyruvate to patients with congestive heart failure resulted in improved hemodynamics and myocardial function.\textsuperscript{5} In addition, it was recently shown that the expression of the monocarboxylate transporter (MCT1) is upregulated in a rat model of congestive heart failure.\textsuperscript{6} This mechanism might contribute to the salutary effects of supplying pyruvate to patients with heart failure. Thus, pyruvate exhibits the profile of an inotropic agent, which warrants further investigation for its clinical application in the treatment of acute myocardial failure.

Accordingly, the present study was performed to extend findings in animal myocardium reported previously (eg, in postischemic hearts)\textsuperscript{2} to the failing human heart. The effects on systolic and diastolic function and the underlying subcellular mechanism of action of pyruvate were evaluated in isolated myocardium from end-stage failing human hearts. In particular, we tested the hypothesis that the subcellular effects of pyruvate include an increase in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} load, with a subsequent increase in intracellular Ca\textsuperscript{2+} transients and an increase in intracellular pH.

Methods

Muscle Strip Preparation

Experiments were performed in ventricular myocardium from hearts obtained from 48 patients with end-stage failing dilated (n=41) or ischemic (n=7) cardiomyopathy undergoing heart transplantation.

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Mean age of the patients was 52±4 years, and mean ejection fraction was 27±3%; 13 patients were women. Immediately after cardiectomy, a portion of the left ventricular myocardium consisting of normal-appearing tissue (with the least fibrotic tissue) was excised and stored in cardioplegic solution, cooled, and oxygenated by bubbling with 95% O2:5% CO2 as previously described.7,9,10 The cardioplegic solution contained an additional 30 mmol/L 2,3-butanedione monoxime to protect the myocardium during transportation and from injury as a result of cutting at the time of muscle strip dissection.8 For the mechanical measurements, the muscle strips were transferred to the muscle chamber and connected to the force gauge by using fine steel hooks (F30 type 372, Hugo Sachs Elektronik). The muscles were submerged in modified oxygenated Krebs-Henseleit buffer (KHB) at 37°C containing (in mmol/L): Na+ 152, K+ 3.6, Cl− 135, HCO3− 25, Mg2+ 0.6, H2PO4− 1.3, SO4− 0.6, Ca2+ 2.5, glucose 11.2, and insulin 10 IU/L. To perform aequorin measurements and rapid cooling experiments, muscles were mounted in special chambers between miniature clamps and connected to an isometric force transducer (OPT1L, Scientific Instruments) and superfused with KHB.9,10 Isometric twitches were evoked with stimulation voltage 20% above threshold (duration, 5 ms) at a length at which maximum steady-state twitch force was reached (Lmax).7,9,10 Cross-sectional area for normalization of force values was calculated as the ratio of blotted weight to Lmax. Average cross-sectional area of the muscles investigated was 0.48±0.03 mm2.

The study protocols were reviewed and approved by the ethics committee and were in accordance with institutional guidelines.

Experimental Protocols

Protocol 1: Concentration-Dependent Effects of Pyruvate on Isometric Contractions

Twitch force, rates of force rise and fall, and timing variables were measured from the recordings (WR 3310, Graphtec). During steady-state stimulation (0.5 Hz) in KHB to which propranolol and prazosin (1 μmol/L) were added, isometric contractions were recorded. Thereafter, pyruvate was applied at concentrations of 1, 5, 10, and 20 mmol/L in 6 muscle strips. For comparison, isometric force was measured with increasing concentrations of glucose (5, 10, and 20 mmol/L; n=4; not shown).

Protocol 2: Influence of Pyruvate on Aequorin Light Emission

After steady-state force values had been reached at Lmax, electrical stimulation was switched off for 5 minutes, and the Ca2+-regulated bioluminescent photoprotein aequorin was microinjected into the quiescent muscle just beneath the endocardium as described previously.9 Aequorin measurements were performed at 1-Hz stimulation frequency before and after application of pyruvate at concentrations of 5, 10, and 20 mmol/L. Aequorin light emission was analyzed as the amplitude of the signal between peak systolic light emission and diastolic baseline values (mV amplifier output) in 6 muscle strips. In addition, the influence of isoproterenol (10 μmol/L) on isometric force and aequorin light emission was studied for comparison (n=7).

Protocol 3: Influence of Pyruvate on Rapid Cooling Contractures

To investigate SR Ca2+ content, rapid cooling contractures (RCCs) were elicited at steady-state conditions essentially as previously described.9,10 On cooling from 37°C to 1°C, all Ca2+ from the SR is released, which leads to a stable contracture of the muscles because all Ca2+ transport systems are blocked by the low temperature. The amplitude of the contracture reflects SR Ca2+ content. Twitch force and RCCs were measured during control and at pyruvate concentrations of 5, 10, and 15 mmol/L under steady-state conditions at 1-Hz stimulation frequency in 11 muscle strips.

Protocol 4: Effects of Pyruvate With SR Ca2+ Storage Function Blocked

To investigate the role of the SR in the contribution to the inotropic effect of pyruvate, we used a protocol in which SR Ca2+ storage function was blocked. After assessment of the inotropic response (n=8; 10 mmol/L pyruvate), pyruvate was washed out, and contractility returned to baseline conditions. Thereafter, 1 μmol/L ryanodine and 10 μmol/L cyclopiazonic acid were added to the perfusate to pharmacologically block the SR.10,11 RCCs were performed to check the effectiveness of this protocol. Approximately 20 minutes after application of ryanodine and cyclopiazonic acid, force stabilized on a new baseline that was characterized by decreased developed force, increased diastolic force, and prolonged twitch-timing. The absence of any RCCs confirmed a complete blockage of SR Ca2+ handling. Pyruvate was applied again, and the inotropic response was recorded under blocked SR Ca2+ storage function.

Protocol 5: Influence of Pyruvate on Intracellular pH

To investigate intracellular pH, muscle strip preparations were loaded with membrane-permeable BCECF-AM. The loading solution was made by adding BCECF-AM to the KHB to give a final concentration of 15 μmol/L. The muscles were incubated in this solution for 2 hours. After loading, the muscles were attached in a specially designed setup (Scientific Instruments) and illuminated by a 100-W mercury lamp (Ushio). The light was passed alternatively through 440- and 495-nm band-pass filters, rotating at 125 Hz. Excitation light was focused on the muscle, and the BCECF fluorescence light emitted from the muscle at each excitation wavelength was directed through a 535-nm band-pass filter. The fluorescence intensities at each excitation wavelength were measured by a photomultiplier, and the fluorescence ration F495/F440 was calculated. To minimize photobleaching, sampling intervals were selected during the protocol (20 seconds in duration, every minute for the first 10 minutes, than every fifth minute for the following 20 minutes). Twitch force and BCECF fluorescence were measured during control and at pyruvate concentrations of 10 mmol/L under steady-state conditions at 1-Hz stimulation frequency in 6 muscle strips from 6 end-stage failing hearts. At the end of each experiment, fluorescence emission was calibrated by the high-K+ nigericin method.12 The calibration solution contained (mmol/L): KCl 140, MgCl2 1.2, HEPES 5.0, nigericin 0.01, and 2,3-butanedione monoxime 30. Buffer pH was adjusted with KOH to 5 different values ranging from 6.8 to 7.6.

Statistical Analysis

Data are expressed as mean±SEM. Differences between control and measurements taken after interventions were compared by repeated-measures ANOVA followed by Tukey’s test, or a t test followed by Bonferroni-Holm transformation was applied. Statistical significance was taken as P<0.05.

Results

Pyruvate resulted in a concentration-dependent increase in systolic force and decrease in diastolic force (Figure 1A). The decrease in diastolic force occurred at 1 mmol/L, and the increase in systolic force was statistically significant at pyruvate concentrations of 5 mmol/L and higher (P<0.05). Pyruvate was associated with increased time to peak force at concentrations ≥5 mmol/L and increased time to 50% relaxation at concentrations ≥10 mmol/L (Table; P<0.05). In contrast to pyruvate, increasing concentrations of glucose were not associated with an increase in contractile force. At 5 mmol/L glucose, developed force was 9.7±0.7 mN/mm2 and at 20 mmol/L it was 10.0±0.7 mN/mm2 (n=4; P=NS; not shown). There were no significant differences in contractile behavior between myocardium from hearts with dilated or ischemic cardiomyopathy.

In rapid cooling experiments, developed force increased by 67% from control (8.9±2.4 mN/mm2) with 15 mmol/L pyruvate (P<0.05). The increase in developed force was associated with a concentration-dependent increase in rapid
cooling contractures, which amounted to 51% at 15 mmol/L pyruvate \((P<0.05)\). Similar to the results in Figure 1A, there were no significant differences in twitch force or RCCs between myocardium from hearts with dilated or ischemic cardiomyopathy: Basal twitch force in muscles from dilated hearts was 9.1\(\pm\)3.1 mN/mm\(^2\) and 8.6\(\pm\)4.0 mN/mm\(^2\) in ischemic hearts \((P=0.94)\). In the presence of 15 mmol/L pyruvate, developed force increased by 71% in dilated hearts and by 52% in ischemic hearts \((P=0.55)\), whereas RCCs increased by 53% and 47%, respectively \((P=0.83)\). This suggests that pyruvate concentration-dependently increases SR Ca\(^{2+}\) content in ischemic and dilated cardiomyopathy (Figure 1B).

As shown in Figure 2, the inotropic effect of pyruvate was associated with an increase in aequorin light signals. The increase in developed force by 96% (from 7.0\(\pm\)1.5 mN/mm\(^2\)) with pyruvate (20 mmol/L) was associated with an increase in aequorin light signal by 85%, indicating increased Ca\(^{2+}\) transients \((P<0.05)\). In comparison, isoproterenol (10 \(\mu\)mol/L) increased contractile force by 118%, which was associated with an increase in aequorin light emission by 406% \((P<0.05)\). The increase in aequorin light emission was significantly higher with isoproterenol compared with pyruvate, whereas the increase in force with both interventions was not significantly different.

To study the contribution of the SR to the effects of pyruvate, SR function was blocked. Before SR block, 10 mmol/L pyruvate significantly increased twitch force and RCC amplitudes (Figure 3; \(P<0.05\)). On washout, both force

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>1 mmol/L</th>
<th>5 mmol/L</th>
<th>10 mmol/L</th>
<th>20 mmol/L</th>
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</thead>
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<tr>
<td>Developed force, mN/mm(^2)</td>
<td>10.0(\pm)2.4</td>
<td>10.9(\pm)2.7</td>
<td>17.3(\pm)3.4*</td>
<td>20.7(\pm)3.6*</td>
<td>25.2(\pm)3.9*</td>
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<tr>
<td>Time to peak force, ms</td>
<td>222(\pm)13</td>
<td>221(\pm)13</td>
<td>252(\pm)14*</td>
<td>275(\pm)16*</td>
<td>293(\pm)16*</td>
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<td>Time to 50% relaxation, ms</td>
<td>137(\pm)6</td>
<td>136(\pm)6</td>
<td>148(\pm)9</td>
<td>155(\pm)9*</td>
<td>165(\pm)10*</td>
</tr>
<tr>
<td>Maximum rate of force rise, mN/mm(^2)/s</td>
<td>63(\pm)13</td>
<td>67(\pm)15</td>
<td>97(\pm)17*</td>
<td>109(\pm)17*</td>
<td>127(\pm)18*</td>
</tr>
<tr>
<td>Maximum rate of force fall, mN/mm(^2)/s</td>
<td>51(\pm)12</td>
<td>58(\pm)15</td>
<td>86(\pm)17*</td>
<td>101(\pm)17*</td>
<td>115(\pm)18*</td>
</tr>
</tbody>
</table>

Time to 50% relaxation indicates time from peak force to 50% relaxation. \(n=6. \) *\(P<0.05\) vs control.

In Figure 1, A, Graph shows concentration-dependent effects of pyruvate on systolic (○) and diastolic (□) force \((n=6)\). Experiments were performed in presence of propranolol and prazosin (1 \(\mu\)mol/L). B, Influence of pyruvate on developed force (○) and rapid cooling contractures (□). The latter reflects sarcoplasmic reticulum Ca\(^{2+}\) load. Basal developed twitch force without pyruvate was 8.9\(\pm\)2.4 mN/mm\(^2\) \((n=11)\). *\(P<0.05\) vs control.

Figure 2. Influence of pyruvate and isoproterenol on isometric twitch force and aequorin light emission. Upper part: Left, original recordings of force and aequorin light emission before and after application of pyruvate (20 mmol/L); right, original recordings of force and aequorin light emission before and after application of isoproterenol (10 \(\mu\)mol/L). Lower part: Change in developed force and aequorin light emission after application of pyruvate \((n=6)\) or isoproterenol \((n=7)\) compared with control conditions before addition of pyruvate or isoproterenol (100%). Basal developed twitch force without pyruvate was 7.0\(\pm\)1.5 mN/mm\(^2\). *\(P<0.05\) vs control.
and RCCs returned to baseline conditions (P = NS). Under a full SR block, developed force decreased by 36% (P < 0.05) and RCCs were completely absent (P < 0.05). Diastolic force increased by 33% (P < 0.05). Pyruvate under SR block increased twitch force by 324 ± 67% of baseline (P < 0.05). Most interestingly, without SR block, diastolic force decreased from 4.6 ± 0.7 to 4.2 ± 0.7 mN/mm² after application of pyruvate (P < 0.05). When the SR was blocked, pyruvate did not result in a decrease of diastolic force but increased diastolic force in each muscle strip, with an average increase from 5.8 ± 1.8 to 6.1 ± 1.9 mN/mm² (P < 0.05).

Figure 4 shows a representative experiment of a muscle strip loaded with the pH fluorescence indicator BCECF. Addition of pyruvate resulted in an initial dip in twitch force, followed by a sustained increase in twitch force that was paralleled by an increase in pH, represented by the increase in the F<sub>690/F<sub>484 ratio. At the end of the experiment, the typical calibration procedure is shown. In this muscle strip, isometric twitch force increased from 5.3 to 8.9 mN, and pH increased from 7.24 to 7.36. Figure 5 summarizes experiments from 6 muscle preparations from 6 failing hearts. After an initial dip in twitch force (decline from 16.9 ± 2.6 to 23.7 ± 3.7 mN/mm² (P < 0.05) and pH, from 7.31 ± 0.03 to 7.40 ± 0.04 (P < 0.05).}

**Discussion**

The present study shows that pyruvate concentration-dependently increases developed force and decreases diastolic force in failing human myocardium. At a subcellular level, increased developed force after pyruvate application was associated with increased Ca<sup>2+</sup> activation of contractile proteins, as indicated by increased aequorin light signals. For a given increase in force, the increase in the aequorin light signal with pyruvate was significantly smaller than that observed with the catecholamine isoproterenol. The increased Ca<sup>2+</sup> transients probably resulted from increased SR Ca<sup>2+</sup> load with subsequent increased SR Ca<sup>2+</sup> release. This is indicated from the rapid cooling contracture measurements. Accordingly, one may speculate that the effects of pyruvate on contractile function resulted solely from increased SR Ca<sup>2+</sup> uptake, as recently suggested: (1) decreased diastolic force may be the consequence of decreased diastolic Ca<sup>2+</sup> levels and decreased diastolic activation of contractile proteins, and (2) increased developed force may be due to increased SR Ca<sup>2+</sup> content and subsequent increased Ca<sup>2+</sup> release and systolic activation of contractile proteins.

However, the inotropic effect of pyruvate was also observed after blockade of SR function. This indicates that other mechanisms besides increased SR Ca<sup>2+</sup> release contribute to the increase in developed force observed with pyruvate. One possibility is increased Ca<sup>2+</sup> sensitivity, which could result from increased intracellular pH. BCECF measurements in the present study exhibited a pyruvate-mediated, stable rise of intracellular pH above baseline levels after a transient decline shortly after the application of pyruvate. Pyruvate enters the myoplasm together with one proton through the sarcolemmal monocarboxylate-proton symporter. This may transiently acidify the myoplasm, thus reducing Ca<sup>2+</sup> sensitivity of the release and systolic activation of contractile proteins.
myofilaments and twitch force. In the second phase, the increase in pH may result from pyruvate uptake into the mitochondria through the monocarboxylate-proton symporter. The subsequent increase of intracellular pH may result in increased Ca\textsuperscript{2+} sensitivity of the myofilaments. Increased Ca\textsuperscript{2+} sensitivity could explain the prolongation of relaxation times at higher pyruvate concentrations and the rise of developed force when pyruvate was applied in the presence of blocked SR Ca\textsuperscript{2+} storage function. It should be noted that an increase in pH has been shown to induce a prolongation of twitches due to increased myofilament Ca\textsuperscript{2+} sensitivity.

Of note, in the pH experiments, baseline developed force was higher as compared with baseline force in the other experiments of the study (Figures 1 through 3). However, the percent increase in twitch force in all experiments was similar (eg, at 10 mmol/L pyruvate used in pH experiments). Furthermore, an increase in pH (after an initial decrease) has been observed in each muscle strip independent from the absolute value of baseline force. Therefore, we believe that these differences in baseline force do not invalidate our interpretation that an increase in pH with pyruvate significantly contributes to the inotropic effect.

The transient decrease in pH during the first few minutes after pyruvate application may contribute to the subsequent inotropic effect and the increased Ca\textsuperscript{2+} transients: The increase in proton concentration may stimulate the Na\textsuperscript{+}/H\textsuperscript{+} antipporter with a subsequent increase in intracellular Na\textsuperscript{+} concentration and activation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, leading to net Ca\textsuperscript{2+} influx by reversal mode of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger.

The effects of pyruvate on diastolic force is intriguing and can be explained by lower diastolic Ca\textsuperscript{2+} caused by increased SR Ca\textsuperscript{2+} accumulation. Furthermore, the pyruvate-induced decrease in diastolic force indicates that in the failing human heart, active force generation is present and contributes to disturbed diastolic function of the myocardium. The combination of prolonged relaxation and yet reduced diastolic force observed with pyruvate may be explained by increased Ca\textsuperscript{2+} sensitivity and decreased diastolic Ca\textsuperscript{2+} levels. Interestingly, the decrease in diastolic force already occurred at lower pyruvate concentrations than the increase in developed force (Figure 1A). Of note, at coronary arterial concentrations of 3 to 6 mmol/L, pyruvate exhibited a significant decrease in pulmonary capillary wedge pressure, whereas stroke volume was increased in a recent clinical study.

Pyruvate has numerous molecular effects that may contribute to its actions on contractile force and Ca\textsuperscript{2+} cycling. These include (1) an increase in phosphorylation potential, (2) a reduction of inorganic phosphate that could affect force development independent from its effect on phosphorylation potential, (3) a decrease in hydrogen ion concentration, and (4) a modulation of the cytosolic redox state. The common mechanism relevant for inotropic stimulation and the decrease in diastolic force may be an increase in phosphorylation potential and an increase in free energy of ATP hydrolysis with subsequent energetic stimulation of SR Ca\textsuperscript{2+}-ATPase. The SR Ca\textsuperscript{2+}-ATPase has a high free energy requirement and is known to be sensitive to changes in the free energy of ATP hydrolysis after changes in phosphorylation potential. Pyruvate increases phosphorylation potential predominantly by its effects on the Krebs cycle as a substrate. In addition, by anaplerotic carboxylase pathways, pyruvate increases the total tricarboxylic acid cycle pool size, resulting in increased flux through the Krebs cycle.

These effects of pyruvate have been suggested from a recent nuclear magnetic resonance study in isolated rabbit hearts. In this study, it was shown that pyruvate increases phosphocreatine and decreases inorganic phosphate, resulting in increased free energy available from ATP hydrolysis. Furthermore, by using a F-nuclear magnetic resonance method to measure ionized Ca\textsuperscript{2+}, the authors showed that SR Ca\textsuperscript{2+} gradient was increased with pyruvate.

In summary, the present findings suggest that metabolic intervention with pyruvate may represent an important, previously unrecognized principle to improve systolic and diastolic function in failing human myocardium. This principle may be applicable for the future treatment of patients with acute heart failure and cardiogenic shock.

**References**


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