Potentiation of beta-adrenergic inotropic response by pyruvate in failing human myocardium

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Abstract

Background: Pyruvate has been shown to increase contractile function in isolated myocardium and to improve hemodynamics in patients with congestive heart failure. We tested the hypothesis that pyruvate potentiates the inotropic response to β-adrenergic stimulation and to elevated extracellular calcium, since this may be of potential therapeutic value in the clinical setting of acute heart failure in order to circumvent deleterious effects on energy demand as can occur during catecholamine therapy.

Methods and Results: We investigated isometrically contracting isolated multicellular muscle preparations from terminal failing human hearts at 37 °C, pH 7.4, and a stimulation frequency of 1 Hz. At an extracellular calcium concentration of 1.25 mM, pyruvate (10 mM) alone increased developed force ($F_{dev}$) from 9.0±2.3 to 21.1±4.3 mN/mm ($n=9$, $P<0.001$) and isoproterenol (1 μM) alone increased $F_{dev}$ from 9.5±2.0 to 31.3±5.4 mN/mm ($P<0.001$), whereas the combination of pyruvate and isoproterenol increased $F_{dev}$ over-proportionally from 9.0±2.3 to 47.4±6.4 mN/mm ($P<0.01$). In a separate series we assessed the combination of pyruvate and calcium. Although $F_{dev}$ did not increase from 12 to 16 mM [Ca$^{2+}$]o, 10 mM pyruvate further increased $F_{dev}$ from 25.8±5.0 to 30.6±4.7 mN/mm ($P<0.01$). Rapid cooling contractures revealed that altered myofilament responsiveness and/or sarcoplasmic reticulum (SR) calcium load must underlie the positive inotropic effect of pyruvate. Conclusion: A combination of pyruvate and β-adrenergic stimulation may be of therapeutic value in acute heart failure by reducing the concentrations of potential deleterious catecholamines that are currently necessary to maintain adequate tissue perfusion.

Keywords: Heart failure; Adrenergic (ant)agonists; Calcium (Cellular); Contractile function; Inotropic agents

1. Introduction

Congestive heart failure still remains a therapeutic dilemma, which frequently demands the administration of potential harmful adrenergic agents to maintain adequate tissue perfusion. Enhancement of cardiac contractility is most commonly achieved by means of β-adrenergic stimulation using intravenous catecholamines, i.e. dopamine, dobutamine, epinephrine, norepinephrine, or combinations thereof. The therapeutic goal of restoring adequate tissue perfusion by increasing cardiac output may be partially offset by increased oxygen consumption and induction of potential harmful arrhythmias. It has been reported that for a variety of inotropic agents including catecholamines, calcium-sensitizing agents, phosphodiesterase-inhibitors, and calcium, enhanced inotropy is achieved via increased ATP-consumption [1–3]. If the additional force/pressure production is gained in parallel with an over-proportional ATP-cost, this will result in a worsening of economy of contraction. This scenario may be of particular importance in medium- to high-dose catecholamine therapy of terminal congestive heart failure or ischemic-stunned myocardium.

It has been reported that the glycolytic intermediate pyruvate exerts positive-inotropic effects and improves contractile function in healthy and diseased canine and swine hearts in vivo [4–7], in in vitro perfused rabbit hearts [8], in isolated rat myocytes [9] and in isolated multicellular muscle preparations from failing human myocardium.

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hearts [10]. Furthermore, pyruvate improves hemodynamics in patients with congestive heart failure [11]. Several mechanisms have been postulated underlying the positive inotropic effect of pyruvate. The predominant actions of pyruvate include an increase in the cytosolic phosphorylation potential \([\text{ATP}] / ([\text{ADP}] \times [\text{Pi}])\), a modulation of pH and cytosolic redox state and a reduction of inorganic phosphate [8,12–14]. Pyruvate therefore may act via an increased thermodynamic driving force of the sarcoplasmic (SR) calcium pump, leading to an increased SR calcium gradient [15] and hence an increased calcium-release during contraction.

It has been previously shown that in stunned guinea pig myocardium [16] and in healthy rabbit myocardium, pyruvate potentiates \(\beta\)-adrenergic inotropism [17]. However, presently no data are available from diseased human myocardium and it is unknown whether the results from healthy rabbit myocardium are applicable to the failing human heart. A potentiated \(\beta\)-adrenergic response may be of particular importance in heart failure which is well characterized by a severely impaired \(\beta\)-adrenergic response [18–20]. As a new approach to potentially reduce the clinical need for exogenous adrenergic stimulation and to avoid potential harmful dose-dependent side-effects of catecholamine therapy, we tested the inotropic response of a combination of pyruvate and the \(\beta\)-adrenergic agonist isoproterenol or calcium in isolated multicellular preparations from end-stage failing human hearts.

2. Methods

2.1. Myocardial preparation

Failing human heart tissue was obtained from explanted hearts of patients undergoing heart transplantation for end-stage congestive heart failure: four patients suffered from ischemic cardiomyopathy and five from idiopathic dilative cardiomyopathy. A total of 14 preparations were taken from the left ventricle, and 13 from the right ventricle. Patient characteristics (seven male, two female) were (average±S.D.): age 52.9±8.8 years; weight 76.0±14.8 kg; LV-ejection fraction 22±7\% ; pulmonary capillary wedge pressure 18±10 mmHg; and cardiac index 2.0±0.54 l/(min×m\(^2\)). Medication (number of patients) included angiotensin converting enzyme (ACE)-inhibitors/angiotensin \(\text{AT}\(_1\) \) receptor antagonists (5), digitalis (5), diuretics (7), \(\beta\)-adrenoceptor antagonists (3), calcium channel antagonists (1), and statins (2). All patients had given written informed consent and the investigations conformed to the principles outlined in the Declaration of Helsinki. Hearts were dissected and superfused at room temperature with a modified Krebs-Henseleit (K-H) solution containing (in mM): 116 NaCl, 5 KCl, 1.2 MgCl\(_2\), 2 Na\(_2\)HPO\(_4\), 1.2 Na\(_2\)SO\(_4\), 20 NaHCO\(_3\), 10 glucose, and 0.25 CaCl\(_2\), with the addition of 20 mM 2,3-butanedione monoxime (BDM) as a cardioprotective agent [21]. This solution was in equilibrium with 95\% \(\text{O}\(_2\)/5\% \(\text{CO}\(_2\), resulting in a pH of 7.4. From these hearts, small, free running and unbranched trabeculae were dissected \((n=27)\) as previously described [22,23]. With the aid of a stereo microscope the dimensions of the preparations were measured at 40\(×\) magnification (resolving power ~10 \(\mu\)m). Preparations were mounted in the experimental set-up in the BDM-containing K-H solution, which was immediately switched to a K-H solution without BDM. Average dimensions of the preparations included in the data were 374±31 \(\mu\)m in width, 305±24 \(\mu\)m in thickness, and 2439±179 \(\mu\)m in length (\(n=27\)).

2.2. Experimental apparatus and protocol

Muscles were mounted using two blocks of ventricular tissue at both ends of the longitudinal preparation axis in the experimental set-up between a basket-shaped extension [22,24] of a force transducer (KG-4, Scientific Instruments, Heidelberg, Germany) and a hook connected to a micro-displacement device. Following mounting of the muscle, superfusion with K-H solution (at 37°C) was started and the extracellular calcium concentration ([Ca\(^{2+}\)]\(_{\text{e}}\)) was raised from 0.25 to 1 mM in steps of 0.25 mM every 2–5 min. At the 1.0-mM concentration, stimulation was started through 3–5-ms asymmetric pulses at 20% above threshold voltage (typically 4–6 V) at frequency of 1.0 Hz (reflecting 60 beats per minute). The [Ca\(^{2+}\)]\(_{\text{e}}\) was then further increased to either 1.25 or 2.5 mM depending on the protocol. Next, the muscle was carefully stretched in several small steps until active developed force (\(F_{\text{dev}}\)) did not increase or only slightly further increased upon lengthening, or until diastolic force (\(F_{\text{dia}}\)) exceeded 5 mN/mm\(^2\). This muscle length reflects a sarcomere length of ~2.1–2.2 \(\mu\)m [24,25]. Under these conditions time-dependent deterioration of contractility could be minimized. The muscles were left contracting under these conditions for at least an additional 45 min to equilibrate. After equilibration, a concentration response curve of isoproterenol was measured. From a 10\(^{-4}\) M stock solution (containing 0.3 mM ascorbic acid to prevent oxidation of isoproterenol), the isoproterenol concentration was sequentially set (after stabilizing at the last given concentration) to 1 nM, 10 nM, 100 nM, and finally 1 \(\mu\)M. At this concentration, 10 mM pyruvate was given (from a 1-M stock solution), and after contractile parameters had stabilized the preparation was superfused with fresh K-H solution, without isoproterenol or pyruvate at the same [Ca\(^{2+}\)]\(_{\text{e}}\) to wash out these compounds. After completion of the wash-out (stabilization of contractile parameters), 10 mM pyruvate was again applied. This dose was used because it has shown near maximal inotropic effects in previous investigations [9,10]. The pH of the superfusate did not change upon addition of 10 mM pyruvate. Now, in the presence of pyruvate, the isoproterenol concentration—
within 1±2 s with custom designed heat/cold-exchangers. Contractility. Application of pyruvate (10 mM) in the
simultaneously rapidly cooling the superfusate to

from each heart was included in the final statistical development after addition of 10 mM pyruvate under 1.25 mM \([Ca^{2+}]_o\). o

during the experiment. Per protocol, only one preparation Fig. 1. Original registration of the time course of isometric force
relaxation (RT, in ms). The program contained an on-line50

in ms), to 50%- (TT, in ms) and to 90%-relaxation50

in mN / mm ), time from stimulation to peak tension (TTP,

Data analysis and statistics

2.3. Data analysis and statistics

Preparations were discarded (n=3, out of 30) when
either maximal developed force during the protocol did not
reach at least 20 mN/mm² or time dependent loss of force
(run-down) during the experiment exceeded 15% per hour.
Data were both collected (1 kHz/channel) and analyzed
off-line with a custom-designed data-acquisition program
written in LabView (National Instruments). From twitch
contractions the following parameters were analyzed:
diastolic force (F_diast, in mN/mm²), developed force (F_dev,
in mN/mm²), time from stimulation to peak tension (TTP,
in ms), to 50%- (TT_50, in ms) and to 90%-relaxation (TT_90, in ms) and time from peak tension to 50%
relaxation (RT_50, in ms). The program contained an on-line
analysis mode to quantify these contractile parameters
during the experiment. Per protocol, only one preparation
from each heart was included in the final statistical analysis. Statistical significance was determined by Stu-
dent’s t-test for paired or unpaired data where applicable.
EC_{50} was calculated from the individual experiments and
was subjected to a paired Student’s t-test, i.e. the fit
parameters were treated statistically as if they were ob-
tained by direct measurement [28]. If normality test failed,
a Wilcoxon signed rank test was applied. For analyzing
dose-response measurements (for isoproterenol or cal-
cium) in the presence and absence of pyruvate, repeated
measurements ANOVA (multifactorial) was performed.
Two-tailed values of \(P<0.05\) were accepted as significant.

3. Results

The contractile parameters of the multicellular prepara-
tions were sufficiently stable over time to perform con-
secutive concentration–response curves. The time-depen-
dent deterioration of contractile performance of the prepar-
ations was analyzed by comparing contractile parameters
under identical conditions throughout the protocol. \(F_{\text{dev}}\)
was measured during a protocol at 1.25 mM \([Ca^{2+}]_o\), 10
mM pyruvate and 1 \(\mu M\) isoproterenol at intervals of 1.5–2
h and remained nearly unchanged (from 9.5±2.0 to
9.0±2.3 mN/mm², \(P=\text{NS}\)).

First, we investigated the influence of pyruvate on basic
contractility. Application of pyruvate (10 mM) in the
presence of 1.25 mM \([Ca^{2+}]_o\) resulted in a bi-phasic
shaped force development as depicted in Fig. 1. After an
initial transient decrease of \(F_{\text{dev}}\) to ~50–60% of the
starting value after ~4–5 min, \(F_{\text{dev}}\) constantly increased
until a new, higher steady state was reached. Pyruvate
increased \(F_{\text{dev}}\) by 134% from 9.0±2.3 to 21.1±4.3 mN/
mm² \(\langle P<0.05\rangle\); the maximum force development was
reached after 20–30 min.

Second, we investigated the effects of a combination of
pyruvate and isoproterenol on contractility. Fig. 2A shows
the isoproterenol concentration–response curve in the
absence and presence of 10 mM pyruvate (at 1.25 mM

Fig. 1. Original registration of the time course of isometric force
development after addition of 10 mM pyruvate under 1.25 mM \([Ca^{2+}]_o\).
Arrow indicates addition of pyruvate.
Addition of pyruvate resulted in an increase of $F_{dev}$ at each isoproterenol concentration. Pyruvate alone increased $F_{dev}$ by $12.2 \pm 2.6$ mN/mm$^2$ ($P<0.05$) and isoproterenol alone (1 $\mu$M) by $21.9 \pm 4.1$ mN/mm$^2$ ($P<0.05$), whereas the combination of these resulted in an increase of $38.4 \pm 5.2$ mN/mm$^2$ (from $9.0 \pm 2.3$ to $47.4 \pm 6.4$ mN/mm$^2$), which was higher than the addition of the individual effects ($P<0.05$). Thus, the isoproterenol-induced enhancement of contractility was potentiated by pyruvate. Addition of 10 mM pyruvate to saturating isoproterenol concentrations (1 $\mu$M) still induced a significant increase in $F_{dev}$, as shown in Fig. 2B. To exclude concentration-dependent effects of pyruvate, we repeated the experiments with the addition of 3 mM pyruvate ($n=4$) and observed less pronounced but still potentiating effects of pyruvate on isoproterenol-enhanced inotropy: pyruvate (3 mM) increased $F_{dev}$ alone by $3.6 \pm 1.8$ mN/mm$^2$ ($P=NS$) and isoproterenol (1 $\mu$M) alone by $15.1 \pm 4.0$ mN/mm$^2$ ($P<0.05$), whereas the combination increased $F_{dev}$ by $20.8 \pm 4.9$ mN/mm$^2$ ($P<0.05$). Furthermore, we did experiments under elevated baseline calcium of 2.5 mM ($n=4$), which resulted in equal increases of developed force: pyruvate alone resulted in $8.4 \pm 2.0$ mN/mm$^2$ ($P<0.05$), isoproterenol alone in $10.1 \pm 2.5$ mN/mm$^2$ ($P<0.05$), and a combination of pyruvate and isoproterenol in $22.6 \pm 5.4$ mN/mm$^2$ ($P<0.05$).

The contractile and twitch timing parameters are given in Table 1. Isoproterenol decreased time to peak tension and relaxation times significantly. In contrast, pyruvate increased twitch timing parameters, while the combination of pyruvate and isoproterenol counter-balanced the effects on twitch timing to a certain extent which is shown in Fig. 3. Remarkably, diastolic force/tension decreased slightly with pyruvate application alone or in combination with isoproterenol ($P<0.05$). Sensitivity of the preparations to isoproterenol stimulation was calculated from the concentration–response

**Table 1**

<table>
<thead>
<tr>
<th>Calcium (mM)</th>
<th>Iso (mM)</th>
<th>Pyruvate (mM)</th>
<th>$F_{dev}$ (mN/mm$^2$)</th>
<th>$F_{max}$ (mN/mm$^2$)</th>
<th>TTP (ms)</th>
<th>$dF/dt_{max}$ (mN mm$^{-2}$ s$^{-1}$)</th>
<th>$dF/dt_{max}$ (mN mm$^{-2}$ s$^{-1}$)</th>
<th>TT$_{50}$ (ms)</th>
<th>RT$_{50}$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>0</td>
<td>0</td>
<td>9.0 ± 2.3</td>
<td>2.4 ± 0.5</td>
<td>118 ± 6.4</td>
<td>65 ± 16</td>
<td>140 ± 30</td>
<td>394 ± 19</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>1.25</td>
<td>0</td>
<td>10</td>
<td>21.1 ± 4.3*</td>
<td>2.1 ± 0.4*</td>
<td>255 ± 11*</td>
<td>140 ± 30*</td>
<td>141 ± 32*</td>
<td>464 ± 27*</td>
<td>131 ± 7*</td>
</tr>
<tr>
<td>1.25</td>
<td>1</td>
<td>0</td>
<td>31.3 ± 5.4</td>
<td>1.4 ± 0.3</td>
<td>170 ± 5</td>
<td>309 ± 51</td>
<td>305 ± 55</td>
<td>310 ± 9</td>
<td>84 ± 3</td>
</tr>
<tr>
<td>1.25</td>
<td>1</td>
<td>10</td>
<td>36.1 ± 6.0*</td>
<td>1.2 ± 0.2*</td>
<td>198 ± 2*</td>
<td>311 ± 56</td>
<td>313 ± 60</td>
<td>363 ± 6*</td>
<td>98 ± 5*</td>
</tr>
<tr>
<td>1.25</td>
<td>1</td>
<td>10</td>
<td>47.4 ± 6.4</td>
<td>2.2 ± 0.5</td>
<td>197 ± 3</td>
<td>404 ± 62</td>
<td>370 ± 62</td>
<td>372 ± 24</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>10</td>
<td>46.6 ± 7.5</td>
<td>1.9 ± 0.6</td>
<td>197 ± 8</td>
<td>398 ± 63</td>
<td>305 ± 44</td>
<td>396 ± 23</td>
<td>112 ± 11</td>
</tr>
</tbody>
</table>

$\text{dF/dt}_{\text{max}}$, maximal derivative of force; $\text{dF/dt}_{\text{max}}$, minimal derivative of force; $F_{max}$, maximal developed force; $F_{max}$, diastolic force; Iso, isoproterenol; RT$_{50}$, time from $F_{dev}$ to 50% relaxation; TTP, time to peak tension; TT$_{50}$, time from stimulation to 90% relaxation.

* Significant difference compared to the paired control ($P<0.05$).
curves of the individual experiments (Fig. 4). Pyruvate sensitized the effect of isoproterenol on $F_{\text{dev}}$, significantly: the EC$_{50}$ (concentration where isoproterenol exerts 50% of its maximal effect) was 47.6±18.6 nM without pyruvate and shifted slightly to the left to 36.6±20.9 nM in the presence of pyruvate ($P<0.05$).

Next we investigated the interaction between calcium and pyruvate regarding the positive inotropic effect (Table 2). We measured force development and rapid cooling contractures at different calcium concentrations, i.e. calcium concentration–response curves before and after addition of 10 mM pyruvate (Figs. 5A and 6). Application of pyruvate again induced an increase in $F_{\text{dev}}$ for each calcium concentration compared to before addition of pyruvate ($P<0.05$). However, compared with the effects of pyruvate on isoproterenol-induced enhancement of contractility, the combination of pyruvate and elevated calcium seemed to exhibit additive, not potentiating, effects on contractile force. Furthermore, the amplitude of the rapid cooling contractures was not dependent on pyruvate longed, but only at high calcium concentrations (12 and 16 mM) again could further increase the amplitude of rapid cooling contractures ($P<0.05$ vs. control). Pyruvate increased the ratio of $F_{\text{dev}}$/RCC-amplitude at low [Ca$^{2+}$]$_o$ of 1.25 mM from 0.65±0.13 to 2.21±0.41 ($P<0.05$), whereas at high [Ca$^{2+}$]$_o$ of 16 mM the ratio did not significantly change. Time from stimulation to peak tension (TTP) was not affected by increasing calcium concentrations but was significantly increased with addition of pyruvate. Time from peak stimulation to half-relaxation (RT$_{50}$) was prolonged, but only at high calcium concentrations (12 and 16 mM) in the absence of pyruvate, indicating impaired diastolic calcium removal; addition of pyruvate at [Ca$^{2+}$]$_o$ of 1.25 mM induced similar changes.

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**Table 2**

<table>
<thead>
<tr>
<th>Calcium (mM)</th>
<th>Pyruvate (mM)</th>
<th>$F_{\text{dev}}$ (mN/mm$^2$)</th>
<th>$F_{\text{dia}}$ (mN/mm$^2$)</th>
<th>RCC (mN/mm$^2$)</th>
<th>TTP (ms)</th>
<th>$dF/dt_{\text{max}}$ (mN mm$^{-2}$ s$^{-1}$)</th>
<th>$dF/dt_{\text{min}}$ (mN mm$^{-2}$ s$^{-1}$)</th>
<th>TT$_{90}$ (ms)</th>
<th>RT$_{50}$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>0</td>
<td>3.1±1.2</td>
<td>4.1±1.0</td>
<td>4.9±1.1</td>
<td>180±5</td>
<td>29±10</td>
<td>-17±7</td>
<td>521±18</td>
<td>133±3</td>
</tr>
<tr>
<td>1.25</td>
<td>10</td>
<td>16.4±4.4*</td>
<td>4.4±0.9</td>
<td>7.4±1.3*</td>
<td>259±15*</td>
<td>111±36*</td>
<td>-103±34*</td>
<td>527±36</td>
<td>145±9</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>22.4±4.5</td>
<td>4.3±1.1</td>
<td>10.0±2.1</td>
<td>209±7</td>
<td>178±39</td>
<td>-121±25</td>
<td>463±21</td>
<td>141±8</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>25.8±5.0</td>
<td>4.6±1.1</td>
<td>12.6±1.9</td>
<td>216±9</td>
<td>195±38</td>
<td>-131±26</td>
<td>484±27</td>
<td>156±11</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td>30.6±4.7*</td>
<td>5.3±1.2</td>
<td>15.8±2.5*</td>
<td>226±13</td>
<td>219±35*</td>
<td>-166±29*</td>
<td>501±37</td>
<td>152±10</td>
</tr>
</tbody>
</table>

$dF/dt_{\text{max}}$, maximal derivative of force; $dF/dt_{\text{min}}$, minimal derivative of force; $F_{\text{dev}}$, maximal developed force; $F_{\text{dia}}$, diastolic force; RT$_{50}$, time from $F_{\text{dev}}$ to 50% relaxation; TTP, time to peak tension; TT$_{90}$, time from stimulation to 90% relaxation.

* Significant difference compared to the paired control ($P<0.05$).
Fig. 5. Influence of calcium and pyruvate on force development and rapid cooling contractures. (A) Average data of developed force during a calcium concentration–response curve before and after addition of 10 mM pyruvate (n=9). Pyruvate significantly increased but did not potentiate the calcium response at each concentration and shifted the curve upwards. (B) Average data from rapid cooling contractures demonstrated increased amplitude of contracture with increasing [Ca\(^{2+}\)]. However, pyruvate induced only non-significant additional effects on RCC amplitudes. (C) Increasing [Ca\(^{2+}\)], from 12 to 16 mM did not result in additional force increase, but application of pyruvate (10 mM) could significantly increase developed force under these conditions (16 mM [Ca\(^{2+}\)]). * Denotes a significant increase compared to baseline measurements without application of pyruvate (P<0.05).

4. Discussion

The present study indicates that a combined inotropic stimulation of failing human myocardium with the β-adrenergic agonist isoproterenol and the glycolytic intermediate pyruvate results in an overproportional increase in contractile performance than can be calculated by a mere addition of the individual inotropic effects. This indicates that pyruvate is capable of potentiating the β-adrenergic inotropic response in failing human heart, whereas the combination of elevated extracellular calcium and pyruvate seems to exhibit additive, not potentiating, effects. These results may be of particular interest in the clinical setting of acute heart failure, where inotropic stimulation with medium- to high-dose catecholamines is frequently inevitable despite their well known side-effects including worsening of energy demand, increasing oxygen need and induction of potentially harmful arrhythmias [2,3]. Co-administration of catecholamines and pyruvate could therefore reduce the necessary therapeutic dose of adrenergic agents which in turn would imply less frequent side-effects.

Under conditions of maximal β-adrenergic stimulation, addition of pyruvate could further increase developed force. This indicates that pyruvate acts independently of β-receptor-G-protein-adenylate cyclase pathway. Addition of pyruvate at higher inotropic baseline conditions resulted in enhanced rapid cooling contractures. Instantaneous cooling of the preparation induces a complete release of the calcium from the SR into the cytosol, resulting in a steady state activation of the myofilaments [26,27]. The increase observed in these rapid cooling contractures indicates that part of the positive inotropic effect of pyruvate is mediated either via (i) increased calcium release of the SR (either absolute or fractional increase), (ii) increased myofilament calcium sensitivity, or (iii) alterations in cross bridge kinetics of the myofilaments.

Previous work has shown that increased SR calcium load is associated with increased calcium transients [9,10]. Together with increased isometric force development at maximal inotropic calcium concentrations, this may be explained by an increased \( V_{\text{max}} \) of the SR calcium pump and subsequently enhanced trans-SR gradient [15]. Previous work has suggested that pyruvate acts in part through thermodynamic stimulation of the SR calcium pump due to an increase of the phosphorylation potential \([\text{ATP}] / ([\text{ADP}] \times [\text{P_i}])\) and free energy available from ATP hydrolysis (\( \Delta G_{\text{ATP}} \)) [12–14]. From the increased ratio of isometric force development to rapid cooling contracture we conclude that the inotropic effect of pyruvate is not exclusively mediated via elevated SR calcium content. Either pyruvate causes an increase of fractional SR cal-
to the healthy rabbit myocardium where we observed a similar overproportional increase in oxygen consumption and processes are achieved by an increased ATP-cost, reflected by energy depletion under catecholamine stimulation [34]. These potentiating effects, which are consistent with recent work on stunned guinea pig myocardium, where pyruvate blunted the energy depletion under increased cAMP levels, adding to the 'wasted' energy under catecholamine stimulation. Additionally, increased futile cycling may occur with our finding that part of the inotropic effect could be mediated via a modulation of calcium kinetics and/or cross bridge cycling kinetics.

The potentiating increase in force development with the combination of isoproterenol and pyruvate is interpreted as a potentiation of β-adrenergic response by pyruvate. Furthermore, we demonstrated a slight increase in isoproterenol-sensitivity after pyruvate administration. These data are consistent with recent work on stunned guinea pig myocardium where pyruvate blunted the energy depletion due to high concentrations of isoproterenol [16], and on healthy rabbit myocardium where we observed a similar potentiation of the β-adrenergic response [17]. These results are even more remarkable in light of the reduced β-adrenergic response in failing human heart due to β-receptor downregulation and/or internalization [19,20]. Compared to data from healthy animal myocardium with the intact β-adrenergic system as the most powerful inotropic mechanism, it could have been expected that the interaction of isoproterenol and pyruvate would, at best, result in an additive effect. Consequently, pyruvate-induced potentiation of inotropic β-adrenergic effects in the setting of heart failure with impaired β-adrenergic system points again to a more downstream mode of action.

Another observation concerning the time course of force development after addition of pyruvate (10 mM) needs to be addressed: a bi-phasic force behavior with an initial decrease to 50–60% of the baseline value over several minutes was observed in all experiments. Subsequently, $F_{dev}$ slowly but continuously increased over the next 10–25 min to a new plateau higher than the initial baseline force value. This initial drop in contractility may be potentially detrimental in patients with acute heart failure, however, in a recent clinical study in patients with stable chronic congestive heart failure, we observed no negative impact of pyruvate administration on invasive hemodynamics [11]. The transient negative inotropic effect could be explained by temporary acidifying of the cytoplasm due to co-transport of $H^+$ and pyruvate by the monocarboxylate symporter [30] thereby reducing force development [31]. Reversal of intracellular acidosis via subsequent mechanisms (e.g. sodium-proton-exchanger, and co-transport of $H^+$ and pyruvate into the mitochondria), may partly explain the transient character of this phenomenon which may be counteracted by the slower positive inotropic effect. During wash-out of pyruvate, the opposite effect on force development was observed; $F_{dev}$ increased by ~10–40% before declining to baseline levels, most likely by the reversal of the mechanism responsible for the wash-in effect.

Inotropic stimulation induces additional energy requirements (i.e. ATP utilization) reflected by increased oxygen consumption for oxidative phosphorylation [1]. If the additional force/pressure generation is achieved via an over-proportional amount of ATP consumption, economy of contraction worsens. This scenario is an inherent drawback of catecholamine treatment in acute heart failure. Beta-adrenergic stimulation induces a desensitization of the myofilaments for calcium through phosphorylation of troponin-I [32] and removes the SR calcium ATPase inhibition through phosphorylation of phospholamban [33]. Thus, a substantial fraction of the increased calcium is ‘wasted’ to overcome the desensitization of the myofilaments. Additionally, increased futile cycling may occur under increased cAMP levels, adding to the ‘wasted energy’ under catecholamine stimulation [34]. These processes are achieved by an increased ATP-cost, reflected by an overproportional increase in oxygen consumption and/or heat production in relation to the accompanying increase in developed force/pressure as has been shown in animal [2,35] and human myocardium [36]. Pyruvate, as a prototype of an energetic stimulating agent, affects energy metabolism by its input into the tricarboxylic-acid cycle (Krebs cycle). The effects of pyruvate seem unique and possibly unrelated to the fact that it is a metabolic fuel; administration of other substrates (acetate, lactate) does not exert strong inotropic effects [17,37]. The beneficial action of glucose-insulin-potassium in patients with myocardial infarction may be partly attributed to increased glycolytic flux with subsequent generation of pyruvate [38–40].

5. Clinical implications

A combination of pyruvate with β-adrenergic agents could possibly ameliorate the deleterious energetic effects of high dose catecholamines and result in a better economy of contraction than is the case with hemodynamically equivalent therapeutic doses of a β-agonist monotherapy. Co-administration of pyruvate could allow reduced catecholamine doses without attenuating the therapeutic efficacy. It may be speculated that the addition of pyruvate might even help to avoid tolerance phenomena of continuous high dose catecholamine treatment.

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