Effects of augmented delivery of pyruvate on myocardial high-energy phosphate metabolism at high workstate

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Ochiai, Koichi, Jianyi Zhang, Guangrong Gong, Yi Zhang, Jingbo Liu, Yun Ye, Xiaoyun Wu, Haiying Liu, Yo Murakami, Robert J. Bache, Kamil Ugurbil, Arthur H. L. From. Effects of augmented delivery of pyruvate on myocardial high-energy phosphate metabolism at high workstate. Am J Physiol Heart Circ Physiol 281: H1823–H1832, 2001.—This study was performed to determine whether the fall in myocardial high-energy phosphates (HEP) that occurs during high workstates can be ascribed to either inadequate glycolytic pyruvate generation and conversion to acyl-CoA or limitation of long-chain fatty acid transport into the mitochondria. This was tested by using infusions of either pyruvate or butyrate in anesthetized dogs. Pyruvate was used because it bypasses the glycolytic sequence of reactions, activates pyruvate dehydrogenase, and increases mitochondrial NADH concentration ([NADHm]) in isolated myocardium, whereas butyrate enters the mitochondria without need for transport by the rate-limiting, palmityl-carnitine transporter. Increasing blood pyruvate from 0.16 ± 0.016 mM to >3 mM did not alter baseline HEP levels determined with 31P nuclear magnetic resonance, but caused an increase in the rate-pressure product and a modest increase in myocardial oxygen consumption (MV˙O2). Infusion of dobutamine + dopamine (each 20 \( \mu \)g·kg\(^{-1}\)·min\(^{-1}\)·iv) increased MV˙O2 and caused decreases of myocardial phosphocreatine (PCr)/ATP. Pyruvate partially reversed the decrease of HEP levels produced by catecholamine stimulation, whereas butyrate had no effect. Neither pyruvate nor butyrate caused an increase of MV˙O2 during catecholamine infusion. Deoxymyoglobin was not detected by \( ^1 \)H magnetic resonance spectroscopy in any group. The data demonstrate that carbon substrate availability to the mitochondria is not the only cause of the reduction of PCr/ATP that occurs at high workstates. Supplemental pyruvate (but not butyrate) attenuated the reduction of PCr/ATP during the high workstates; this may have resulted from direct effects on intermediary metabolism or from other effects such as the free radical scavenging activity of pyruvate.

catecholamines; oxygen consumption; magnetic resonance spectroscopy

IN THE IN VIVO CANINE HEART high-energy phosphate (HEP) levels do not change during moderate increases of cardiac workload produced by catecholamine infusion, i.e., when rate-pressure products (RPP) are <35,000 mmHg·beats·min\(^{-1}\) (1, 26, 36). However, catecholamine infusions that produce RPP >45,000 mmHg·beats·min\(^{-1}\) do cause decreases of myocardial phosphocreatine (PCr) and loss of ATP (36). Furthermore, when a period of high cardiac workstate is terminated by discontinuing catecholamine infusion, PCr levels quickly return to control, whereas ATP levels remain depressed, indicating that a portion of the adenine nucleotide pool has been lost during the high workstate (36). Lastly, during catecholamine-induced high cardiac workstates, pharmacological coronary vasodilation causes a significant increase of myocardial oxygen consumption (MV˙O2), but HEP levels do not recover (36). These observations suggested that demand-induced ischemia might be present during high workstates. However, using \( ^1 \)H magnetic resonance spectroscopy to detect myocardial deoxymyoglobin (Mb-\( \delta \)), we found that the reductions of HEP at very high workstates were not associated with detectable myoglobin desaturation (37). Hence, ischemia, if defined as oxygen limitation of metabolism, was not present at the very high workstates.

Because the decreased myocardial HEP content during high workstates could not be ascribed to oxygen limitation, other explanations must be considered including limitation of production of mitochondrial NADH concentration ([NADHm]), which is primarily generated by the tricarboxylic acid cycle from acyl-CoA, which, in turn, is generated by carbohydrate or fatty acid metabolism. It possible that the metabolic pathways leading to 1) pyruvate generation and/or its conversion to acyl-CoA and/or 2) transport of long-chain fatty acids into the mitochondria and/or their rate of conversion to acyl-CoA are sufficiently limited to cause a decrease of steady-state acyl-CoA concentration and, in consequence, [NADHm] during high workstates. On the basis of previous studies of isolated and in vivo myocardium, we speculated that the reductions of cardiac high-energy phosphate metabolism at high workstate...
of HEP might be prevented by the administration of supplemental carbon substrate (5, 7, 14, 17, 23, 30). We further speculated that if the postulated high workload associated [NADHm] reduction was severe enough to limit the rate as well as the kinetics of oxidative phosphorylation, then carbon substrate supplementation during a high workstate might also increase MnO2.

Pyruvate and butyrate were chosen as the carbon substrates to be administered for the following reasons: Pyruvate, which bypasses the entire glycolytic sequence of reactions including glucose transport into the cell, activates pyruvate dehydrogenase (PDH), and increases [NADHm] in isolated mitochondria, has also been shown to increase HEP in vivo myocardium (5, 17, 30). Short-chain fatty acids enter mitochondria without need for transport by the palmitoyl-carnitine transporter by the palmitoyl-carnitine transporter and have been shown to increase myocardial acyl-CoA levels, to prevent the reduction of acyl-CoA with increased workstates, and to increase [NADHm] in vitro myocardium. They also increase HEP in vivo myocardium (5, 14, 23, 30). The present experiments sought to determine whether the administration of high concentrations of either pyruvate or butyrate could prevent or reverse the reductions in PCR/ATP associated with high workstates and/or cause MnO2 to increase during high workstates.

**METHODS**

Studies were performed in 30 normal mongrel dogs weighing 18–27 kg. All experimental procedures were approved by the University of Minnesota Animal Care Committee and conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1985).

**Experimental Preparation**

After sedation with ketamine (10 mg/kg im), animals were anesthetized with α-chloralose (100 mg/kg iv bolus followed by an infusion of 20 mg/kg−1·h−1), intubated, and ventilated with a respirator with supplemental oxygen to maintain arterial blood gases within the physiological range. A heparin-filled polyvinyl chloride catheter was introduced into the right femoral artery and advanced into the ascending aorta. A filled magnetic resonance (NMR) surface coil (22, 37) was conformed to the 18–27 kg. All experimental procedures were approved by the National Institutes of Health, 1985).

**General Methods of NMR Spectroscopy**

Measurements were performed in a 40-cm bore 4.7-T magnet interfaced with a computer console (SISCO; Fremont, CA). The LV pressure signal was used to gate MR data acquisition to the cardiac cycle, whereas respiratory gating was achieved by triggering the ventilator to the cardiac cycle between data acquisitions (22, 37). 31P and 1H NMR frequencies were 81 and 200.1 MHz, respectively. During each intervention, after steady-state conditions had been reached, Mb-δ data and HEP data were acquired by 1H magnetic resonance spectroscopy and 31P magnetic resonance spectroscopy, respectively.

**Spatially Localized 31P NMR Spectroscopic Technique**

31P spectra were recorded in late diastole with a pulse repetition time of 6–7 s. This repetition time allowed full relaxation for ATP and inorganic phosphate (Pi) resonances, and ~90% relaxation for the PCR resonance. PCR resonance intensities were corrected for this minor saturation (22, 37). Radiofrequency transmission and signal detection were performed with the 28-mm diameter surface coil. A capillary containing 15 μl of 3 M phosphonoacetic acid was placed at the coil center to serve as a reference. The proton signal from water detected with the surface coil was used to homogenize the magnetic field, to adjust the position of the animal in the magnet so that the coil was at or near the magnet and gradient isocenters, and to determine the spatial coordinates for spectroscopic localization (22, 37). Chemical shifts were measured relative to PCR, which was assigned a chemical shift of -2.55 ppm relative to 85% phosphoric acid at 0 ppm.

Spatial localization across the LV wall was performed with the RAPP-ISIS/PSW method. Detailed data with regard to voxel profiles, voxel volume, and documentation of the accuracy of the spatial localization have been published elsewhere (11, 12, 27). Briefly, signal origin was restricted using B0 gradients and adiabatic inversion pulses to a column coaxial with the surface coil and perpendicular to the LV wall; column dimensions were 17 mm × 17 mm. Within this column, the signal was further localized using the B1 gradient to five voxels centered about 45, 60, 90, 120, and 135° spin rotation. The position of the voxels relative to the coil was set using the B1 magnitude at the coil center that was experimentally determined in each case by measuring the 90° pulse length for the phosphonoacetic acid reference located in the coil center. Each set of spatially localized transmural spectra consisted of a total of 96 scans accumulated in a 10-min block. Resonance intensities were quantified using integration routines provided by SISCO software. The numerical values for PCR and ATP in each voxel were expressed as ratios of PCR to ATP (PCR/ATP). Pi levels were measured as change from baseline values (ΔP), using integrals obtained in the region covering the Pi resonance and reported as ΔP/PCR.

**2H NMR Spectroscopic Technique**

2H NMR methods for measuring Mb-δ have been previously described (6, 37). In brief, radiofrequency transmission and signal detection were performed with the dual-tuned, 28-mm diameter surface coil. A single-pulse collection sequence with a 1-ms frequency selective Gaussian excitation pulse was used to selectively excite the Mb-δ resonance. A short repetition time (25 ms) was used, due to the short T1 of Mb-δ. Each spectrum was acquired in 5 min (10,000 free induction decays). Although the short T1 of Mb-δ and fast
acquisition prevent gating to the cardiac cycle, signal loss due to motion was negligible due to the inherently broad line width of the MB-5 peak. Resonance intensities were quantified using integration routines provided by the SISCO software. To verify that MB-5 could be detected when it was known to be present, a total coronary occlusion was produced at the end of the experiment and a large MB-5 resonance was observed. For the purpose of estimating intracellular $P_{O2}$, a $d$ at the end of the experiment and a large MB-5 resonance was assumed and $P_{O2}$ was calculated from the Hill equation (37). It was assumed that at baseline when no MB-5 resonance could be detected, myoglobin was $\sim 10\%$ desaturated and during coronary occlusion was $\sim 95\%$ desaturated (6).

**Myocardial Blood Flow and $MV_\dot{O}2$ Measurements**

Myocardial blood flow and $MV_\dot{O}2$ were measured with radioactive microspheres, 15 $\mu$m in diameter, labeled with $^{141}$Ce, $^{51}$Cr, $^{85}$Nb, $^{85}$Sr, or $^{46}$Sc (New England Nuclear, Boston, MA) as previously described (37). $MV_\dot{O}2$ was calculated from the $PO2$ determination.

**Tissue Preparation**

At the end of the study, the heart was fixed in 10% buffered formalin. The atria, right ventricle, aorta, and large epicardial vessels were dissected from the LV. The region of myocardium beneath the surface coil was sectioned into three transmural layers from epicardium to endocardium, weighed, and placed into vials for counting.

**Experimental Protocol**

Aortic and LV pressures were measured with pressure transducers positioned at midchest level and recorded on an 8-channel direct writing recorder (Coulbourne Instruments, Lehigh Valley, PA). LV pressure was recorded at normal and high gain for measurement of end-diastolic pressure. Hemodynamic measurements and magnetic resonance spectroscopy spectra were first obtained under basal conditions. Mid-wall myocardium (MID) and subendocardium (ENDO), respectively.

Data obtained during different experimental conditions within the same group were compared using one-way ANOVA with replications. A value of $P < 0.05$ was required for significance. When a significant result was found, individual comparisons were made using the Scheffé method. All values are expressed as means $\pm SE$.

**RESULTS**

Tables 1, 2, and 3 summarize hemodynamic data, myocardial blood flow data, and the HEP data, respec-

<table>
<thead>
<tr>
<th>Table 1. Hemodynamic data</th>
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<tbody>
<tr>
<td>$n$</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Group I</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Pyruvate</td>
</tr>
<tr>
<td>DbDp + pyruvate</td>
</tr>
<tr>
<td>Group II</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>DbDp</td>
</tr>
<tr>
<td>DbDp + pyruvate</td>
</tr>
<tr>
<td>Group III</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>DbDp</td>
</tr>
<tr>
<td>DbDp + butyrate</td>
</tr>
</tbody>
</table>

Values are means $\pm SE$; $n$ = number of dogs. LV, left ventricle; Db, dobutamine; Dp, dopamine. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. pyruvate.
pressure, and RPP. Mean myocardial blood flow increased LV systolic pressure, heart rate, mean aortic pressure (RPP), while the arterial pyruvate concentration caused modest, but significant, increases of LV systolic pressure (Fig. 1). The baseline spectra of the animals in each group. Table 4 summarizes the MV$_{O2}$ data in the subsets of animals in each group.

**Group I.** In the animals in group I, pyruvate infusion caused modest, but significant, increases of LV systolic pressure and the heart rate times systolic blood pressure (RPP), while the arterial pyruvate concentration increased from a baseline value of 160 ± 16 µM to 3.5 ± 0.4 mM before the onset of catecholamine infusion (P < 0.01). Catecholamine infusion markedly increased LV systolic pressure, heart rate, mean aortic pressure, and RPP. Mean myocardial blood flow increased from a baseline value of 0.55 ± 0.07 to 0.86 ± 0.12 (P < 0.05) and 2.62 ± 0.69 ml·min·g$^{-1}$·100 g$^{-1}$·min$^{-1}$ during pyruvate infusion (P < 0.05) and then markedly increased to 34.1 ± 4.3 ml·100 g$^{-1}$·min$^{-1}$ during catecholamine infusion.

Baseline 31P magnetic resonance spectra were characterized by high PCr and ATP, whereas Pi was too low to be detected (Table 3). Pyruvate did not significantly alter the baseline spectra. Catecholamine infusion caused significant increases of Pi and decreases of PCr and ATP in every myocardial layer (Fig. 1). The baseline PCr/ATP were 2.25 ± 0.07, 2.14 ± 0.06, and 1.90 ± 0.05 for EPI, MID, and ENDO, respectively, and were unchanged during pyruvate infusion (Table 3). When catecholamine was infused during the pyruvate infusion, PCr/ATP fell significantly in all myocardial layers (to 1.96 ± 0.07, 1.75 ± 0.08, and 1.69 ± 0.09 for EPI, MID, and ENDO, respectively). ΔP/PCr was undetectable at baseline and during pyruvate alone but increased to 0.28 ± 0.04, 0.26 ± 0.05, and 0.21 ± 0.05 for EPI, MID, and ENDO, respectively, during catecholamine infusion.

**Group II.** Catecholamine infusion resulted in significant increases of LV systolic pressure, heart rate,

Table 2. *Myocardial blood flow*

<table>
<thead>
<tr>
<th>Group</th>
<th>EPI</th>
<th>MID</th>
<th>ENDO</th>
<th>Mean</th>
<th>ENDO/EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.54 ± 0.07</td>
<td>0.54 ± 0.07</td>
<td>0.58 ± 0.08</td>
<td>0.55 ± 0.07</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.83 ± 0.11*</td>
<td>0.84 ± 0.11*</td>
<td>0.92 ± 0.16*</td>
<td>0.86 ± 0.12*</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td>DbDp + pyruvate</td>
<td>2.51 ± 0.57†</td>
<td>2.39 ± 0.53†</td>
<td>2.96 ± 0.99†</td>
<td>2.62 ± 0.69†</td>
<td>1.08 ± 0.13</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.57 ± 0.11</td>
<td>0.63 ± 0.11</td>
<td>0.70 ± 0.11</td>
<td>0.64 ± 0.11</td>
<td>1.25 ± 0.09</td>
</tr>
<tr>
<td>DbDp</td>
<td>1.39 ± 0.23*</td>
<td>1.61 ± 0.24*</td>
<td>1.87 ± 0.28*</td>
<td>1.63 ± 0.23*</td>
<td>1.45 ± 0.24</td>
</tr>
<tr>
<td>DbDp + pyruvate</td>
<td>1.69 ± 0.24*</td>
<td>1.74 ± 0.21*</td>
<td>1.71 ± 0.25*</td>
<td>1.71 ± 0.21*</td>
<td>1.06 ± 0.14</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.73 ± 0.12</td>
<td>0.77 ± 0.14</td>
<td>0.90 ± 0.14</td>
<td>0.79 ± 0.13</td>
<td>1.29 ± 0.11</td>
</tr>
<tr>
<td>DbDp</td>
<td>1.94 ± 0.29*</td>
<td>1.88 ± 0.24*</td>
<td>1.94 ± 0.24*</td>
<td>1.93 ± 0.24*</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>DbDp + butyrate</td>
<td>2.88 ± 0.45*</td>
<td>3.12 ± 0.44‡</td>
<td>3.12 ± 0.48‡</td>
<td>3.05 ± 0.46‡</td>
<td>1.06 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. baseline; †P < 0.05 vs. pyruvate; ‡P < 0.05 vs. DbDp.

Table 3. *Myocardial HEP and Pi data*

<table>
<thead>
<tr>
<th>Group</th>
<th>∆P/PCr</th>
<th>EPI</th>
<th>MID</th>
<th>ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td>2.25 ± 0.07</td>
<td>2.14 ± 0.06</td>
<td>1.90 ± 0.05§</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2.29 ± 0.07</td>
<td>2.15 ± 0.05</td>
<td>2.04 ± 0.05</td>
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<tr>
<td>DbDp + pyruvate</td>
<td>0.28 ± 0.04†</td>
<td>0.26 ± 0.05†</td>
<td>0.21 ± 0.05†</td>
<td>1.96 ± 0.07†</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>2.29 ± 0.12</td>
<td>2.02 ± 0.07</td>
<td>1.92 ± 0.06§</td>
</tr>
<tr>
<td>DbDp</td>
<td>0.27 ± 0.16</td>
<td>0.22 ± 0.12</td>
<td>0.20 ± 0.10</td>
<td>1.72 ± 0.15*</td>
</tr>
<tr>
<td>DbDp + pyruvate</td>
<td>0.19 ± 0.11</td>
<td>0.18 ± 0.10</td>
<td>0.16 ± 0.07</td>
<td>1.90 ± 0.09*</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>2.21 ± 0.11</td>
<td>2.14 ± 0.10</td>
<td>1.99 ± 0.10</td>
</tr>
<tr>
<td>DbDp</td>
<td>0.23 ± 0.06*</td>
<td>0.22 ± 0.06*</td>
<td>0.22 ± 0.06*</td>
<td>1.76 ± 0.09*</td>
</tr>
<tr>
<td>DbDp + butyrate</td>
<td>0.21 ± 0.05*</td>
<td>0.19 ± 0.05*</td>
<td>0.20 ± 0.05*</td>
<td>1.82 ± 0.09*</td>
</tr>
</tbody>
</table>

Values are means ± SE. ∆P, change in inorganic phosphate; PCr, phosphocreatine. *P < 0.05 vs. baseline; †P < 0.05 vs. pyruvate; ‡P < 0.05 vs. EPI. §P < 0.05 vs. EPI.
mean aortic pressure, and RPP. Mean myocardial blood flow increased from a baseline value of 0.64 ± 0.11 to 1.63 ± 0.23 ml·min⁻¹·g⁻¹ during catecholamine infusion (P < 0.05). MVO₂ (in a subset of six dogs in which it was measured) increased from 7.0 ± 1.0 to 13.4 ± 2.4 ml·100 g⁻¹·min⁻¹ during catecholamine infusion (P < 0.05). Infusion of pyruvate during continuing catecholamine infusion did not cause further significant increases in either of these variables, although arterial blood pyruvate levels were averaged ~4.6 mM at the time of the measurements.

Baseline PCr/ATP were 2.29 ± 0.12, 2.02 ± 0.07, and 1.92 ± 0.06 for EPI, MID, and ENDO, respectively. During catecholamine infusion, PCr/ATP fell in all myocardial layers to 1.72 ± 0.15, 1.56 ± 0.10, and 1.52 ± 0.06 for EPI, MID, and ENDO, respectively (P < 0.05 vs. baseline for each layer), and ΔP/ΔCr became detectable with values of 0.27 ± 0.16, 0.22 ± 0.12, and 0.20 ± 0.10 for EPI, MID, and ENDO, respectively (Fig. 1). When the pyruvate infusion was initiated while the catecholamine infusion continued, PCr/ATP rose in all myocardial layers (1.90 ± 0.09, 1.85 ± 0.09, and 1.80 ± 0.06 in EPI, MID, and ENDO, respectively; P < 0.05 for MID and ENDO). During pyruvate, ΔP/ΔCr trended downward, but this was not significant. Of note, the magnitude of the reduction of PCr/ATP produced by catecholamine infusion in the group I hearts that were pretreated with pyruvate was significantly less than in the group II hearts that had not been pretreated with pyruvate (mean PCr/ATP values for the LV wall were 1.91 ± 0.06 vs. 1.68 ± 0.09, respectively; P < 0.01), and the decrease in PCr/ATP for individual myocardial layers also tended to be lower in the group I hearts (P = 0.08).

Group III. In the animals in group III, catecholamine infusion resulted in significant increases of LV systolic pressure, heart rate, mean aortic pressure, and RPP. Mean myocardial blood flow increased from a baseline value of 0.79 ± 0.13 to 1.93 ± 0.24 ml·min⁻¹·g⁻¹ during catecholamine infusion (P < 0.05). MVO₂ (in a subset of five dogs in which it was measured) increased from 7.6 ± 1.0 to 20.8 ± 2.9 ml·100 g⁻¹·min⁻¹ during catecholamine infusion (P < 0.05). The infusion of butyrate during continuing catecholamine infusion did not cause significant changes in any hemodynamic variable. However, myocardial blood flow increased to 3.05 ± 0.46 ml·min⁻¹·g⁻¹ (P < 0.05 vs. both baseline and catecholamine) and MVO₂ increased to 30.4 ± 5.7 ml·100 g⁻¹·min⁻¹, although this was not significant.

At baseline, PCr/ATP was 2.21 ± 0.11, 2.14 ± 0.10, and 1.99 ± 0.10 for EPI, MID, and ENDO, respectively. During catecholamine infusion, PCr/ATP fell in all myocardial layers (to 1.76 ± 0.09, 1.74 ± 0.09, and 1.77 ± 0.08 for EPI, MID, and ENDO, respectively; P < 0.05 vs. baseline for each layer), and ΔP/ΔCr became detectable with values of 0.23 ± 0.10, 0.22 ± 0.06, and 0.22 ± 0.06 for EPI, MID, and ENDO, respectively (Fig. 1). When butyrate infusion was initiated with continuing catecholamine infusion, neither PCr/ATP nor ΔP/ΔCr were significantly affected.

Myoglobin saturation. No Mb-δ resonance was detected in any heart of any group under basal conditions or during catecholamine and substrate infusions. During coronary artery occlusion (done as a positive control), a prominent Mb-δ resonance appeared at 71 ppm downfield of the water resonance with a high signal/noise ratio (data not shown).

**DISCUSSION**

The main findings of this study are that 1) supplemental pyruvate partially (but significantly) ameliorated the reductions of PCr/ATP that occurred during the high cardiac workstates, 2) supplemental butyrate did not blunt the reductions of PCr/ATP that occurred at the high workstate, and 3) neither pyruvate or butyrate significantly increased MVO₂ during the high workstate. The lack of Mb-δ confirms our earlier finding that limited oxygen availability is not the cause of the high workstate-associated HEP reductions (37). Taken together, the data imply that the availability of carbon substrate directly utilizable by mitochondria did not limit the rate of ATP synthesis at the high workstate. However, the modest but significant effect of pyruvate on PCr/ATP during the high workstate suggests that supraphysiological concentrations of pyruvate may affect the kinetics of oxidative phosphorylation.

**Implications of the PCr/ATP fall during catecholamine infusion.** Creatine kinase is a near equilibrium enzyme and myocardial PCr levels are roughly inversely proportional to calculated cytosolic ADP concentration ([ADP]) levels if total cellular creatine remains constant and there are not marked changes in ATP levels (33). We have previously shown that these conditions are met in in vivo canine myocardium and that calculated free [ADP] is elevated during high workstates, whereas both PCr concentration ([PCr]) and [ATP] are reduced (36). Cytosolic [ADP] is primarily determined by mitochondrial ATP synthesis kinet-
Fig. 1. Typical $^{31}$P magnetic resonance (MR) spectra showing myocardial high-energy phosphate (HEP) and Pi resonances from single heart in groups I–III (GI–GIII), under baseline conditions (A), during pyruvate infusion (GI, B), during high cardiac workstates (GII and GIII, B), and during high cardiac workstate with the supplemental carbon substrate infusion of pyruvate (GI and GII, C) or butyrate (GIII, C). During high cardiac workstates, the phosphocreatine (PCr) to ATP ratio decreased across the left ventricular wall, which was accompanied by accumulation of Pi (*). These changes cannot be corrected by supplemental infusion of pyruvate (groups I and II) or butyrate (group III). EPI, subepicardium; MID, midwall myocardium; ENDO, subendocardium.
ics (7), and changes in cytosolic [ADP] are reflected by changes in cytosolic [PCr] and the PCr/ATP (33). Thus we can conclude that during moderately increased workstates (not associated with HEP changes), mechanisms other than ADP elevation mediate the increased rate of ATP synthesis (27), but at the higher workstates observed in the present study increases of [ADP] are also required to drive the ATP synthetic rate.

Cytosolic O2 during catecholamine stimulation. Myoglobin desaturation was not detected during catecholamine infusion, indicating there was no evidence of inadequate convective or diffusive oxygen transport at high workstates. As discussed in a previous report (37), we assume that in the absence of a detectable Mb-δ resonance ~10% desaturation is present; this level of desaturation predicts an intracellular Po2 >20 mmHg (using the Hill equation and a P50 value of 2.38 mmHg) (29). This Po2 value far exceeds the Michaelis constant of cytochrome oxidase with regard to oxygen and should not affect oxidative phosphorylation kinetics or limit ATP synthesis. Thus the current data confirm our previous finding that inadequate oxygen availability per se does not cause the high workstate-associated reduction of PCr/ATP.

Effects of pyruvate infusion. Pyruvate, when infused during basal workstates, caused modest but significant increases of RPP and MVo2. However, pyruvate had no effect on PCr/ATP. These observations are in contrast to findings in the perfused heart utilizing only glucose; in that model the addition of pyruvate markedly increased PCr/ATP and NADHm (7). The modest nonsignificant response to pyruvate in the basal state is not surprising because the in vivo heart has access to abundant alternative carbohydrate and lipid substrates so that [NADHm] is presumably high and non-limiting. The current data differ somewhat from earlier in vivo canine data reported by Laughlin et al. (17), who also reported a modest but significant increase of PCr/ATP (but not of MVo2) when a comparable amount of pyruvate was infused during basal workstate conditions. However, it should be noted that the physiological changes in both studies were small. The modest in vivo inotropic response to pyruvate under basal conditions in the present study suggests that the much more pronounced inotropic response to pyruvate in isolated myocardium and perfused hearts may be due to the less physiological state of the experimental preparation.

When pyruvate was infused before the initiation of the high workstate (group I), the subsequent infusion of catecholamine induced the typical hemodynamic response and a reduction of PCr/ATP occurred. However, the decrease of PCr/ATP tended to be less than in the group II hearts (which had no preceding pyruvate infusion). Pyruvate administered after the induction of a high work state in the group II hearts caused no significant hemodynamic change and no change of MVo2. However, in these hearts, pyruvate did cause an increase of PCr/ATP. Hence, pyruvate modestly (but significantly) limited the decrease in PCr/ATP produced by catecholamine infusion. However, in neither case was pyruvate supplementation capable of maintaining PCr/ATP at normal levels during the increased workstate.
Based on studies in perfused hearts, a high concentration of pyruvate in the blood and myocytes should have increased [NADH$_m$] and largely corrected the reduced PCr/ATP if the HEP reduction was caused primarily by limited flux of acetyl-CoA into the tricarboxylic acid cycle via PDH (5, 7, 16, 30). The inability of a high concentration of pyruvate to prevent or fully reverse the PCr/ATP reductions or to significantly increase MV$_{O2}$ during the high workstate supports the view that carbohydrate substrate availability and metabolism cannot be the only cause of the PCr/ATP reductions and that carbohydrate substrate availability does not limit the rate of ATP synthesis. However, the modest but significant amelioration of the HEP changes in response to supraphysiological concentrations of pyruvate suggests that this intervention can affect the kinetics of oxidative phosphorylation.

There is evidence that oxidative phosphorylation is kinetically regulated by the concentrations of its primary substrates, which include O$_2$, ADP, P$_i$, and NADH (7). Over a broad range of workstates in the perfused heart (in which the availability of P$_i$ and O$_2$ concentrations are not limiting), the relationship between MV$_{O2}$ and RPP is relatively independent of the carbon substrate utilized (7). In contrast, the relationship between ADP and MV$_{O2}$ is highly sensitive to the specific substrate employed; thus ADP levels are lowest with substrates that induce high NADH$_m$ levels (supraphysiological concentrations of pyruvate or octanoate) and are highest when glucose or glucose plus insulin are the substrate (7). In the latter cases, NADH$_m$ levels are relatively low (5, 16, 30). Hence, if in the current study [NADH$_m$] was within the kinetic regulatory range for oxidative phosphorylation at high workstates, then the administration of supraphysiological concentrations of pyruvate could have increased acyl-CoA levels by means of PDH activation and/or a mass action effect on PDH. In this regard, in an in vivo porcine model, flux through PDH was increased during dobutamine infusion, although the PDH activation state was not increased or decreased (unpublished data cited in Ref. 10). It is of interest that the substantial increase of fatty acid uptake induced by dobutamine might have been expected to cause inhibition of PDH (10). The authors suggested that this increase of flux through PDH was likely mediated by increased pyruvate availability resulting from increased lactate and glucose uptake. These findings support the view that increased pyruvate levels can increase the rate of conversion of pyruvate to acyl-CoA during dobutamine infusion, which could, in turn, cause [NADH$_m$] to increase. An increase of [NADH$_m$] would be expected to result in lower [ADP] and higher [PCr]. As indicated above, if at high workstates [NADH$_m$] was not sufficiently low to limit the rate of oxidative phosphorylation, then the postulated increase in [NADH$_m$] produced by pyruvate would not be expected to increase MV$_{O2}$ despite the fact that it could reduce [ADP] (5, 7, 16, 30).

Effects of butyrate infusion. Butyrate infused during the high workstate had no significant effect on hemo-

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ment for an elevation of cytosolic ADP (31). Importantly, these data indicate that at least in the in vivo right ventricle, alterations of substrate mix can alter the kinetics of oxidative phosphorylation dramatically without reducing the rate of ATP synthesis.

**Alternative mechanisms for pyruvate effects on PCr/ATP.** In addition to its metabolic properties, pyruvate has been shown to act as an antioxidant (2, 34, 35). Therefore, it is possible that the effect of pyruvate on PCr/ATP may depend on antioxidant or other properties rather than effects on [NADHm]. For example, the function of the electron transport chain is facilitated by increases of mitochondrial matrix volume (19), and the terminal component cytochrome oxidase is regulated allosterically by ATP/ADP (13) and also by nitric oxide (NO), which competitively inhibits the binding of O2 to cytochrome oxidase (4, 8). It has recently been reported that the rate of free radical generation by mitochondria is increased by NO and that exercise, which is mimicked by dobutamine infusion, increases both NO and free radical production by the heart (3, 28). Inhibition of the electron transport chain or of its terminal component cytochrome oxidase by NO or by increased levels of other free radical species might reduce the mitochondrial proton motive force sufficiently to require an increase of cytosolic [ADP] to maintain a given rate of ATP synthesis. These effects might be limited by free radical scavenging by pyruvate (2, 34, 35).

**Limitations.** A critical question is whether the pyruvate infusion increased intracellular [pyruvate] sufficiently to induce activation of pyruvate dehydrogenase. It has recently been reported that a 1 mM coronary artery blood concentration of pyruvate [pyruvate] failed to increase intracellular [pyruvate] in an in vivo porcine model (25). However, earlier work in perfused hearts demonstrated that a [pyruvate] perfusate of 2 mM or greater resulted in a substantial increase in the active fraction of PDH (24). In the current study, arterial plasma [pyruvate] was 3.5–4.6 mM at the time HEP measurements were obtained. Laughlin et al. (17) demonstrated that plasma pyruvate levels of 2.86 ± 0.27 mM increased myocardial pyruvate extraction and markedly decreased glucose and lactate extraction in open-chest dogs; the effects on glucose and lactate uptake likely resulted from the well-known intracellular inhibitory effect of pyruvate on glycolysis and the effects of increased extracellular pyruvate on trans-sarcolemmal lactate transport (17). Furthermore, blood [pyruvate] of ~3 mM was able to increase glycoen accumulation rates sixfold in the canine heart when glucose uptake was maintained by glucose and insulin infusion; again, this could only result from inhibition of glycolysis by pyruvate entry into the myocyte (17). Although we were unable to measure the pyruvate uptake rate or intracellular [pyruvate] in the present study, our observations, together with the data cited above, indicate that it is probable that the coronary blood [pyruvate] achieved in the present study resulted in an increased rate of pyruvate uptake and a significant elevation of the cytosolic pyruvate level. An additional limitation of the present study is that we were not able to determine myocardial [NADHm]. Therefore, our discussion of the effects of pyruvate on [NADHm] are speculative, although based on reasonable inferences from studies of isolated myocardium and perfused hearts.

The present findings demonstrate that the reductions of PCr/ATP that occur at high workstates in the canine heart do not indicate limitation of the rate of ATP synthesis as the result of carbon substrate inadequacy or limitation of O2 availability. Hence, other explanations must be sought. High workstate-associated functional limitations at the level of the electron transport chain and especially at its terminal component, cytochrome oxidase, or possibly of ATP synthase could profoundly affect the kinetics of oxidative phosphorylation and are candidates for future study.

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