Relation between phosphate metabolites and oxygen consumption of heart in vivo

LAWRENCE A. KATZ, JULIE A. SWAIN, MICHAEL A. PORTMAN, AND ROBERT S. BALABAN (With the Technical Assistance of S. James): Laboratory of Cardiac Energetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

KATZ, LAWRENCE A., JULIE A. SWAIN, MICHAEL A. PORTMAN, AND ROBERT S. BALABAN. Relation between phosphate metabolites and oxygen consumption in heart in vivo. Am. J. Physiol. 256 (Heart Circ. Physiol. 25): H265-H274, 1989.—The relation between induced increases in cardiac work and phosphate metabolites was investigated in the canine heart in vivo to evaluate the role of ATP hydrolysis products, ADP and inorganic phosphate (Pi), in the control of myocardial oxygen consumption (MV02). In these studies, myocardial blood flow and oxygen consumption were simultaneously measured with the 31P-nuclear magnetic resonance (NMR)-detected phosphate metabolites. Three protocols were used to increase myocardial work: pacing, epinephrine, and phenylephrine infusions. When these protocols were used, no or only slight changes in myocardial ATP, Pi, and creatine phosphate were observed with a greater than threefold increase in MV02. The calculated intracellular free Mg concentration, ADP, and pH were also only slightly affected by these increases in work. These data indicate that a simple model involving the feedback of cytosolic ADP and Pi to the mitochondria regulating respiration is inadequate to explain respiratory control in vivo. These data suggest that some other parameters or cooperativity effects involving the phosphate metabolites must play a role in the feedback between respiration and work in the heart in vivo.

Some of the earliest studies on the energy metabolism of the heart established that over normal work loads the heart is capable of matching its rate of energy conversion, through the production of ATP, with the rate of energy utilization by work (12, 14). Under normal conditions, the heart produces most of its ATP via oxidative phosphorylation in the mitochondria (12, 14). Therefore, this energy conversion process must be closely regulated by the work output or ATP utilization of the heart. The mechanism by which this orchestration of respiration and work occurs is still unclear and is an area of active research (9, 12, 14).

The earliest models dealing with this problem have focused on the ATP hydrolysis products, ADP and inorganic orthophosphate (Pi), produced by the work-generating ATPases (12, 14). Simply put, these models state that the ATP hydrolysis products serve as feedback regulators of intermediary metabolism. As work increases, the rate of ATP hydrolysis increases, resulting in a decrease in ATP and a rise in ADP and Pi, which in turn stimulate the production of ATP at various levels of intermediary metabolism. The control strengths of different steps of the respiratory chain and mitochondrial function have been extensively described in mitochondria in vitro (for reviews see Refs. 10, 12, and 14), but how this potentially complex control network may work in vivo has not been evaluated. Several specific models of in vivo respiratory control have been proposed that involve the ADP or Pi concentrations alone or various combinations of the ATP/ADP, ATP/ADP × P ratios. Models have also been proposed that claim that the control is based on a putative near equilibrium between ATP hydrolysis and the redox state of the respiratory chain (10, 14). The potentially important interaction between respiratory control and creatine kinase in the cytoplasm and on the mitochondrial membrane has also been enumerated (26). However, whether this enzyme system, involving creatine phosphate (CrP) and creatine, plays a role as a cytosolic buffer or "shuttle" of ATP or ADP, the actual active components of respiratory control are still believed to be ATP and its hydrolysis products.

Regardless of which model is correct, all of these models suggest that ATP, ADP, and Pi play an important role in the control of respiration in the intact cell. Therefore, the determination of the relation between respiration and these phosphate compounds helps clarify their role in the regulation of in vivo mitochondrial respiration.

Many in vitro studies have demonstrated that appropriate changes in these phosphate metabolites are not observed with alterations in work (4, 6, 11, 18, 19, 25). Some studies do claim that the control of respiration is explained by changes in the phosphorylation potential (13) or that this process is very dependent on the substrate regime used in vitro (10, 19, 25). In vivo studies have also demonstrated that changes in work are not associated with significant changes in ATP or the calculated ADP (3, 17, 24, 30). These findings suggest that the simple ADP and Pi model described above may not be operational in the heart in vivo. However, the relationship between myocardial oxygen consumption (MV02) and the free concentration of these phosphates in vivo has not been established.

The purpose of this study was to determine the relation...
between MVO₂ and the phosphate metabolites detected by ³¹P-nuclear magnetic resonance (NMR) in vivo. This was accomplished with a canine heart model in vivo where the phosphate metabolites were monitored with ³¹P-NMR simultaneously with coronary blood flow and MVO₂ using a coronary sinus thermodilution catheter. Myocardial work was altered by using electrical pacing or graded infusions of epinephrine or phenylephrine to vary the inotropic state of the heart during the alterations in work.

MATERIALS AND METHODS

Animal Preparation

Eleven- to 20-kg male and female beagles were used in this study. The animals were initially sedated with 20 mg/kg intravenous thiopental and intubated. The animals were then ventilated (Siemens D-900 pediatric ventilator) and anesthetized with 80% O₂-20% N₂O with 0.75-1.0% halothane. Positive end-expiratory pressure of 5 cmH₂O was used in all experiments. Muscle relaxation was maintained with hourly intravenous doses of 10 mg/kg pancuronium bromide. A central arterial line was placed via the femoral artery to monitor arterial blood pressure and blood gases. Arterial blood pH was kept at 7.35-7.45 by adjusting the ventilatory tidal volume. Metabolic acidosis, if present, was corrected with sodium bicarbonate infusions before the start of the experimental measurement periods. The arterial Po₂ was maintained in excess of 100 mmHg. After the blood pressure, arterial pH, and PCO₂ had stabilized, a left thorocotomy was performed exposing the left ventricle. The pericardium was not opened. A coronary sinus thermodilution catheter (Webster) was then manipulated into the coronary sinus via the left external jugular vein. Direct visualization of the catheter tip during pacing or epinephrine infusions demonstrated that catheter movement did not occur with these perturbations. After satisfactory placement of the coronary sinus catheter, a specially designed NMR surface coil (3 cm OD), constructed of 12-gauge copper wire, was directly sutured to the pericardium just above the apex of the heart. This coil was coated with a biocompatible polymer (Caschem Polycin/Vorite) to insulate the coil from the pericardium. This type of coil attachment permitted normal cardiac motion while keeping the coil in close reproducible proximity to the heart. The reproducible nature of this placement was directly assessed using this coil to collect ¹H-NMR gradient recalled echo images of the heart gated to the cardiac and respiratory cycles (20). These images demonstrate that the motion was reproducible to better than 0.2 mm within the cardiac and respiratory cycle. After the placement of the coil, the thoracotomy opening was sealed with plastic wrap to minimize water loss. A constant infusion of Ringer lactate maintained the animal’s fluid balance. The animal was placed in a custom-designed Lucite cradle, which permitted the centering of the heart in the x and y dimensions before it was placed in the magnet. In the cradle, the animals were wrapped in a water circulating blanket (American Hospital) and a “space blanket” (Boy Scouts of America) to maintain body temperature (37 ± 0.5°C). Core body temperature was monitored with a fiber-optic thermocouple (Luxatron).

NMR Measurements

The NMR probe was tuned to ~81 MHz and matched to 50 Ω to detect ³¹P at 4.7 T. The animal was then transferred into a 26-cm clear-bore 4.7 T magnet (Oxford Instruments), and the heart was positioned in the center of the magnetic field using Z, X and Y gradient projections of the ¹H-NMR signal from the coil. Ventilation was maintained via 12 ft of Tygon tubing attached to the inspiratory and expiratory ports of the ventilator. Normal blood gases demonstrated that adequate ventilation was achieved using this apparatus. Expiratory halothane and oxygen concentrations were continuously monitored by using the sensors in the Siemens ventilator. Blood pressure was monitored with pressure transducers (Gould) located 6 ft from the magnet to avoid interferences by the fringe magnetic fields.

All of the NMR data were collected with a General Electric (Fremont, CA) CSI spectrometer. Shimming of the magnetic field was performed by using the ¹H-NMR free-induction decay. All acquisitions of data were gated to the respiratory cycle using the begin-inspiration sync pulse from the D-900 ventilator, which was processed through a devoted triggering device (MRI, Hershey, PA) to control the CSI spectrometer. This ensured that the NMR signals were always being acquired during the start inspiration phase of the respiratory cycle to maintain a consistent magnetic homogeneity around the heart during data acquisition. The variable capacitors of the probe were also adjusted to tune (81 MHz) and match (50 Ω) the coil at the start-inspiratory phase of the respiratory cycle. In some experiments, the heart rate was paced to a precise harmonic of the respiratory rate to improve the magnetic field shimming around the heart (20), using a laboratory computer (Digital, 11/73) interfaced to the CSI spectrometer. The respiratory rate used in all of these studies was ~30 beats/min, resulting in an interpulse delay of 2 s in the shimming routine as well as the ³¹P-NMR data acquisitions.

Proton line widths of 40–70 Hz were routinely obtained after 10–20 min of manual shimming the static magnetic field. ³¹P-NMR spectra were collected using the same gating sequence as in ¹H shimming. Experiments were conducted to determine the pulse width to attain the maximum CrP signal with the 100-W amplifier used in these studies. The maximum CrP signal occurred with a pulse width between 40 and 50 μs. Previous studies demonstrated that this pulse width resulted in the acquisition of signal weighted over the center of the myocardiue (20).

Saturation Transfer Experiments

Saturation transfer experiments were conducted to determine the CrP-tu-ATP flux via creatine kinase (i.e., forward flux) as described elsewhere (15). Basically, this was accomplished by selectively irradiating the γ-phosphate of ATP resonance for 16 s and observing the effect on the magnitude of the CrP resonance. The control for this experiment was a 16-s irradiation of the region
equidistant in frequency from the CrP as the γ-phosphate of ATP but on the higher frequency side. CrP spin-lattice relaxation times were corrected for the exchange with ATP as described previously (15). The reverse reaction (i.e., ATP to CrP) was not determined in this study because of the complications introduced by competing reactions (1, 15). However, it is established that the creatine kinase reaction rate can be reliably determined from the forward rate of the reaction with the use of classic saturation transfer techniques (5, 15).

Animal Protocols

Pacing protocols. Cardiac pacing was achieved in this study with a coronary sinus catheter that contained bipolar pacing leads (Webster). On each animal 31P-NMR data, myocardial blood flow, and oxygen extraction data were simultaneously monitored over a 20 min control period without cardiac pacing and during two subsequent 20-min periods in which the heart rate was paced at −200 and 250 beats/min. 31P-NMR data were collected in 2-min blocks of 60 averaged acquisitions with 2-s interpulse delays (controlled by the respiratory rate). NMR data were analyzed by averaging the last seven blocks from each period, and processing the data as described in Data Analysis. Coronary blood flow was measured 5 and 15 min after the start of each period. Immediately after each flow determination, coronary sinus blood samples were taken to measure coronary sinus oxygen (Radiometer blood gas analyzer and Lexo-2-Con) and lactate content. Arterial blood samples were also taken to determine arterial lactate and oxygen content. Values reported are the average of these two determinations in each period. Rate pressure product (RPP) was calculated in these and all other studies using the heart rate (beats/min) × the mean arterial blood pressure (mmHg). Mean pressure was used in this calculation because this was the most reliable pressure measurement. The compliance of the 6 ft of tubing required to monitor the pressure in the magnet with the present apparatus made the systolic pressure and rate of pressure development (dP/dt) measurements inaccurate.

Epinephrine infusion protocol. Epinephrine was administered via the central venous line in doses of 0.4, 1.0, and 2.0 μg·min⁻¹·kg⁻¹. In preliminary experiments, it was found that steady-state increases in cardiac performance and coronary blood flow were maintained for only 10 min after the start of and after each increment of epinephrine infusions. Therefore, the protocol timing was different in these experiments when compared with the pacing protocol resulting in a lower signal-to-noise ratio in the epinephrine NMR data. Each epinephrine infusion period lasted 10 min, which consisted of 5 NMR acquisition blocks as described in the pacing protocol. During the last three NMR block acquisitions of a period in which stable hemodynamics were recorded, the coronary sinus and arterial blood were sampled between two determinations of coronary blood flow. The two blood flows were averaged, and only the last three NMR data acquisition blocks were averaged for analysis. In some studies, the epinephrine infusion was stopped, and a recovery measurement was made 60 min later.

Phenylephrine infusion protocol. In these experiments, 4–10 μg·min⁻¹·kg⁻¹ of phenylephrine were infused via the central venous line. The kinetics of the phenylephrine effects on blood pressure and coronary blood flow were similar to those observed for epinephrine. Therefore, the protocol used was identical to the epinephrine protocol described above. In these studies, recovery periods 10 and/or 60 min after the infusions were collected on all of the animals.

Data Analysis

Blood flow and MVO₂ were normalized to the left ventricle heart wet weight measured at the end of an experiment. We have chosen to report our MVO₂ values as micromolar O₂ per minute per gram to compare these values on a molar basis with the lactate production as well as with data from other preparations and techniques. The classic unit of cardiac MVO₂ is milliliters O₂ per minute per 100 grams ventricle based on an early Warburg type measurements. When these units were used, our average control MVO₂ was 4.1 ml O₂·min⁻¹·100 g wet wt⁻¹ of left ventricle.

31P spectra were collected and averaged as described above. Each resultant spectrum was analyzed using the GEMCAP lorentzian line-fitting routine resident in the GEMCSI software. This type of fitting permitted quantization of overlapping resonances, which was particularly important in determining the Pᵢ intensity (20). All NMR data were normalized to the β-phosphate ATP resonance in the control spectra. All data were corrected for the relative spin lattice relaxation times (T₁) of the individual spins as determined in our previous studies on the preparation at this magnetic field strength (20).

Because the low concentration of ADP in this tissue prevents the direct determination by either NMR or extraction techniques (2), the concentration of ADP is generally estimated using the creatine kinase equilibrium reaction (2, 6, 11, 29, 25)

\[
[ADP] = \frac{[ATP] [CrP]}{K_{eq} [CrP] [H^+]}
\]

To use this technique, the tissue creatine ([Cr]), CrP, ATP, and magnesium contents must be known as well as the pH and the equilibrium constant (Kₑq) of the reaction. The Kₑq was assumed to be 1.66 × 10⁹ (23). When 31P-NMR is used, the ATP, CrP, and pH can be monitored. The control ATP concentration [6.6 mM ATP·1 cell water⁻¹ (28)] and the total creatine concentration [27.3 mM total creatine·1 cell water⁻¹ (33)] were taken from the literature. Using the reasonable assumption that the total creatine pool does not change over the time course of these protocols, the changes in free ADP concentrations were estimated with Eq. 1 and the T₁ corrected 31P NMR data. Because of the multiple variables in this analysis, we only consider this determination an estimate of the absolute free intracellular [ADP]. However, we assume that the relative concentrations of ADP within the same preparation can be accurately monitored using this approach.

Statistical analyses were performed with paired t tests using each animal as its own control compared with the
highest work-load maintained by each animal. Statistical tests and computer models were performed with the use of the RS1 (BBN) statistical graphics package running on an IBM AT computer. Statistical significance was defined at the 95% confidence level. Rate constants and magnetic relaxation times are reported as geometric means and the range of values.

RESULTS

Control Physiological Parameters and Relative Concentrations of $^{31}$P Metabolites

The mean values for the phosphate metabolites and physiological parameters are presented in Table 1 for animals under control conditions. Assignments of $^{31}$P NMR resonances have been made in a previous publication from this laboratory (20).

Effect of Cardiac Pacing

The $^{31}$P-NMR data from a representative pacing experiment is shown in Fig. 1. No significant change in the ATP, CrP, or Pi, was detected with a twofold increase in oxygen consumption induced by increased heart rate. The data from these pacing experiments are summarized in Table 2.

With the increase in heart rate and MVO$_2$, no consistent change in intracellular pH, estimated from the chemical shift of P, (20), was observed when compared with control.

The free Mg$^{2+}$ concentration was estimated from the difference in the $\alpha$- and $\beta$-phosphate resonances of ATP according to Wu et al. (34). The chemical shift difference under control conditions was 8.54 ± 0.04 corresponding to a free Mg$^{2+}$ concentration of 2.5 mM. No significant change from control in the chemical shift difference between the $\beta$- and $\alpha$-phosphates of ATP were detected at the maximum respiratory rates obtained in this study. This result suggests that changes in intracellular Mg are not a major factor in the calculation of ADP using the creatine kinase reaction equilibrium described in MATERIALS AND METHODS. However, because of the insensitivity of the free Mg concentration using the chemical shifts of the ATP phosphates (34), this conclusion is limited to only large (>0.25 mM) changes in free Mg.

The important parameters for the purposes of this study were the relative changes in ADP and Pi, during increases in MVO$_2$. In Fig. 2, normalized ADP and Pi data are plotted as a function of MVO$_2$ in these pacing experiments. Comparison of control values for ADP and Pi with values obtained after the maximal increase in heart rate (250 beats/min) by a paired $t$ test revealed no significant difference ($P > 0.05$). Also presented in Fig. 2 is the CrP/ATP ratio as a function of MVO$_2$. As found in our earlier studies on closed-chest dogs using a catheter coil (3), no significant ($P < 0.05$) change in CrP/ATP was observed at the highest respiratory rates obtained when compared with control. It should be noted that the scatter was the greatest for in the Pi measure-

![In Vivo Heart - Pacing Protocol](image)

**FIG. 1.** Selected example of $^{31}$P-nuclear magnetic resonance (NMR) data from an experiment in pacing protocol. Spectra were collected as described in MATERIALS AND METHODS. Assignments and chemical shifts of the $^{31}$P-NMR resonances are found in a previous publication from this laboratory (20). Each spectrum is average of 420 acquisitions with 2-s interpulse delays (14 min total time). Control is basal heart rate (HR) (119 beats/min) myocardial O$_2$ consumption (MVO$_2$) = 1.72 μM O$_2$·min$^{-1}$·g$^{-1}$. Upper spectrum is after heart was paced up to 250 beats/min. MVO$_2$ was 5.56 μM O$_2$·min$^{-1}$·g$^{-1}$. This corresponds to a 320% increase in MVO$_2$. Difference spectrum is the experimental minus the control spectrum. Intensity scale has been multiplied by 2 in the difference scale to visualize level of noise more closely.

### TABLE 1. Averaged control values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>120 ± 4.0</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>67 ± 2.0</td>
</tr>
<tr>
<td>Heart rate × pressure, beats·min$^{-1}$·mmHg</td>
<td>8,027 ± 300</td>
</tr>
<tr>
<td>Oxygen consumption, μM·min$^{-1}$·g$^{-1}$</td>
<td>1.60 ± 0.12</td>
</tr>
<tr>
<td>Lactate consumption, μM·min$^{-1}$·g$^{-1}$</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>Coronary flow, ml·min$^{-1}$·g$^{-1}$</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>CrP/ATP</td>
<td>2.09 ± 0.03</td>
</tr>
<tr>
<td>CrP/Pi</td>
<td>12.5 ± 1.0</td>
</tr>
<tr>
<td>P, chemical shift</td>
<td>4.90 ± 0.01</td>
</tr>
<tr>
<td>Intracellular pH</td>
<td>7.10 ± 0.01</td>
</tr>
<tr>
<td>ADP, μM</td>
<td>55.2 ± 4.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 18 measurements. ADP calculated assuming 6.6 mmol ATP/l cell water, 27.1 mmol total creatine/l cell water, and creatine kinase equilibrium constant of 1.66 × 10$^{-6}$ M (see MATERIALS AND METHODS). P, inorganic phosphate.

### TABLE 2. Pacing protocol results

<table>
<thead>
<tr>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Rate Pressure Product</th>
<th>Coronary Blood Flow, ml·min$^{-1}$·g$^{-1}$</th>
<th>Oxygen Consumption, μM·min$^{-1}$·g$^{-1}$</th>
<th>Lactate Consumption, μM·min$^{-1}$·g$^{-1}$</th>
<th>[Pi]</th>
<th>[CrP]/[ATP]</th>
<th>[ADP]</th>
<th>pH</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>121 ± 4</td>
<td>71 ± 2</td>
<td>8,635 ± 383</td>
<td>0.40 ± 0.03</td>
<td>1.8 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>24 ± 3</td>
<td>1.9 ± 0.1</td>
<td>7.1 ± 0.01</td>
<td>56 ± 6</td>
<td>6</td>
</tr>
<tr>
<td>200 ± 2</td>
<td>71 ± 2</td>
<td>14,114 ± 404</td>
<td>0.67 ± 0.05</td>
<td>3.4 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>28 ± 7</td>
<td>1.8 ± 0.1</td>
<td>7.1 ± 0.01</td>
<td>58 ± 6</td>
<td>6</td>
</tr>
<tr>
<td>246 ± 4*</td>
<td>70 ± 5</td>
<td>15,927 ± 960*</td>
<td>0.78 ± 0.05*</td>
<td>4.8 ± 0.5*</td>
<td>1.6 ± 0.6*</td>
<td>22 ± 5</td>
<td>1.8 ± 0.1</td>
<td>7.1 ± 0.02</td>
<td>56 ± 6</td>
<td>5</td>
</tr>
</tbody>
</table>

All values are means ± SE. All nuclear magnetic resonance (NMR) data were corrected for differential saturation in $T_1$. [Pi] is the area of inorganic phosphate (Pi) resonance relative to control $\beta$-phosphate of ATP = 100; CrP, creatine phosphate; pH, intracellular pH. * $P < 0.06$ as analysed by a paired $t$ test with control condition. Only the highest work loads were analyzed for significance.
TABLE 3. Epinephrine protocol results

<table>
<thead>
<tr>
<th>Dose, pg·min⁻¹·kg⁻¹</th>
<th>Heart Rate, beat/min</th>
<th>Mean Arterial Blood Pressure, mmHg</th>
<th>Rate Pressure Product</th>
<th>Coronary Blood Flow, ml·min⁻¹·g⁻¹</th>
<th>Oxygen Consumption, μM·min⁻¹·g⁻¹</th>
<th>Lactate Consumption, μM·min⁻¹·g⁻¹</th>
<th>[P₄]</th>
<th>[CrP]/[ATP]</th>
<th>pHᵢ</th>
<th>[ADP] μM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>128±6</td>
<td>65±3</td>
<td>8,314±430</td>
<td>0.34±0.1</td>
<td>1.2±0.2</td>
<td>0.4±0.1</td>
<td>20±2</td>
<td>1.9±0.1</td>
<td>7.1±0.05</td>
<td>59±3</td>
<td>7</td>
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<tr>
<td>0.4</td>
<td>134±6</td>
<td>103±12</td>
<td>14,043±1,441</td>
<td>0.94±0.3</td>
<td>2.7±0.7</td>
<td>1.1±0.2</td>
<td>23±3</td>
<td>1.8±0.1</td>
<td>7.1±0.00</td>
<td>61±4</td>
<td>7</td>
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<tr>
<td>1.0</td>
<td>162±19</td>
<td>136±7</td>
<td>20,527±2,163</td>
<td>0.97±0.2</td>
<td>4.3±0.7</td>
<td>1.4±0.3</td>
<td>29±2</td>
<td>2.0±0.1</td>
<td>7.1±0.01</td>
<td>64±8</td>
<td>6</td>
</tr>
<tr>
<td>2.0</td>
<td>189±16*</td>
<td>143±6*</td>
<td>26,682±2,274*</td>
<td>1.50±0.3*</td>
<td>5.5±1.0*</td>
<td>1.8±0.3*</td>
<td>29±4</td>
<td>1.9±0.1</td>
<td>7.1±0.01</td>
<td>57±5</td>
<td>7</td>
</tr>
<tr>
<td>Recovery (60 min after infusion)</td>
<td>123±8</td>
<td>56±4</td>
<td>6,832±396</td>
<td>0.29±0.1</td>
<td>0.6±0.1</td>
<td>0.4±0.1</td>
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<td>2.1±0.1</td>
<td>7.1±0.01</td>
<td>51±4</td>
<td>3</td>
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</table>

All values are means ± SE. All NMR data were corrected for differential saturation in T₁, [P₄] is area of P₄ resonance relative to control, & phosphate of ATP = 100. For other abbreviations see Table 2. * P < 0.05 analyzed by a paired t test with control condition. Only the highest work loads were analyzed for significance.

**FIG. 2.** Plots of normalized (i.e., to control values = 1) [ADP], inorganic phosphate concentration ([P₄]), and creatine phosphate concentration ([CrP]/[ATP]) as a function of myocardial O₂ consumption (MV₀₂) in pacing protocols. Only experiments in which an increase in MV₀₂ was recorded are plotted here. MV₀₂ is reported as μM O₂·min⁻¹·g⁻¹. Each symbol represents different animal.

**FIG. 3.** Selected example of the ³¹P-NMR data from an experiment in epinephrine protocol. Data were collected as described in MATERIALS AND METHODS. Each spectrum is average of 180 acquisitions with 2 s interpulse delays (6 min of data acquisition). Control spectrum myocardial O₂ consumption (MV₀₂) = 1.12 μM O₂·min⁻¹·g⁻¹. Experimental spectrum is during infusion of 1 μg·min⁻¹·kg⁻¹ of epinephrine, MV₀₂ = 3.2 μM O₂·min⁻¹·g⁻¹. This corresponds to a 286% increase in respiration. Difference spectrum is of epinephrine spectrum minus control. Difference intensity scale has been multiplied by 2 to help visualize the noise.

CrP and ATP resonances that were used to calculate the free [ADP].

**Effect of Epinephrine Infusions**

Epinephrine infusions were used to increase the inotropic state of the heart and examine higher increases in MV₀₂ than obtained with pacing alone. We found that increasing MV₀₂ approximately three- to fourfold with epinephrine induced arrhythmias (corresponding to doses >2 μg·min⁻¹·kg⁻¹) (35), which caused a decrease in cardiac performance (mean pressure and RPP) and MV₀₂. A representative ³¹P-NMR experiment using epinephrine at 1 μg·min⁻¹·kg⁻¹ is shown in Fig. 3. Little or no change in phosphates was observed with a threefold increase in MV₀₂. The data from these protocols are presented in Table 3. Although reversible increases in ADP and P₄ were observed in some experiments, paired analyses of control data and values obtained at the highest epinephrine infusion levels resulting in an increase in respiration indicate that no significant change was observed (P < 0.05). Even in those individual experiments in which ADP and P₄ did apparently increase (2 animals), these increases were well below those required...
<table>
<thead>
<tr>
<th>Dose, µg·min⁻¹·kg⁻¹</th>
<th>Heart Rate, beat/min</th>
<th>Mean Arterial Blood Pressure, mmHg</th>
<th>Rate Pressure Product, ml·min⁻¹·g⁻¹</th>
<th>Coronary Blood Flow, ml·min⁻¹·g⁻¹</th>
<th>Oxygen Consumption, µM·min⁻¹·g⁻¹</th>
<th>Lactate Consumption, µM·min⁻¹·g⁻¹</th>
<th>[P]</th>
<th>[CrP]/[ATP]</th>
<th>pHₚ</th>
<th>[ADP], µM</th>
<th>n</th>
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<tbody>
<tr>
<td>0</td>
<td>105±9</td>
<td>64±2</td>
<td>6,636±424</td>
<td>0.47±0.1</td>
<td>1.8±0.2</td>
<td>1.0±0.1</td>
<td>20±2</td>
<td>1.9±0.1</td>
<td>7.1±0.01</td>
<td>62±4</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>120±10</td>
<td>131±5</td>
<td>17,009±1,988</td>
<td>1.15±0.1</td>
<td>4.8±0.6</td>
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<td>29±3</td>
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<td>7.1±0.01</td>
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<tr>
<td>6</td>
<td>125±8</td>
<td>167±4</td>
<td>21,494±1,912</td>
<td>2.02±0.4</td>
<td>7.2±1.7</td>
<td>3.7±0.7</td>
<td>36±5</td>
<td>1.8±0.1</td>
<td>7.1±0.02</td>
<td>68±8</td>
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</tr>
<tr>
<td>10</td>
<td>145±11*</td>
<td>174±7*</td>
<td>24,743±1,998*</td>
<td>2.70±0.6*</td>
<td>9.6±2.0*</td>
<td>3.5±2.0*</td>
<td>38±2*</td>
<td>1.8±0.2</td>
<td>7.1±0.02</td>
<td>74±10</td>
<td>4</td>
</tr>
<tr>
<td>Recovery (60 min after infusion)</td>
<td>106±6</td>
<td>77±4</td>
<td>8,051±509</td>
<td>0.43±0.1</td>
<td>1.9±0.4</td>
<td>0.4±0.4</td>
<td>22±2</td>
<td>2.0±0.1</td>
<td>7.1±0.02</td>
<td>65±6</td>
<td>4</td>
</tr>
</tbody>
</table>

All values are means ± SE. All NMR data were corrected for differential saturation in T₁. Pi is reported as the area of the Pi resonance relative to control Pₚ-phosphate of ATP = 100. For other abbreviations see Table 2. *P < 0.05 as analyzed by a paired t test with control condition. Only the highest work loads were analyzed for significance.

**FIG. 4.** Plots of normalized (i.e., to control values = 1) [ADP], [Pₚ], and [CrP]/[ATP] as a function of MVₐO₂ in epinephrine protocols. Only data in which an increase in MVₐO₂ was recorded are reported here. No recovery data is plotted. MVₐO₂ is reported as µM·min⁻¹·g⁻¹. A: [ADP] as a function of induced changes in MVₐO₂. B: [Pₚ] as a function of MVₐO₂. C: [CrP]/[ATP] as a function of MVₐO₂. Each symbol represents a different animal. See Fig. 2 for abbreviations.

**FIG. 5.** Selected example of the 3¹P-NMR data from a phenylephrine protocol. Data were collected as described in MATERIALS AND METHODS. Each spectrum is average of 180 acquisitions with 2 a interpulse delays (6 min of data acquisition). Control spectrum MVₐO₂ = 1.03 µM O₂·min⁻¹·g⁻¹. Experimental spectrum is during infusion of 6 µg·min⁻¹·kg⁻¹ of phenylephrine, MVₐO₂ = 6.31 µM·min⁻¹·g⁻¹. This corresponds to an increase in respiration of 612%. Difference spectrum intensity scale has been multiplied by 2 and filtered to 10 Hz exponential line broadening to help visualization of difference. See Fig. 1 for abbreviations.

*Effect of Phenylephrine Infusions*

Phenylephrine infusions were used to increase MVₐO₂ with minimal direct β-adrenergic stimulation. Figure 5 presents the 3¹P-NMR data from a phenylephrine experiment where a small change in phosphates occurred with
FIG. 6. Plots of normalized (i.e., to control values = 1) [ADP], [P_i], and [CrP]/[ATP] as a function of M_{\text{VO}}_2 in phenylephrine protocols. Only experiments in which an increase in M_{\text{VO}}_2 was recorded are presented. No recovery data are plotted. M_{\text{VO}}_2 is reported as \mu M \cdot min^{-1} \cdot g^{-1}. A: [ADP] as a function of induced changes in M_{\text{VO}}_2. B: [P_i] as a function of M_{\text{VO}}_2. C: [CrP]/[P_i] as a function of M_{\text{VO}}_2. Each symbol represents a different animal. See Fig. 2 for abbreviations.

a very large increase in M_{\text{VO}}_2. Only small changes in CrP and in the P_i region were observed. Table 4 presents the physiological and NMR data for these studies, whereas Fig. 6 presents the normalized ADP and P_i data. Paired statistical analysis of the highest respiratory rates obtained in this study revealed that a significant increase in P_i (P < 0.05), but not ADP, was observed. However, this increase in P_i was again far below what is predicted if P_i were the sole feedback between respiration and work in the myocardium (see APPENDIX).

The Creatine Kinase Unidirectional Flux

The unidirectional flux of creatine kinase was measured to determine the influence of M_{\text{VO}}_2 on this reaction rate to establish whether the creatine kinase reaction is truly near equilibrium under these conditions. Figure 7A

is the control spectrum where a constant low-power (1.0 W) irradiation was applied as a control for radiofrequency bleed over by the low-power pulse. In Fig. 7B, the low-power irradiation was moved to the \gamma-phosphate of ATP. With the irradiation on the \gamma-ATP, the \gamma-ATP was saturated, and a significant decrease in CrP was observed. This is most clearly seen in the difference spectrum (Fig. 7C). A summary of the data from all of the saturation transfer experiments conducted on hearts under both control conditions and when paced at ~200 beats/min is presented in Table 5. Similar pseudo-first-order rate constants for the creatine kinase reaction rate were obtained under both work loads. With the rate constant obtained in these studies, the rate of ATP exchange can be calculated through the creatine kinase reaction to be ~114 \mu M \cdot min^{-1} \cdot g^{-1}. With the use of an ATP/O_2 ratio of 5.6, the ATP flux via oxidative phosphorylation can be estimated from the M_{\text{VO}}_2 values to be 9.0 \mu M \cdot min^{-1} \cdot g^{-1} at the work loads used in these saturation transfer studies. Thus the creatine kinase flux is greater than 10 times the oxidative phosphorylation flux, indicating that this reaction is indeed near equilibrium in the canine heart in vivo.

DISCUSSION

These results demonstrate that \textsuperscript{31}P-NMR data can be obtained simultaneously with measurement of coronary blood flow and oxygen consumption in the canine heart in vivo. The data collected from this preparation demonstrate that over a physiological range (two- to threefold increases over resting rates) of ATP hydrolysis rates and M_{\text{VO}}_2 the hydrolysis products of ATP, ADP and P_i do not significantly accumulate in the cytosol.
Electrical pacing was used in these studies to increase M\(\text{VO}_2\) with only minor inotropic effects (6) and to validate our previous results with pacing in closed-chest animals using a catheter coil (3). Pacing to 200 or 250 beats/min resulted in a two- to threefold increase in M\(\text{VO}_2\) over the basal heart rate condition. The relationship between the rate pressure product and M\(\text{VO}_2\) in these experiments was linear \((r = 0.82, \text{slope} = 2.84 \times 10^{-4})\); see Fig. 8. These values are in close agreement with previous pacing studies on the perfused canine heart in vitro (Ref. 6; see Fig. 8). These results substantiate the assumptions used in our previous work on closed-chest animals in which M\(\text{VO}_2\) was estimated from the rate pressure product alone (3). No detectable changes in any of the phosphates were observed with the maximum M\(\text{VO}_2\) obtained in these studies (using paired \(t\) tests with each animal serving as its own control). These results are in agreement with our earlier in vivo studies (3) as well as with those on the isolated perfused rat heart (4, 11, 18, 19, 25) in which two- to threefold increases in M\(\text{VO}_2\) are not associated with large changes in the phosphate metabolites.

Because the signal-to-noise ratio was in excess of 100:1 for ATP in these pacing studies, it is reasonable to assume that any changes in ATP and P\(_i\) that did occur would have to be much lower than 10%. Because ADP is derived from the measurement of multiple parameters, it is difficult to evaluate the accuracy of this calculation. However, the precision of the values obtained with each animal suggests that 10–15% changes would be detected easily. Calculations of the magnitude of change in ADP and P\(_i\), expected if these metabolites are controlling respiration are presented in APPENDIX. Based on this analysis, one would predict a three- to fivefold increase in ADP and P\(_i\) if respiration was solely regulated by these substrates in a simple Michaelis-Menten fashion. Clearly this did not occur in these studies.

Epinephrine was used to increase M\(\text{VO}_2\) by combined \(\alpha\) - and \(\beta\)-adrenergic stimulation to induce more inotropic effects than pacing alone. It was found that the oxygen consumption of the heart could only be increased approximately fourfold before arrhythmias occurred, which resulted in hemodynamic instability and a decrease in cardiac performance, blood flow, and M\(\text{VO}_2\). This observation is consistent with previous studies on the canine heart in which high levels of epinephrine and halothane have been found to induce arrhythmias (35). However, using the data from the lower epinephrine doses in which arrhythmias did not occur but in which physiological increases in M\(\text{VO}_2\) were maintained, there was no significant increase in ADP and P\(_i\) when the data showing maximum respiratory rates were analyzed using a paired \(t\) test. In some experiments, a reversible increase in ADP and P\(_i\) was occasionally detected, but this increase was small compared with the changes in M\(\text{VO}_2\) observed.

Phenytoin infusions were used to induce an increase in M\(\text{VO}_2\) with minimal \(\beta\)-adrenergic effects. Phenyltoin infusions resulted in the highest M\(\text{VO}_2\) observed in this study. No significant overall changes occurred in ADP content despite the fact that some animals did have small reversible changes in ADP content. In contrast, significant reversible increases in P\(_i\) were observed in these studies. These small increases in P\(_i\) and, possibly, in ADP, were still too small to explain the large increase in respiration induced in these studies (see APPENDIX). These results imply that as very high levels of work are approached, ADP and P\(_i\) may begin to play a direct stimulating role on respiration. Because no changes were observed with the epinephrine infusions, this increase in ADP and P\(_i\) may only occur in the absence of \(\beta\)-adrenergic stimulation or may be related to the high pressures generated by phenyltoin, potentially resulting in subendocardial ischemia.

Lactate consumption was monitored in these studies to estimate the amount of anaerobic glycolysis induced by these work increases. The control rate of lactate consumption was 0.69 \(\mu\text{M}\cdot\text{min}^{-1}\cdot\text{g}^{-1}\). If all of this lactate was oxidized and not converted to glycogen, then this lactate consumption could account for all of the oxygen consumption observed in this study using 3 mol of oxygen to oxidize 1 mol of lactate. This is in agreement with other open-chest canine studies (8). Thus it is reasonable to assume that a major oxidative substrate under these conditions was lactate. Consistent with this notion was the observation that lactate consumption generally increased with increases in respiration. The increase in lactate consumption with work also implies that the work levels induced did not grossly overcome the aerobic capabilities of the tissue (31) and that anaerobic glycolysis was not significantly induced.

Overall, these data suggest that a simple feedback control mechanism of ADP and P\(_i\) cannot explain the feedback regulation of oxidative respiration by ATPase activity in the myocardium over physiological levels of work. These data are also inconsistent with the notion that the control of coronary blood flow is controlled by the overall phosphorylation potential of the myocardium (32). Other processes or cooperativity effects must be occurring to permit such large variations in respiration and coronary blood flow with little or no change in the overall concentrations of ADP and P\(_i\). The three most obvious candidates are the tissue concentrations of oxygen, the mitochondrial redox state, and membrane potential or cooperative kinetics between ADP, P\(_i\), and all of the parameters above.

Oxygen delivery is a potentially rate-limiting step in
oxidative phosphorylation in the heart (12, 14, 32). Although several studies have indicated that oxygen may be rate limiting based on optical measurements of myoglobin or cytochrome $a_d$ (27), these results are still controversial because of the difficulties of monitoring these optical parameters of the heart in vivo. Thus coronary blood flow and subsequent oxygen delivery is still a possible controlling step in this process.

Recent studies have suggested that the mitochondrial redox state may be an important parameter in the regulation of mitochondrial respiration (18, 19). In isolated mitochondria, it has been demonstrated that the redox state of mitochondrial NADH can control the rate of respiration without requiring changes in ADP and $P_i$ (22). Therefore, it is possible that activation of dehydrogenase activity by increasing work could increase the mitochondrial NADH and thereby cause an increase in ATP production without alterations of cytosolic ADP and $P_i$. Activation of pyruvate dehydrogenase (PDH) occurs with either $\beta$-adrenergic stimulation (7) or increased afterload in the isolated perfused heart (21). Denton and McCormack (7) have proposed that this is a key event in the stimulation of cardiac metabolism by hormonal stimulation. Because lactate was the most likely dominant oxidative substrate in the present studies, regulation of reducing equivalent delivery at the level of PDH could be a mechanism of respiratory control in these hearts.

In summary, these data demonstrate that in the physiological range of $M\text{VO}_2$ only small or no changes occur in intracellular contents of CrP, ATP, or ADP and $P_i$. This implies that the feedback mechanism between the ATPase activity in the myofibrils and the mitochondria is highly buffered and does not rely on a simple mechanism using the kinetic effects of ADP and $P_i$ alone on mitochondrial respiration. Other parameters, such as the mitochondrial redox state and membrane potential, as well as the delivery of oxygen to the tissue should also be considered.

APPENDIX

The major purpose of this study was to investigate the role of the ATP hydrolysis products, ADP and $P_i$, on the control of myocardial respiration in vivo. Using the in vitro Michaelis constant ($K_m$) values of ADP and $P_i$, in the regulation of in vitro mitochondrial respiration, we can estimate how much ADP and $P_i$ would have to change to result in the two- to threefold increase in respiration observed in the various protocols used in this study. The simplest model to account for a shared control of respiration by ADP and $P_i$ would involve a random bireactant kinetic model. Control rate (i.e., $M\text{VO}_2 = 1 ADP$ and $P_i = 1$) is the $M\text{VO}_2$ driven by having ADP and $P_i$ at their respective Michaelis constant ($K_m$) values (i.e., $25\% V_{max}$). This is considered the control respiratory rate. Subsequent curve was generated by increasing ADP and $P_i$, proportionally and calculating the resultant $M\text{VO}_2$. A fivefold increase in both ADP and $P_i$ only results in an approximate threefold increase in $M\text{VO}_2$. See Fig. 2 for abbreviations.

These values in Eq. 1 we can calculate the change in ADP and $P_i$, required to induce a given change in respiration using this simple model of respiratory control. Because both metabolites are near their respective $K_m$ values, the most effective way to increase respiration is to change both the ADP and $P_i$ concentrations together. The combined effect of increases in both ADP and $P_i$ on respiration is plotted in Fig. 9. These data are plotted using the same graphic orientation as in the body of the manuscript. A fourfold combined increase in both ADP and $P_i$ is required to increase respiration 2.5-fold, the approximate average increase in $M\text{VO}_2$ observed in this study. A fourfold increase in ADP and $P_i$ should have been easily observed with the signal-to-noise ratio and resolution of the $^31$P NMR data collected in this study. It should be noted that the actual ADP and $P_i$ values determined in vivo were above the in vitro $K_m$ values, which would make the $M\text{VO}_2$ even more insensitive to changes in these compounds. Analysis of Eq. 1 where only ADP and $P_i$ alone are allowed to change indicates that the largest increase in $M\text{VO}_2$ with reasonable increases in ADP and $P_i$ (10-fold) is only 2.5- to threefold.

These results suggest that a simple Michaelis-Menten type interaction between ADP, $P_i$, and the mitochondria derived from in vitro mitochondria data cannot explain the regulation of respiration observed in the heart in vivo. These results imply that other parameters must be involved in the regulation of respiration or the reaction kinetics involving the ATP hydrolysis products must be different in vivo than obtained under classic in vitro conditions.

REFERENCES


