ATP Synthesis Kinetics and Mitochondrial Function in the Postischemic Myocardium as Studied by $^{31}$P NMR*

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The effects of ischemia on mitochondrial function and the unidirectional rate of ATP synthesis ($P_i \rightarrow ATP$ rate) were studied using a Langendorff-perfused heart preparation and $^{31}$P NMR spectroscopy. There was significant postischemic depression of mechanical function assessed as the heart rate pressure product, and the myocardial oxygen consumption rate at a given rate pressure product was elevated. Experiments performed on glucose- and pyruvate-perfused hearts demonstrated the presence of a large contribution to the unidirectional $P_i \rightarrow ATP$ rate catalyzed by glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase. This rate was much greater than the maximal glucose utilization rate in the myocardium, demonstrating that the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase reactions are near equilibrium both before and after ischemia. In the pyruvate-perfused postischemic hearts, the glycolytic contribution was eliminated and the net rate of ATP synthesis by oxidative phosphorylation was measurable. Despite the reduced mechanical function and increased myocardial oxygen consumption rate, the ratio of the net rate of ATP synthesis by oxidative phosphorylation to oxygen consumption rate (the P:O ratio) was not altered subsequent to ischemia (2.34 ± 0.12 and 2.36 ± 0.09 in normal and postischemic hearts, respectively). Therefore, mitochondrial uncoupling cannot be the cause of postischemic depression in mechanical function; instead, the data suggest the existence of ischemia-induced inefficiency in ATP utilization.

Myocardial contractile function is depressed and recovers very slowly following a sublethal period of ischemia (1, 2). Although the precise molecular mechanisms responsible for this dysfunction are not fully understood, abnormalities in the contractile apparatus, excitation-contraction coupling, cellular membrane integrity, and myocardial bioenergetics have all been proposed as potential contributors (for reviews, Refs. 3–6). Among these possibilities, the central importance of energy metabolism to cardiac function has made it one of the primary foci of studies concerned with consequences of ischemia.

Evidence for postischemic damage in high energy phosphate production has been provided by numerous experiments conducted on isolated mitochondria (7–13). It is well recognized, however, that the tissue homogenization and organelle isolation procedures can be injurious, and mitochondria subjected to long periods of ischemia may be especially susceptible to damage by these methods (7, 12, 14, 15). Consequently, it is difficult to evaluate the effects of a reversible ischemic insult on mitochondrial performance in vivo by extrapolating from the isolated mitochondria data.

Attempts to analyze postischemic mitochondrial function in the intact heart have invariably been based on measurements of myocardial oxygen consumption ($\text{MVO}_2$). When reversible periods of ischemia have been studied, postischemic $\text{MVO}_2$ has been within the normal range or even elevated despite a reduction of mechanical function (16, 17). These observations are consistent with the hypothesis that mitochondrial uncoupling is induced by the ischemic insult. This hypothesis can now be evaluated directly in the intact heart using NMR spectroscopy techniques. We have previously demonstrated that the net rate of ATP synthesis by oxidative phosphorylation (18, 19) and the mode of mitochondrial respiratory regulation in the normal myocardium (19, 20) can be studied using $^{31}$P NMR. In this paper, we present the results of NMR studies on ATP synthesis kinetics and the efficiency of coupling between ATP synthesis and oxygen consumption in the postischemic heart.

MATERIALS AND METHODS

Isolated Perfused Heart Preparation—Details of the preparation of Langendorff-perfused hearts and the experimental arrangement that allowed continuous and simultaneous measurement of left ventricular pressure (LVP), heart rate, and $\text{MVO}_2$ were described previously (21). Hearts from Sprague-Dawley rats weighing 350–450 g were perfused with a modified phosphate-free Krebs-Henseleit buffer (119 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl$_2$, 1.8 mM CaCl$_2$, 28 mM NaHCO$_3$, 0.1 mM EDTA equilibrated with 95% O$_2$, 5% CO$_2$ gas mixture).

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1 The abbreviations used are: $\text{MVO}_2$, myocardial oxygen consumption; LVP, left ventricular pressure; $\text{LVSP}$, left ventricular systolic pressure; RPP, rate pressure product; IA, iodococetate; FID, free induction decay; $dp/dt$, time derivative of LVP; CP, creatine phosphate.


3 For a reaction $A \rightleftharpoons B$, net rate of formation of compound B equals the unidirectional $A \rightarrow B$ conversion rate minus the unidirectional $B \rightarrow A$ conversion rate. Similarly, net rate of formation of compound A is the unidirectional $B \rightarrow A$ rate minus the unidirectional $A \rightarrow B$ rate.
Mechanical performance was evaluated as the rate pressure product (RPP, mm Hg · min⁻¹), the product of peak left ventricular systolic pressure (LVSP) and heart rate. LVF was measured via an intraventricular balloon, the volume of which could be adjusted to set the end diastolic pressure. Measurement of effluent O₂ content and coronary flow allowed calculation of MVO₂.

Fully relaxed 31P NMR measurements were obtained using a Nicolet wide-bore 960 spectrometer at 146.1 MHz using a single-turn solenoidal coil. Unidirectional ATP synthesis rates were measured by conventional two-site saturation transfer by saturating the ATP, resonance and monitoring the Pi, peak as described previously (18). Eight to ten spectra were recorded using 90° pulses, 64 ms acquisition time, and a list of different pulse repetition times, cycling through this list 24 times and accumulating 12 FIDs for each repetition time during each cycle. One of the spectra was obtained with full relaxation of the Pi resonance and in the absence of ATP, saturation.

The remaining spectra were acquired while saturating ATP, with repetition times ranging from 0.3 to 3.5 s; these spectra constituted a progressive saturation sequence executed while ATP, spins were nulled. The Pi, intensities obtained from this sequence were fitted to the equation $M(T) = M'(1 - e^{-(-T/T*)})$ to determine the parameters $M'$ and $T*$, the Pi, intensity and the spin-lattice relaxation time, respectively, when ATP, spins are nullled. From $M'$ and the spectra recorded with ATP, saturation, the fractional reduction in ATP, content (ΔM/M') in Pi, intensity upon irradiation of the ATP, spins was calculated. The pseudo-first order unidirectional rate constant $k_i$ was calculated as $k_i = (ΔM/M')/T*$. The Pi, → ATP rate was $k_i/P_i$.

Fully relaxed 31P NMR spectra (40 FIDs) were recorded before and after administration of kinetic data for each heart using a 15 s pre-pulse and a 15 s interpulse delay. The Pi, ATP, and CP contents were determined for each heart from these fully relaxed spectra using a 100 mM phenyl phosphonate solution in the left ventricular balloon as a standard.

Glycolysis Contribution to Pi, → ATP Rate—The glycolytic contribution to the unidirectional Pi, → ATP rate must be eliminated prior to NMR measurements of the ATP synthesis rate by oxidative phosphorylation (18). This can be accomplished by using pyruvate as the sole carbon substrate and either depleting the heart of its endogenous glycogen or infusing isocitrate (IA) to inhibit glyceraldehyde-3-phosphate dehydrogenase (15). In control hearts, glycogen depletion was accomplished by a brief period of substrate-free perfusion (18, 22, 23). This was not necessary in the postischemic hearts because their glycogen stores are already depleted (6). The IA exposure protocol was based on studies conducted on normal hearts where maximal glyceraldehyde-3-phosphate dehydrogenase inhibition was achieved. With reperfusion, IA exposure was initiated 10 min after the postischemic hearts had resumed beating and was continued for 10 min. Subsequently, the IA concentration was reduced to 0.025 mM and maintained at this concentration for the kinetic measurements.

Because of the necessity to examine and eliminate the glycolytic contribution to the NMR-measurable Pi, → ATP rate, four separate postischemic groups that differed on their exogenous carbon source and IA exposure were studied. These groups were: (a) perfusion with 11 mM glucose, (b) perfusion with 1 mM pyruvate, (c) perfusion with 1 mM pyruvate plus exposure to IA, and (d) perfusion with 10 mM pyruvate. The use of 1 and 10 mM pyruvate was required because in nonischemic control hearts, 10 mM pyruvate causes a diminution of the cytosolic Pi, to levels that are inadequate for the execution of the saturation transfer experiments (18-20). Therefore, the kinetic studies were conducted under conditions of inadequate pyruvate, which is capable of supporting cardiac function at the MVO₂ levels achieved in this study. However, postischemic hearts perfused with 1 mM pyruvate exhibited significantly more depression in mechanical function than the hearts perfused with glucose or 10 mM pyruvate. Since it was feasible to conduct saturation transfer measurements on postischemic hearts perfused with 10 mM pyruvate due to their inherently higher Pi, content, both 1 and 10 mM pyruvate were employed as postischemic carbon substrates.

Experimental Protocol—The NMR kinetic measurements were performed as a function of RPP and MVO₂ achieved using two of the five workstates employed on our previous study (18). These were workstate i, which consisted of maintaining the end-diastolic pressure at 4-8 mm Hg and the heart rate at 300 beats/min by pacing if needed, and workstate ii, where end-diastolic pressure and heart rate were set at 4-5 mm Hg and 400 beats/min, respectively, and the heart was exposed to an isotropic agent (Dobutamine, 90 ng/ml).

To ensure equivalent insults during ischemia, all hearts were initially perfused with 11 mM glucose and operated at workstate i in the preischemic period. During this preischemic interval, RPP and MVO₂ of the heart at workstate i were measured, and a fully relaxed NMR spectrum was recorded.

The ischemic period consisted of 18 min of no flow at a monitored temperature of 38 °C. Under these perfusion conditions, this was the maximum insult compatible with recovery of a significant postischemic developed pressure (at least 20 mm Hg). An ischemic period of 25 min resulted in no recovery. All hearts underwent ischemic contracture, as noted by a rise in LVP of at least 10 mm Hg, beginning at an average time of 8.5 ± 2.6 min following the onset of ischemia. The left ventricular balloon volume was maintained during ischemia at the preischemic level and was reduced during reperfusion in order to reduce transmural pressure and facilitate postischemic coronary flow. The balloon volume was readjusted to achieve end-diastolic pressure of 4-8 mm Hg after the hearts resumed regular rhythm following reperfusion.

Following ischemia, the reperfusion substrate was glucose or pyruvate, depending on the substrate group under study. With reperfusion, typically the hearts fibrillated for a brief period followed by resumption of rhythmic beating; this transition from fibrillation to beating was usually assisted by electrical defibrillation. The time from the onset of reflow to beginning of beating was 11.5 ± 1.7 (N = 25), 8.6 ± 0.7 (N = 36), and 7.2 ± 2.8 (N = 8) min for the glucose, 1 mM pyruvate, and 10 mM pyruvate groups. Hearts were considered to have recovered if they began beating within 20 min and maintained a pressure of at least 20 mm Hg during the collection of NMR spectra. Hearts that did not beat, could not achieve sufficient pressure, or fibrillated irreversibly were not considered to have recovered. Hearts that fibrillated briefly during data collection; if they could resume a regular rhythm within 2 min by electrical stimulation, they were included in the recovered group. By these criteria, 76, 56, and 75% of hearts reperfused with glucose, 1 mM pyruvate, and 10 mM pyruvate, respectively, recovered. It is important to note that these hearts are ischemic and thus do not have pump fluid; therefore, the criteria for recovery are different from those for a heart in the working mode.

Within ~20 min following resumption of beating, a new steady state of mechanical function and MVO₂ was achieved. All postischemic functional, metabolic and NMR data reported were obtained during this new steady state. Measurements of LVP, heart rate, coronary flow, and CP content were made every 15 min during the time required (80-85 min) for the collection of the kinetic data, and average values of MVO₂ and RPP were determined. Statistical analysis was performed using a two-sample t test. The relationship of RPP to MVO₂ was carried out by one-way analysis of variance.

It should be emphasized that the control (nonischemic) and the postischemic measurements were collected on separate groups of hearts. The time required for the acquisition of the necessary NMR kinetic data is too long to conduct two such measurements on a single heart, one before and the other after the ischemic insult. The data accumulation procedure on the postischemic hearts described above was identical to that used for the controls except for the presence of the ischemia and recovery periods. The data on control hearts have previously been reported as part of a more comprehensive study on the ATP ⇔ Pi, exchange in the rat myocardium (18).

RESULTS

The typical effect of an 18 min ischemic insult on LVP and its first derivative (dp/dt) at workstate i is illustrated in Fig. 1. Shortly after the onset of ischemia, contraction ceased. Approximately 15 min later, a rise in pressure was noted due to ischemic contracture. With reflow, there was an initial period of fibrillation with eventual return to a regular rhythm though with substantially decreased LVSP and dp/dt.

Fully relaxed 31P NMR spectra recorded before and after ischemia at workstate i from a typical glucose-perfused heart are shown in Fig. 2. The postischemic decrease in ATP levels is clearly observed in these spectra (Fig. 2, A and B). The reduced ATP level was maintained throughout the duration of the kinetic measurements without further significant decline (Fig. 2C). The decrease in the NMR detected phosphates due to the large loss in ATP following ischemia was not compensated by changes in the Pi, and CP intensities. Overall,
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FIG. 1. Tracing of left ventricular pressure (LVP) and the first derivative of left ventricular pressure with respect to time (dp/dt) during a typical ischemia episode in a glucose-perfused heart.

A

B

C

D

FIG. 2. Fully relaxed $^3$P NMR spectra of a glucose-perfused heart at workstate i. A, preischemic; B, postischemic, prior to commencing the saturation transfer experiment; and C, subsequent to completion of the saturation transfer measurement. $R$ represents the reference signal from the intraventricular balloon containing 100 mM phenyl phosphonate; $P_i^{cy}$, cytosolic inorganic phosphate; CP, creatine phosphate; ATP$\gamma$, $\gamma$-phosphate of ATP. 40 FIDs were collected for each spectrum using 90° pulses and 15-s interpulse delay.

~30% of the total $^3$P signal was lost subsequent to ischemia. This loss may arise due to the leakage of phosphate from the cell and/or to incorporation of $P_i$ into NMR-invisible, large molecular weight complexes, such as Ca$^{2+}$-P$\gamma$ precipitates.

Fig. 3 illustrates typical spectra obtained during the kinetic measurements at workstates i and ii on the glucose-perfused postischemic hearts. The two spectra shown for each workstate were taken from a series of spectra recorded in a time-averaged fashion for the determination of the $P$ → ATP rate as described under "Materials and Methods." A large reduction in the $P_i$ intensity was detected at both workstates upon saturation of the ATP$\gamma$ spins. It should be noted that the $P_i$ spin-lattice relaxation time decreases upon saturation of the ATP$\gamma$ spins. Therefore, when ATP$\gamma$ magnetization was nullled, a higher repetition rate was used for increased efficiency. With the repetition times employed, all of the $P_i$ peaks seen in Fig. 3 are fully relaxed; this is not necessarily the case for the rest of the resonances. The reduction in the CP intensity observed upon saturation of ATP$\gamma$ spins is due in part to this phenomenon, but is largely a consequence of the creatine kinase-catalyzed exchange between CP and ATP$\gamma$ phosphates. An example of the complete data acquired with a single glucose-perfused heart for the calculation of the $P_i$ → ATP rate constant $k_i$ is shown in Fig. 4.

The NMR-derived values for $T_i^*$ and $\Delta M/M_0$ obtained from saturation transfer measurements and the high energy phosphate concentrations maintained during the acquisition of the kinetic data are given in Table I. The expected postischemic fall in ATP levels to approximately 50% of control values is seen in all substrate groups and workstates. Table II lists the data on mechanical performance, $\text{MVO}_2$, the unidirectional $P$ → ATP rate measured by saturation transfer, and the ratio of this rate to the oxygen atom consumption rate (MVO).$^4$ To facilitate comparison of data and the discussion presented in the next section, the $P$ → ATP rates measured in control and postischemic hearts and the (P → ATP rate)/MVO ratio measured for the postischemic hearts are repro-

$^4$ MVO = $\text{MVO}_2 \times 2$.  

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\text{MVO} = \text{MVO}_2 \times 2.
\]
ATP Synthesis in the Postischemic Myocardium

Fig. 4. P, signal intensity as a function of pulse repetition time during saturation transfer measurements while ATP, spins were either selectively saturated (O) or not (C). The data were obtained on a post-ischemic glucose-perfused heart at workstate i. From this data the $T_1^*$ and $\Delta M/M^0$ for P, are calculated.

duced as bar graphs in Figs. 5 and 6, respectively. The dashed line in Fig. 6 is the average (P, → ATP rate)/MVO ratio obtained on control hearts at five different workstates (18).

In glucose-perfused hearts, the P, → ATP rate measured by NMR increased after ischemia (Fig. 5). This increase was dependent on the duration of the ischemic period and was not observable after 13 min of ischemia; in 12 hearts subjected to 13 min of ischemia and reperfused with glucose, the measured P, → ATP rate was 7.9 ± 0.7 (S.E.) μmol·s⁻¹ (g dry weight)⁻¹ at workstate i.

The effect of the ischemic insult on mechanical function for a given workstate and substrate condition can be evaluated by comparing RPP of control and postischemic hearts. Because the control and postischemic data were obtained on different groups of animals, and the RPP attained under a given set of conditions vary among different hearts, such a comparison tends to mask the significance of the ischemia-induced alterations in RPP. Nevertheless, a statistically significant decrease in postischemic RPP ($p < 0.01$) was noted for each respective substrate and workstate group except for the workstate i glucose and pyruvate/IA groups. For the workstate i glucose group, however, it is possible to compare pre- and postischemic RPP directly for the same heart, because for this particular group only, the pre- and postischemic substrates and workstates were identical and the only variable was the ischemic insult. In the glucose-perfused workstate i group, every heart displayed a reduction in LVSP and RPP after ischemia and the post- to preischemic RPP ratio was 0.83 ± 0.04 (S.E., N = 13); this is significantly different from 1 ($p < 0.001$). The same type of analysis could not be performed on the pyruvate/IA group because in the ischemic studies IA was used after but not before the ischemic insult, and because IA itself induces a small but non-negligible reduction in LVSP (19).

The postischemic depression in RPP was most prominent at workstate ii in the 1 mM pyruvate group. When a reperfusion pyruvate concentration of 10 mM was employed on a separate group of hearts, the ischemia-induced reduction in RPP was not as pronounced. This was a surprising observation since 1 mM pyruvate is clearly an adequate substrate for control hearts at workstate ii. Therefore, this substrate concentration effect was examined further in several hearts where the postischemic carbon source was switched from 1 to 10 mM pyruvate or vice versa after the hearts resumed steady-state RPP and MVO₂; no significant change in either RPP or MVO₂ was noted upon this switch. This suggests that the dependence

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>ATP, P, and CP content measured during the acquisition of kinetic data on control and postischemic hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>All numbers are mean ± S.E. The number of measurements performed on different hearts is given as N.</td>
<td></td>
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<tr>
<td>Postischemic groups are designated Post-I.</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Workstate ³</th>
<th>N</th>
<th>ATP*</th>
<th>P*</th>
<th>CP*</th>
<th>$T_1^*$</th>
<th>$\Delta M/M^0$</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
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<td></td>
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<tr>
<td>Control</td>
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<td>23.5 ± 2.0</td>
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<td>0.59 ± 0.03</td>
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<td>Post-I</td>
<td>13</td>
<td>15.5 ± 1.4</td>
<td>15.5 ± 1.4</td>
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<td>0.57 ± 0.02</td>
<td>0.41 ± 0.01</td>
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<tr>
<td>Control</td>
<td>9</td>
<td>28.6 ± 1.2</td>
<td>11.9 ± 1.1</td>
<td>41.0 ± 2.3</td>
<td>0.84 ± 0.08</td>
<td>0.32 ± 0.04</td>
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<tr>
<td>Post-I</td>
<td>8</td>
<td>13.6 ± 1.2</td>
<td>13.1 ± 1.1</td>
<td>36.6 ± 3.4</td>
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<td>Pyruvate/IA</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>22.8 ± 1.7</td>
<td>7.4 ± 0.7</td>
<td>38.2 ± 1.8</td>
<td>0.72 ± 0.05</td>
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<tr>
<td>Post-I</td>
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<td>14.3 ± 0.9</td>
<td>11.9 ± 0.9</td>
<td>39.8 ± 3.0</td>
<td>1.07 ± 0.09</td>
<td>0.28 ± 0.03</td>
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<tr>
<td>Workstate ii</td>
<td></td>
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<tr>
<td>Control</td>
<td>10</td>
<td>21.2 ± 0.8</td>
<td>17.2 ± 0.7</td>
<td>23.1 ± 1.7</td>
<td>0.78 ± 0.03</td>
<td>0.32 ± 0.01</td>
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<td>Post-I</td>
<td>12</td>
<td>14.6 ± 1.0</td>
<td>19.4 ± 1.1</td>
<td>32.1 ± 2.7</td>
<td>0.71 ± 0.04</td>
<td>0.40 ± 0.01</td>
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<td>Pyruvate</td>
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<tr>
<td>Control</td>
<td>9</td>
<td>30.4 ± 2.8</td>
<td>14.3 ± 1.4</td>
<td>49.9 ± 4.8</td>
<td>1.13 ± 0.12</td>
<td>0.33 ± 0.04</td>
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<tr>
<td>Post-I(A)</td>
<td>12</td>
<td>11.6 ± 0.6</td>
<td>16.2 ± 1.8</td>
<td>30.3 ± 1.7</td>
<td>1.16 ± 0.06</td>
<td>0.28 ± 0.02</td>
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<tr>
<td>Post-I(B)</td>
<td>8</td>
<td>15.5 ± 1.0</td>
<td>14.7 ± 1.5</td>
<td>37.5 ± 2.2</td>
<td>1.19 ± 0.09</td>
<td>0.37 ± 0.03</td>
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<tr>
<td>Pyruvate/IA</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>21.7 ± 2.4</td>
<td>12.5 ± 1.6</td>
<td>39.4 ± 4.5</td>
<td>0.85 ± 0.07</td>
<td>0.26 ± 0.02</td>
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<td>Post-I(A)</td>
<td>9</td>
<td>13.0 ± 2.1</td>
<td>15.8 ± 1.1</td>
<td>35.0 ± 3.7</td>
<td>1.14 ± 0.10</td>
<td>0.30 ± 0.03</td>
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</table>

* Metabolite contents are in units of micromoles (g dry weight)⁻¹.
³ At workstate i, pyruvate concentration was 0.5 and 1 mM for control and postischemic groups, respectively.
⁴ At workstate ii, pyruvate concentration was 1 and 10 mM in the Post-I(A) and Post-I(B) groups, respectively, irrespective of the use of IA. Control pyruvate concentration was 1 mM.
of postischemic RPP on pyruvate concentration may reflect increased substrate needs for the myocardium during the recovery period immediately following the ischemic insult; however, a detailed analysis of this finding is beyond the scope of this paper and does not affect the primary conclusions reached regarding ATP synthesis rates.

The postischemic reduction in RPP was not accompanied by commensurate alterations in MVO₂. The consequences of the ischemic insult on the MVO₂-RPP relationship are illustrated in Fig. 7. The data given in this figure on normal hearts include workstates i and ii listed in Table II as well as additional workstates which were previously employed for the determination of Pᵢ → ATP and ATP → Pᵢ rates at higher MVO₂ (18). In normal hearts, our data fulfill the expectation that MVO₂ should be linearly dependent on RPP (24); the solid lines in Fig. 7 are the best fits obtained by linear regression (r² = 0.98 and 0.99 for Fig. 7A and B, respectively), and are indistinguishable in their slope and y intercept for the control glucose and the control pyruvate groups. The postischemic points are displaced relative to these lines and illustrate that, at a given MVO₂, the RPP is reduced or, conversely, at a given RPP, the MVO₂ is elevated for the postischemic hearts relative to the controls. For example, the postischemic glucose group at workstate i operates with an ATP rate for the pyruvate-pyruvate/IA glucose-pyruvate/IA ratio of 2.2 ± 0.2 for the control and i mm for the Post-I groups.

At workstate ii, pyruvate concentration was 1 mM for the control and 10 mM for Post-I(A) and Post-I(B) groups, respectively. The pyruvate concentration in the control group was 1 mM.

The data are reproduced from Table II. The dashed line is the corresponding average (Pᵢ → ATP rate)/MVO₂ ratio observed for nonischemic controls over a larger MVO₂ range (18).

FIG. 5. Bar graph illustrating the Pᵢ → ATP rate for the glucose (Glu), pyruvate (Pyr), and pyruvate + IA (Pyr/IA) groups at workstates i and ii. The solid and striped bars represent control and postischemic data, respectively.

FIG. 6. Bar graph showing the ratio of unidirectional ATP synthesis rate to oxygen consumed (the (Pᵢ → ATP rate)/MVO₂ ratio, a dimensionless parameter) at workstates i and ii, in postischemic hearts where the glycolytic contribution was eliminated. The data are reproduced from Table II. The solid line is the corresponding average (Pᵢ → ATP rate)/MVO₂ ratio observed for nonischemic controls over a larger MVO₂ range (18).

### Table II

<table>
<thead>
<tr>
<th>Workstate</th>
<th>N</th>
<th>RPP 10⁶ mm Hg min⁻¹</th>
<th>MVO₂ μmol min⁻¹ (g dry wt)⁻¹</th>
<th>Pᵢ → ATP rate μmol s⁻¹ (g dry wt)⁻¹</th>
<th>(Pᵢ → ATP rate)/MVO₂ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>26.4 ± 2.0</td>
<td>34.0 ± 2.2</td>
<td>7.1 ± 0.8</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>Post-I</td>
<td>13</td>
<td>21.8 ± 2.0</td>
<td>42.7 ± 1.8</td>
<td>11.4 ± 1.1</td>
<td>7.9 ± 0.7</td>
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<tr>
<td>Pyruvate</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>26.5 ± 1.0</td>
<td>36.1 ± 1.5</td>
<td>5.0 ± 0.9</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>Post-I</td>
<td>9</td>
<td>21.6 ± 1.4</td>
<td>41.9 ± 1.7</td>
<td>3.6 ± 0.5</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Pyruvate/IA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>22.1 ± 1.4</td>
<td>39.5 ± 1.5</td>
<td>3.1 ± 0.4</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Post-I</td>
<td>8</td>
<td>19.4 ± 0.9</td>
<td>43.6 ± 1.7</td>
<td>3.1 ± 0.4</td>
<td>2.1 ± 0.3</td>
</tr>
</tbody>
</table>

* Pyruvate concentration at workstate i was 0.5 mM for the control and i mm for the Post-I groups.

* At workstate ii, pyruvate concentration was 1 and 10 mM for Post-I(A) and Post-I(B) groups, respectively. The pyruvate concentration in the control group was 1 mM.
The Origin of NMR-Measurable Pi and ATP Production Rates—

We have recently shown that in normal hearts, a large fraction of the NMR-measurable unidirectional Pi ↔ ATP rate originates from the reactions catalyzed by the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase when the exogenous carbon substrate is glucose (18); the rate of net ATP synthesis by oxidative phosphorylation was measurable only when the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase contribution was eliminated. In nonischemic control hearts, supplying pyruvate as the only carbon source and depleting the glycolytic pathway was sufficient to eliminate the glycolytic contribution to the P → ATP rate measured by NMR at both workstates except at workstate i. The complete suppression of the glycolytic contribution at workstate i, however, required the use of IA.

In postischemic hearts, the unidirectional P → ATP rate decreased at both workstates when the postischemic carbon substrate was switched from glucose to pyruvate (Fig. 5); an additional significant decrease was not noted relative to the pyruvate groups when IA was employed to directly inhibit glyceraldehyde-3-phosphate dehydrogenase (Fig. 5). Therefore, we conclude that, as in controls, there is a large glycolytic contribution to the unidirectional P → ATP rate measured by NMR in postischemic hearts perfused with glucose, and, unlike the controls, pyruvate perfusion alone is sufficient to eliminate this glycolytic contribution at both workstates examined. This difference between control and postischemic hearts may be a consequence of more complete depletion of glycogen stores during ischemia than by transient substrate-free perfusion at workstate i in the nonischemic normal.

In the normal hearts, when the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase component was suppressed with or without the use of IA, the NMR-measurable P → ATP rate was linearly dependent on MVO₂ and satisfied the equation (P → ATP rate)/MVO₂ = K, where K is a constant (18). This is expected if the measured P → ATP rate is the net rate of ATP synthesis by oxidative phosphorylation, in which case K must be the effective P:O ratio. In the postischemic myocardium, the MVO₂ range examined is much higher than those achieved at workstate ii. Nevertheless, for the postischemic pyruvate and pyruvate/IA hearts operating in the MVO₂ range 41.9 ± 1.7 to 57.3 ± 1.8 μmol - min⁻¹ (g dry weight)⁻¹, the (P → ATP rate)/MVO₂ ratio was constant (Fig. 6), indicating that as in the normal hearts, two-site saturation transfer measures the net rate of ATP synthesis by oxidative phosphorylation subsequent to the suppression of the contribution.

Effect of Ischemia—Ischemia-induced alterations in the glycolytic and oxidative components of the NMR-measurable P → ATP rate can be deduced by comparing the controls with the corresponding postischemic group. In glucose-perfused hearts, the post-ischemic P → ATP rate was elevated by ∼50% relative to glucose-perfused controls (Fig. 5). Comparison with rates obtained when the glycolytic component was suppressed (Fig. 5) illustrates that this postischemic increase is entirely due to the glycolytic contribution. The increase was dependent on the duration of the ischemic episode and was not observable with only 13 min of ischemia.

The enhanced contribution to the NMR-measured P → ATP rate in the postischemic heart is not necessarily indicative of an elevated net flux through the glycolysis pathway. This follows from the fact that the NMR-measured rate is a unidirectional rate. In both normal and postischemic hearts, the unidirectional P → ATP rate due to the reactions far exceeds the maximum rate of glucose catabolism by the glycolysis pathway (24). Therefore, the reverse reaction must be significant and the coupled reactions must be near equilibrium. Under these conditions, the unidirectional P → ATP and ATP → P rates can increase commensurately so that the net rate, which is the difference of the two unidirectional rates, remains unaltered. It is also possible that both the unidirectional rates and the net rates are enhanced subse-

**FIG. 7. MVO₂-RPP relationship in glucose-perfused (A) and pyruvate-perfused (B) hearts. O, data obtained with normal (nonischemic) hearts; • and △, represent postischemic hearts. For the pyruvate group (B), the postischemic pyruvate concentration was either 1 mM (•) or 10 mM (△); for the normals, pyruvate concentration was 0.5, 1, 1.5, 10, and 10 mM for the five points with increasing RPP and MVO₂, respectively. Unless error bars are shown, the standard errors for the data points is less than or equal to the size of the symbols used.**
ATP Synthesis in the Postischemic Myocardium

sequent to ischemia. Despite this ambiguity about the precise nature of the changes at the level of glycolaldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase enzymes, the ischemia-induced effect in this rate is indicative of a significant alteration in the glycolytic pathway consequent to the ischemic insult. Unlike the glycolytic component, the P_i → ATP rate due to oxidative phosphorylation at a given MVO_2 was not altered by the ischemic insult in spite of a decreased mechanical output. This is easily seen from Fig 6 where the (P_i → ATP rate)/MVO_2 ratios are illustrated for the pyruvate-perfused postischemic hearts where the glycolytic contribution to the P_i → ATP rate has been suppressed. The dashed line in this graph corresponds to the (P_i → ATP rate)/MVO_2 ratio of 2.34 ± 0.12 obtained from nonischemic controls over a larger MVO_2 range using rate measurements in both the P_i → ATP and ATP → P_i directions (18). The average value obtained for the same ratio from the five pyruvate and pyruvate/IA postischemic groups was 2.36 ± 0.09. It is therefore concluded that there is no uncoupling at the level of oxidative phosphorylation following the ischemic insult.

Is should be emphasized that although we have not observed uncoupling due to a reversible, sublethal ischemic insult, such an insult may produce other deleterious consequences on mitochondrial oxidative function. In fact, we have recently established that even shorter periods of ischemia, which induce less mechanical dysfunction than that reported in the present study, alter the mechanisms of respiratory control and lead to a significant reduction in the apparent mitochondrial V_{O2} for O_2 consumption in the intact myocardium (29). Postischemic ATP Kinetics and Mechanical Function—The absence of postischemic mitochondrial uncoupling indicates that at any given MVO_2, the postischemic heart synthesizes and utilizes ATP at the same rate as the normal heart. This conclusion follows from the fact that ATP content is at a constant value in the ischemic insult in spite of a decreased mechanical output. This is easily seen from Fig. 6 where the (P_i → ATP rate)/MVO_2 ratio of 2.34 ± 0.12 obtained from nonischemic controls over a larger MVO_2 range using rate measurements in both the P_i → ATP and ATP → P_i directions (18). The average value obtained for the same ratio from the five pyruvate and pyruvate/IA postischemic groups was 2.36 ± 0.09. It is therefore concluded that there is no uncoupling at the level of oxidative phosphorylation following the ischemic insult.

The interpretation of the above observation is dependent upon the relationship between RPP and the rate of ATP hydrolysis due to force development by the contractile apparatus. It was shown previously that RPP is linearly dependent on MVO_2 in the isolated perfused rat heart operating in either the working or the Langendorff mode using different exogenous carbon substrates (24, 30). If the efficiency of the contractile apparatus at the level of cross-bridge interactions is unaltered by the ischemic insult, then the data indicate that there must exist increased and competing ATP utilization by noncontractile processes in the postischemic myocardium. Alternatively, the possibility exists that the efficiency of the cross-bridge interaction is reduced. The current data do not allow us to distinguish between these possibilities.

While the above discussion rests on the use of RPP as a measure of ATP utilized by mechanical activity, it should be noted that with this degree of ischemia postischemic mechanical function is depressed irrespective of the particular parameter and the method employed to assess it. The ischemic episode used in this study was approximately the maximum duration compatible with partial recovery of mechanical function. It was recently shown in the intact canine myocardium that a similar transient and sublethal regional ischemic insult induced significant depression in mechanical performance as measured by wall segment shortening while the MVO_2 of the same region was elevated* (31). This finding is strikingly similar to our observations and therefore, supportive of our conclusions.

CONCLUSIONS

Our use of NMR to measure the unidirectional P_i → ATP rate in the glucose-perfused myocardium demonstrates that:

1) there exists a significant glycolaldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase contribution to the NMR-measurable unidirectional P_i → ATP rate subsequent to ischemia;

2) the glycolaldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase contribution is increased by the ischemic insult, and the increase is dependent on the duration of ischemia; and

3) the reactions catalyzed by the glycolaldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase couple are in equilibrium in the postischemic as well as normal cardiac muscle. The primary conclusion concerning oxidative phosphorylation is that a maximal sublethal ischemic insult does not induce mitochondrial uncoupling in the intact heart; therefore, at any given MVO_2, the net ATP synthesis rate by oxidative phosphorylation is unaltered by ischemia. This observation eliminates ischemia-induced uncoupling of mitochondria as the cause of depressed postischemic mechanical function and suggests the existence of inefficiency in ATP utilization in the postischemic myocardium.

REFERENCES

ATP Synthesis in the Postischemic Myocardium