Regulation of bioenergetics in O$_2$-limited isolated rat hearts

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Assessing the factors that acutely regulate myocardial bioenergetics during I or H is critical in understanding ischemic preconditioning (see Ref. 41 for review) and to optimize myocardial protection techniques. When hearts are exposed to acute (20 min) I and H at comparable QO$_2$, temperature, and time of O$_2$ deprivation, they show poorer recovery from H than from I (11); furthermore, the role of nonspecific oxidative injury in determining the extent of reperfusion injury is relatively modest compared with that of metabolic factors (36). We designed this study to gain further insight on the regulation of myocardial bioenergetics during dysoxia. For this purpose, I and H were matched for QO$_2$ to exclude this variable as a factor that affects myocardial bioenergetics. Hearts were electrically paced during part of the experiments to test the hypothesis that a reserve of energy can be made available during H or I to cope with the increased energy demand. Finally, because different coronary flows (CF) are likely to determine different rates of washout of diffusible substances, we evaluated the role of lactate as regulator of myocardial bioenergetics. We showed that I and II elicit different responses and that lactate plays a more critical regulatory role than O$_2$ at least under severely dysoxic conditions.

MATERIALS AND METHODS

Experimental design. We perfused isolated rat hearts by a Langendorff technique and monitored performance, O$_2$ uptake (VO$_2$), and lactate release (J$_L$). Isovolumic hearts were stabilized for 20 min at CF of 15 ml/min and Pao, of 670 Torr (QO$_2$, of 14.1 µmol/min using the reported O$_2$ solubility coefficient; Ref. 32). The volume of the intraventricular balloon was set to yield an end-diastolic pressure (EDP) of 7.0 ± 0.5 mmHg and was kept constant throughout the experiment. After 20 min of baseline, QO$_2$ was reduced to 1.41 µmol/min by either decreasing CF to 1.5 ml/min (I group; n = 6) or Pao, to 67 Torr (II group; n = 6). The time delay for the hypoxic perfusate to reach the heart and that required to set the pump to 1.5 ml/min were <1 min and ~30 s, respectively. Hearts were then allowed to adjust their energy demand and rate (HR) for 20 min, after which hearts were paced at 300 beats/min for 10 min.

Apparatus. The microoxygenator used in this study (Dideco, Mirandola, Italy) consisted of a hollow-fiber polypropylene membrane that provided a total OD surface area of 0.12 m$^2$. In
TABLE 1. Myocardial performance and metabolism during baseline at CF of 15 ml/min, Pao, of 670 Torr, and volume of intraventricular balloon adjusted to yield EDP of 7 mmHg

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDP, mmHg</td>
<td>7.3±0.3</td>
</tr>
<tr>
<td>Developed pressure, mmHg</td>
<td>139±6</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>4.196±0.254</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>243±7</td>
</tr>
<tr>
<td>Coronary sinus Po, mmHg</td>
<td>305±27</td>
</tr>
<tr>
<td>LVDP × HR, mmHg × 1,000 beats/min</td>
<td>38.7±1.5</td>
</tr>
<tr>
<td>O₂ uptake, amol/min</td>
<td>7.7±0.6</td>
</tr>
<tr>
<td>O₂ extraction, %</td>
<td>54±7.6</td>
</tr>
<tr>
<td>CPP, mmHg</td>
<td>80±5</td>
</tr>
<tr>
<td>Coronary resistance, mmHg·min·ml⁻¹·g</td>
<td>4.8±0.3</td>
</tr>
<tr>
<td>Coronary sinus [lactate], mM</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Lactate release, amol/min</td>
<td>0.9±0.05</td>
</tr>
<tr>
<td>H₂O content, %</td>
<td>86±7.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 rats. CF, coronary flow; Pao, arterial Po₂; EDP, end-diastolic pressure; dP/dt max, maximal rate of pressure development; HR, heart rate; LVDP, left ventricular developed pressure; CPP, coronary perfusion pressure; [lactate], lactate concn.

TABLE 2. EDP, CF, CPP, and coronary vascular resistance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ischemia</th>
<th>Hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDP, mmHg</td>
<td>4.6±0.4</td>
<td>21.3±4.6</td>
</tr>
<tr>
<td>CF, ml/min</td>
<td>1.5</td>
<td>15</td>
</tr>
<tr>
<td>CPP, mmHg</td>
<td>10±1</td>
<td>79±6</td>
</tr>
<tr>
<td>Coronary vascular resistance, mmHg·min·ml⁻¹·g</td>
<td>3.8±0.9</td>
<td>3.9±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE where indicated; n = 6 rats in each group.
creased in H hearts ($P = 0.01$) but not in I hearts (NS). In H hearts, $\text{LVDP} \times \text{HR}$ remained constant (NS). In I hearts, $\text{LVDP} \times \text{HR}$ nearly doubled ($P = 0.005$) but remained less than that in H hearts ($P < 0.0005$). Pacing slightly increased the $\text{O}_2$ extraction to $86.5 \pm 2.7\%$ and $88.6 \pm 1.5\%$ in I and H hearts (NS), respectively. Therefore, $\text{PvO}_2$ and $\text{Vo}_2$ in paced hearts were similar to those measured in spontaneous hearts. Pacing increased both venous [lactate] and $J_{\text{lac}}$ ($P = 0.002$) during I but not during H. As an overall result of the above changes, $J_{\text{ATP}}$ increased by $3.3 \pm 0.5 \mu\text{mol} / \text{min}$ during I ($P = 0.001$), whereas it decreased slightly ($1.6 \pm 1.5 \mu\text{mol} / \text{min}$; NS) during H.

Figure 3 shows the contribution of aerobic and anaerobic metabolisms to total $J_{\text{ATP}}$ calculated as explained in MATERIALS AND METHODS.

**DISCUSSION**

**Critique of the experimental model.** Reliability of $Q_{\text{O}_2}$ ($\text{CF} \times \text{PaO}_2$) was critical in this study. In our Langendorff preparations, the aortic flow was quantitatively displaced through the coronary arteries and was controlled with a digital display roller pump. We neglected the small fraction (2%) of left coronary artery inflow that circulates through the thebesian veins into the left ventricle (31). However, to reduce this variable to a minimum, no drainage was applied to the left ventricle. The arterial $\text{O}_2$ content was controlled by the described microoxygenator that yields the desired $\text{Po}_2$ and $\text{PCO}_2$ with little influence of liquid and gas flows. The small compliance of the oxygenator prevented slow flow adjustments secondary to changes of roller pump settings that may cause inconsistencies in the actual CF.

$Q_{\text{O}_2}$ during baseline (14.1 $\mu\text{mol} / \text{min}$ or 12.7 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) was slightly higher than $Q_{\text{O}_2}$ in the heart in vivo ($8.5$–$10.1 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, if we assume CF of 70–85 ml $\cdot$ 100 g$^{-1}$ $\cdot$ min$^{-1}$, hemoglobin concentration of 15.5 g/dl, and 98% $\text{O}_2$ saturated at $\text{PaO}_2$ of 100 Torr). Nevertheless, $\text{Vo}_2$ and $\text{O}_2$ extraction (see Table 1) were near normal (3.6–6.7 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ and 66%, respectively; Ref.
ROLE OF LACTATE IN ISCHEMIC DOWNREGULATION

**FIG. 3.** Contribution of aerobic and anaerobic metabolisms to ATP turnover in our model. Open bars, spontaneous; crosshatched bars, paced. * Significantly different from respective value in ischemic hearts (P < 0.0005; unpaired Student's t-test). # Significantly different from respective value in spontaneous hearts (P < 0.0005; paired Student's t-test). See explanations in text.

8). Furthermore, the venous [lactate] was low and the level of high-energy phosphates compared well with normal values (unpublished observations). Therefore, \( Q_o \) during baseline was adequate to support myocardial activity. We avoided the use of blood to minimize interferences, to retain the accuracy of \( Q_o \) and \( V_o \), and to avoid difficult interpretations of the phenomena related to red blood cell spacing within the capillaries and kinetics of \( O_2 \) unloading from hemoglobin.

The relatively high glucose concentration ([glucose]) fully saturated the glucose transport system (45). The glucose supply during I (190 \( \mu \)mol \( \cdot \) min\(^{-1} \cdot \) g dry wt\(^{-1} \)) exceeded by one order of magnitude the maximal glucose utilization by anoxic Langendorff-perfused isolated rat hearts (14 \( \mu \)mol \( \cdot \) min\(^{-1} \cdot \) g dry wt\(^{-1} \); Ref. 33) excluding glucose shortage during I and H. The use of hyperglycemic perfusate ([glucose] of 11 mM) contributed to minimize the utilization of glycogen (the contribution of fats is ruled out for the absence of fatty acids in the perfusate). Although tissue glycogen decreases by 80 and 75\%, respectively (34), in working hearts exposed to no-flow I and total anoxia, more recent work with anoxic (1 h at 30\(^\circ\)C, 1 Hz) Langendorff-perfused ferret hearts showed that tissue glycogen declines only for perfusate [glucose] of <10 mM (25). Furthermore, computer simulation of the mechanisms underlying the increased glycolytic flux in a work jump (switching from the Langendorff to the left atrial perfusion) showed that after a short initial phase (20 s) of glycogen breakdown the increased glycolytic flux is sustained mainly through increased glucose uptake (1). Finally, the utilization of exogenous glucose appears more important than that of glycogen during H (35) and during I before the development of contracture (28), as in our case (EDP did not increase during I), most likely because of subcellular compartmentation problems. Thus, we have reasonably assumed the ATP-to-lactate ratio of 1.0. Hyperglycemia could not have any significant effect in our model because the glycolytic rate is regulated by enzyme activity rather than intracellular glucose (24) and there was no competition between glucose and other substrates.

**H and I.** Although matched for \( Q_o \), H and I elicited different responses in our model. During H, hearts performed at a higher level than during I despite same \( V_o \). However, \( J_{\text{lac}} \) was also higher, possibly contributing to increase \( J_{\text{ATP}} \) and to account for higher contractility during H (Fig. 4). In skeletal muscle exposed to 25% \( Q_o \) reduction, the increase of \( J_{\text{lac}} \) failed to account for the observed increase of muscle contractility during H (38). Perhaps inexact matching of \( Q_o \) in those experiments may explain the discrepancy as, by the authors' own admission, flow was not constant in their protocol (see Table 1 of Ref. 38).

The maintenance of the coronary vascular resistance during I and H (Table 2) indicates unchanged myocardial perfusion. The increased CPP and resistance in paced I hearts, likely secondary to lactate accumulation, may

**FIG. 4.** Relationship between \( LVDP \times HR \) and \( O_2 \) uptake (\( V_o \); A) or ATP turnover rate (B). All data are shown. Squares and circles are spontaneous and paced hearts, respectively. Best-fit line uses all data points (\( r = 0.975; P < 0.001 \)).
imply some degree of flow heterogeneity in this group. Indeed, skeletal muscle VO$_2$ may decrease because of flow maldistributions even at constant total flow if arterial pressure is low (39). On the other hand, flow heterogeneity observed in epi-, mid-, and endomyocardium as well as during the different phases of the contraction cycle of hypertrophied hearts subjected to low-flow I tends to disappear in nonhypertrophied hearts (5). Also, this problem may be less important in Langendorff-perfused hearts than in other models because of the intraventricular balloon that induces isovolumetric contractions and the fixed CF during I and H. However, we cannot rule out the possibility that downregulation of muscular activity during I was the result not only of lactate accumulation (see below) but also of nonuniform muscle perfusion so that some regions were nonperfused to maintain perfusion in others (22).

Altered Ca$^{2+}$ homeostasis may be critical in our model. However, the heart does not consume Ca$^{2+}$ as it does O$_2$. Thus, the Ca$^{2+}$ influx into the myocyte depends on the Ca$^{2+}$ gradient, i.e., on medium Ca$^{2+}$ concentration that is constant throughout. In fact, a study aimed at assessing the effect of Ca$^{2+}$ washout during low and no-flow I showed that this effect is critical during reperfusion but not during I (40). Therefore, we believe that the role of Ca$^{2+}$ is marginal in our model. The low HR during I may reflect lower K$^+$ washout in I than H hearts (30) with consequent higher intracellular K$^+$ during I. The bradycardic effect may also be exerted by lactate itself (43).

Although many workers have already compared ischemic vs. hypoxic hearts, we are not aware of other studies where the QO$_2$ in the two conditions was matched except in previous studies by Corno and co-workers (11, 12, 36). Thus, we compared our data with those obtained by Hogan et al. (23) and Dodd et al. (16) in moderately dysoxic in situ working dog gasterocnemius muscle. In these studies, no functional differences between matched H and I were found. Because no lactate data are available from either study, it is difficult to explain such discrepancies. Several factors may determine these apparently contradictory findings. First, red blood cells in the perfusing medium may have buffered O$_2$ and lactate stores and blunted our observed effects. Second, the O$_2$ deprivation was 90% in our study, 50% in the study by Hogan et al. (23), and 27% in that by Dodd et al. (16), therefore, a more severe energy supply-demand unbalance was produced in our experiments. Third, the relatively higher amount of mitochondria in heart than in muscle may make hearts more sensitive to perturbations than skeletal muscles.

Despite such discrepancies, all studies agree on reporting lower PV$_{O_2}$ during H than during I. This difference is amplified in our work because of the severe dysoxia and the lack of blood O$_2$ buffering capacity. It is difficult to assess from the present data whether the high PV$_{O_2}$ during I results from vascular collapse leading to a lesser exchange area and lower O$_2$ diffusive conductance (22) or rather from "metabolic arrest" (19). In our experiments, however, the extra workload caused by pacing did not alter PV$_{O_2}$, implying that either the metabolic arrest was insensitive to pacing or that the primary limitation in I was at the level of the microcirculation somehow supporting the former hypothesis. In any case, this study shows that O$_2$ did not appear to be an adequate energy reserve during dysoxia. In fact, Fig. 4 shows that the changes of LVDP × HR were independent of VO$_2$ under all dysoxic conditions. In contrast, LVDP × HR was highly related to $\Delta$ATP ($r = 0.99$), suggesting that components other than O$_2$-dependent metabolism may have regulated myocardial performance. This conclusion is in qualitative agreement with the relationship between $\Delta$O$_2$ and VO$_2$ (Fig. 1B of Ref. 16) where the I point lies above the best-fit line, indicating that factors other than O$_2$ have regulated bioenergetics during I.

**Role of lactate.** The greater contractility during H than during I and the ability of hearts to upgrade their performance during I were linked to non-O$_2$-dependent processes, most likely anaerobic glycolysis. Actually, although anaerobic glycolysis provides a minor fraction of total ATP, it may be quickly stimulated at the level of phosphofructokinase (1) by allosteric factors such as AMP, the concentration of which increases early during I. The relative importance of anaerobic ATP production increases during I or H. Indeed, the observation that pacing did not alter $J_{lac}$ during H suggests that anaerobic glycolysis was already working at maximal or near-maximal levels in H hearts. In fact, the values of $J_{lac}$ during H (9.2–10.8 μmol·min$^{-1}$·g wet wt$^{-1}$) and 60–70 μmol·min$^{-1}$·g dry wt$^{-1}$) are close to the values of 10–14 μmol·min$^{-1}$·g wet wt$^{-1}$ (27) and 60–70 μmol·min$^{-1}$·g dry wt$^{-1}$ (42) reported for contracting anoxic isolated rat hearts.

Depressed anaerobic glycolysis led to lower LVDP × HR during I, but electrical stimulation upgraded LVDP × HR. If we consider venous lactate a reliable index of cell lactate, then the higher [lactate] in the venous effluent of I hearts reflects low washout, in agreement with the observation that perfusing hearts at CF of 1 ml/min results in a >10-fold tissue accumulation of lactate than at CF of 15 ml/min (40). It is difficult to assess whether our data were influenced by lactate or H+. On one hand, lactate-induced acidosis inhibits glycolysis and depresses contractility (27, 33, 44). On the other hand, some authors questioned the possibility that lactate does cause acidosis, with the bulk of H+ generated from ATP breakdown and/or turnover (15) and possibly from the glycolytic flux. However, acidosis is always greater during H than during I, and lactate may directly affect the activity of glycolytic enzymes (33). Acid pH exerts its protective action by inhibiting proteolytic enzymes and other degradative processes (6).

Release of the inhibition of glycolysis by lactate and/or H+ requires shorter times than biosynthesis of new enzymes. This mechanism may thus regulate what Hochachka and Matheson (21) called the "effective enzyme concentration," depending on the needs of the system, whereas fine bioenergetics adjustments still depend on substrate variations. It is therefore possible that the hypothesized "latent" or "inaccessible" pool of enzymes depends in part on the inhibition of the existing enzyme pool by lactate and/or H+. Conversely, the greater availability of these enzymes may be explained, at least for acute phenomena, by lower intracellular lactate levels,
possibly combined with less acidosis and associated release of inhibition. This process would provide a "metabolic" alternative to the autonomic and hormonal mechanisms that were proposed to override protective hibernation in intact organisms (17). Furthermore, it was recently indicated that adenosine-related mechanisms may not fully explain the cardioprotective effects of ischemic preconditioning (3, 18). Finally, this process may also override the emergence of protective features in heat-acclimatized animals that were related to energy-sparing mechanisms originated by myosin switching from the fast to the slow isoform (26).

The downregulating effect by lactate and/or II* may be an active phenomenon by which the myocardial energy demand is spared to preserve the energy stores (4, 7, 14, 20, 37, 44) and to prevent fatigue. Interestingly, McArdle patients develop fatigue before any significant lactate accumulation (29). Furthermore, if downregulation follows fatigue, we should have found worse recovery in postischemic than in posthypoxic hearts. On the contrary, one of the reasons postischemic hearts recover better than posthypoxic hearts (11, 36) may involve downregulation that prevents exhaustion of the energy reserves in ischemic but not in hypoxic hearts, similar to observations made in rabbit skeletal muscles (20).

Conclusions. Ischemic hearts are more downregulated than hypoxic hearts at the same QO2. We hypothesize that in the absence of vascular and hormonal factors, downregulation may at least partially be exerted either by intracellular lactate or by the associated acidosis; high CF during H increases washout and prevents intracellular lactate accumulation, whereas low CF during I leads to high intracellular lactate that in turn depresses glycolysis. The role of lactate and other diffusible metabolites in the process of myocardial preconditioning and in the adaptation to sustained I or H remains to be evaluated.

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REFERENCES


14. Currin, R. T., G. J. Gores, R. G. Thurman, and J. J. Lomas-


18. Canote, C. E., S. Armstrong, and J. M. Downey. Adenosine and AI selective agonists offer minimal protection against isch-


27. Matthews, P. M., D. J. Taylor, and G. K. Rudd. Biological