Regulation of bioenergetics in O_2-limited isolated rat hearts

MICHELE SAMAJA, STEFANIA CASALINI, SONIA ALLIBARDI, ANTONIO CORNO, AND SERGIO L. CHIERCHIA

Department of Biomedical Science and Technology, Scientific Institute San Raffaele, University of Milan, San Donato Hospital, I-20132 Milan, Italy

Samaja, Michele, Stefania Casalini, Sonia Allibardi, Antonio Corno, and Sergio L. Chierchia. Regulation of bioenergetics in O_2-limited isolated rat hearts. *J. Appl. Physiol.* 77(6): 2530–2536, 1994.—Assessing the role of O_2 supply in the regulation of cardiac function in O_2-limited hearts is crucial to understanding myocardial ischemic preconditioning and adaptation to hypoxia. We exposed isolated Langendorff-perfused rat hearts to either ischemia (low coronary flow) or hypoxemia (low P_{O_2} in the perfusing medium) with matched O_2 supply (10% of baseline). Myocardial contractile work and ATP turnover were greater in hypoxemic than in ischemic hearts ($P < 0.05$; $n = 12$). Thus, the energy demand was higher during hypoxemia than during ischemia, suggesting that ischemic hearts are more downregulated than hypoxemic hearts. Venous P_{O_2} was 12 ± 2 and 120 ± 15 Torr ($P < 0.0001$) for ischemic and hypoxemic hearts, respectively, but O_2 uptake was the same. Lactate release was higher during hypoxemia than during ischemia (9.7 ± 0.9 vs. 1.4 ± 0.2 μmol/min, respectively; $P < 0.0001$). Electrical stimulation (300 min^{-1}; to increase energy demand) increased performance in ischemic ($P < 0.005$) but not in hypoxemic hearts without changes in venous P_{O_2} or O_2 uptake. However, venous lactate concentration and lactate release increased in ischemic ($P < 0.002$) but not in hypoxemic hearts, suggesting that anaerobic glycolysis provides the energy necessary to meet the increased energy demand in ischemic hearts only. We conclude that high intracellular lactate or H^+ concentration during ischemia plays a major role as a downregulating factor. Downregulation disappears in hypoxemic hearts secondary to enhanced washout of lactate or H^+.

hypoxemia; ischemia; lactate; oxygen uptake; oxygen supply; energy demand

IN THE MAMMAL MYOCARDIUM, high-energy phosphates are utilized at high rates with respect to their steady-state intracellular concentration (21). Therefore, the myocardial contractile system is critically dependent on energy-yielding metabolic processes and on the continuous supply of O_2 and substrates. If the O_2 supply is low with respect to the needs of the system, a potentially lethal condition may be established due to rapid depletion of high-energy phosphates, leading to cell failure and necrosis. Such a situation is referred to here as "dysoxia," i.e., O_2-limited cytochrome turnover (10). To prevent the effects of dysoxia, contractile systems may adapt by establishing a new equilibrium between energy supply and demand. For example, the capacity of energy-yielding processes may be amplified through increased availability of key enzymes (21). Alternatively, myocardial activity may be downregulated during ischemia (I) to reduce high-energy phosphate utilization (2). The regulators of myocardial activity during dysoxia are not identified, but the O_2 supply ($\dot{Q}O_2$; flow × O_2 content) is a major candidate. Stainsby et al. (38) suggested that the mechanical and metabolic responses of skeletal muscle to I [low flow at normal arterial P_{O_2} (Pa_{O_2})] and hypoxemia (H; low Pa_{O_2} at normal flow) are not the same and thus $\dot{Q}O_2$ may not be the only factor that determines the energy demand. In contrast, Hogan et al. (23) and Dodd et al. (16) showed that in ischemic and hypoxemic gastrocnemius muscles matched for $\dot{Q}O_2$ the bioenergetics of H and I involve similar patterns. Their results imply that $\dot{Q}O_2$ has a major regulatory role during dysoxia, in agreement with the classical hypothesis that ATP synthesis is mainly controlled by O_2, P_i, nicotinamide adenine dinucleotide, and ADP (9).

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 $\dot{Q}O_2$, 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, H and I were matched for $\dot{Q}O_2$ 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 H 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 ($\dot{V}O_2$), and lactate release (J_{lac}). Isovolumic hearts were stabilized for 20 min at CF of 15 ml/min and Pa_{O_2} of 670 Torr ($\dot{Q}O_2$ 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, $\dot{Q}O_2$ was reduced to 1.41 μmol/min by either decreasing CF to 1.5 ml/min (I group; $n = 6$) or Pa_{O_2} to 67 Torr (H group; $n = 6$). The time delay for the hypoxemic 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, Pa_{O_2} of 670 Torr, and volume of intraventicular balloon adjusted to yield EDP of 7 mmHg*

Parameter	Value
EDP, mmHg	7.3±0.3
Developed pressure, mmHg	139±6
dP/dt_{max}, mmHg/s	4,196±254
HR, beats/min	243±7
Coronary sinus Po_2, Torr	305±27
LVDP × HR, mmHg × 1,000 beats/min	33.7±1.5
O_2 uptake, μmol/min	7.7±0.6
O_2 extraction, %	54.4±4.0
CPP, mmHg	80±5
Coronary resistance, mmHg · min · ml^{-1} · g	4.8±0.3
Coronary sinus [lactate], mM	<0.1
Lactate release, μmol/min	<1
ATP turnover, μmol/min	53.5±3.8
Ventricle wt, g	0.90±0.05
H_2O content, %	86.7±0.6

Values are means ± SE; n = 12 rats. CF, coronary flow; Pa_{O_2}, arterial Po_2; 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.

the selected configuration, liquid and gas flow inside and outside the fibers, respectively. The priming volume in the liquid line was 2.5 ml. The oxygenator was housed in a polycarbonate structure immersed in a water bath at 37°C and connected to the appropriate gas cylinders and to the perfusing system through gastight tubing. The medium (in mM: 115.6 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 0.5 EDTA, 1.2 Na_2SO_4, 28.5 $NaHCO_3$, 2.5 $CaCl_2$, 1.2 $MgCl_2$, and 11 glucose; pH 7.40 ± 0.02, 37°C) was equilibrated with either 94% O_2-6% CO_2-0% N_2 or 10% O_2-6% CO_2-84% N_2 partitions (nominal accuracy ±0.1%) to yield Po_2 of 670 or 67 Torr at constant Pco_2 (43 Torr). A roller pump (Gilson, Villiers le Bel, France) delivered the buffer at a flow rate of either 15.0 or 1.5 ml/min to a filter (8-μm pore size, 47 mm diam, Nuclepore, Pleasanton, CA), a preheater, and then to the aortic cannula. The coronary perfusion pressure (CPP) was monitored with a pressure transducer above the aortic cannula.

Male Sprague-Dawley rats (250–280 g) were anesthetized by heparinized thiopental sodium (10 mg/100 g body wt ip). Hearts were excised, and the aorta was cannulated and mounted on the system. I never exceeded 45 s and was typically in the range of 15–30 s. Hearts were immersed in the buffer that was kept at 37°C by an external circulator. The coronary sinus return was collected by the pulmonary artery and a saline-filled latex balloon was introduced into the left ventricle. A square-wave stimulator (Harvard Apparatus, South Natick, MA) with 5-ms pulse duration and 10-V pulse amplitude was used for pacing; electrodes were placed on the aortic cannula and on the apex of the ventricle.

Measurements. Myocardial performance was monitored by a pressure transducer (model 52-9966, Harvard Apparatus) connected to the intraventricular balloon. A Clark-type electrode assembly (model 5300 oxygen monitor, Yellow Springs Instruments, Yellow Springs, OH) was connected to the aortic and coronary sinus lines to monitor Pa_{O_2} and venous Po_2 (Pv_{O_2}), respectively. Data were collected and analyzed by a dedicated LabVIEW system (National Instruments, Austin, TX) running on Macintosh Quadra 700 (Apple, Cupertino, CA). The system provided measurements of HR, EDP, peak systolic pressure, left ventricular developed pressure (LVDP), maximal rate of pressure development (dP/dt_{max}), CPP, and $\dot{V}O_2$ at 60-s intervals. At the end of each experimental phase, lactate concentration ([lactate]) was assayed by standard enzymatic methods (Sigma Diagnostics, St. Louis, MO) in the coronary sinus effluent.

Calculations and statistics. In isovolumic nonejecting hearts, myocardial contractile work is defined as "potential" work and expressed as LVDP × HR. $\dot{V}O_2$ is calculated from Pa_{O_2}, Pv_{O_2}, and CF. O_2 extraction is calculated as $\dot{V}O_2/\dot{Q}O_2$. Net J_{lac} is calculated from venous [lactate] and CF. The ATP turnover rate (J_{ATP}) is calculated as the sum of the two components yielding energy in the form of ATP under the selected experimental conditions anaerobic glycolysis and oxidative metabolism: $(1.0 \times J_{lac}) + (6 \times \dot{V}O_2)$. We assumed that the ATP-to-lactate ratio was 1.0 (glucose as substrate without significant glycogenolysis) and the ATP-to-O_2 ratio was 6 (no mitochondrial uncoupling).

Data are expressed as means ± SE. Parametric Student's t-tests for paired or unpaired observations were used where appropriate. Significance level was set to $P = 0.05$ (two tailed).

RESULTS

To test the performance of the microoxygenator, medium Po_2 was measured when the oxygenator was flowed with gases at Po_2 of 67 or 670 Torr (Pco_2 was always 43 Torr). Accuracy was 6 and 1% at flows of 1.5 and 15.0 ml/min, respectively. No effect of gas flow was observed in the range of 100–750 ml/min. Resistance was reasonable, leading to maximal head pressure of 27 mmHg at flow of 15 ml/min.

Table 1 shows baseline function and metabolism at full $\dot{Q}O_2$. During dysoxia, HR decreased both in I (158 ± 8 beats/min) and in H hearts (190 ± 31 beats/min). However, the change was significant ($P < 0.001$) in I hearts only (n = 6 for both groups). H was associated with diastolic contracture as shown by the increase in EDP (P = 0.02, Table 2). This parameter decreased during I ($P <$ 0.0005) as a result of reduced CPP secondary to low CF. Table 2 also reports the coronary vascular resistance calculated as (CPP − EDP) · CF^{-1} · $ventricle\ weight^{-1}$ (13). Figures 1 and 2 show the changes of contractility, oxygenation, and metabolic parameters as well as the time course of LVDP × HR in spontaneous and paced ischemic and hypoxemic hearts. During I, LVDP (P = 0.001), LVDP × HR ($P < 0.0005$), and dP/dt_{max} ($P < 0.0005$) were less than values during H. In addition, Pv_{O_2} was higher ($P < 0.0005$) but $\dot{V}O_2$ was the same in the two groups. O_2 extraction was also the same in the two groups (81.7 ± 2.8 vs. 82.1 ± 3.1, respectively; NS), but venous [lactate] was higher (P = 0.03) and J_{lac} was lower ($P <$ 0.0005) than during H.

When hearts were paced at 300 beats/min, LVDP de-

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

	Ischemia		Hypoxemia	
Parameter	Spontaneous	Paced	Spontaneous	Paced
EDP, mmHg	4.6±0.4	4.7±0.3	21.3±4.6	26.1±4.5
CF, ml/min	1.5		15	
CPP, mmHg	10±1	21±5	79±6	113±23
Coronary vascular resistance, mmHg · min · ml^{-1} · g	3.8±0.9	11.2±3.2	3.9±0.6	5.8±1.7

Values are means ± SE where indicated; n = 6 rats in each group. Coronary vascular resistance is calculated as (CPP − EDP) · CF^{-1} · ventricular wt^{-1}.

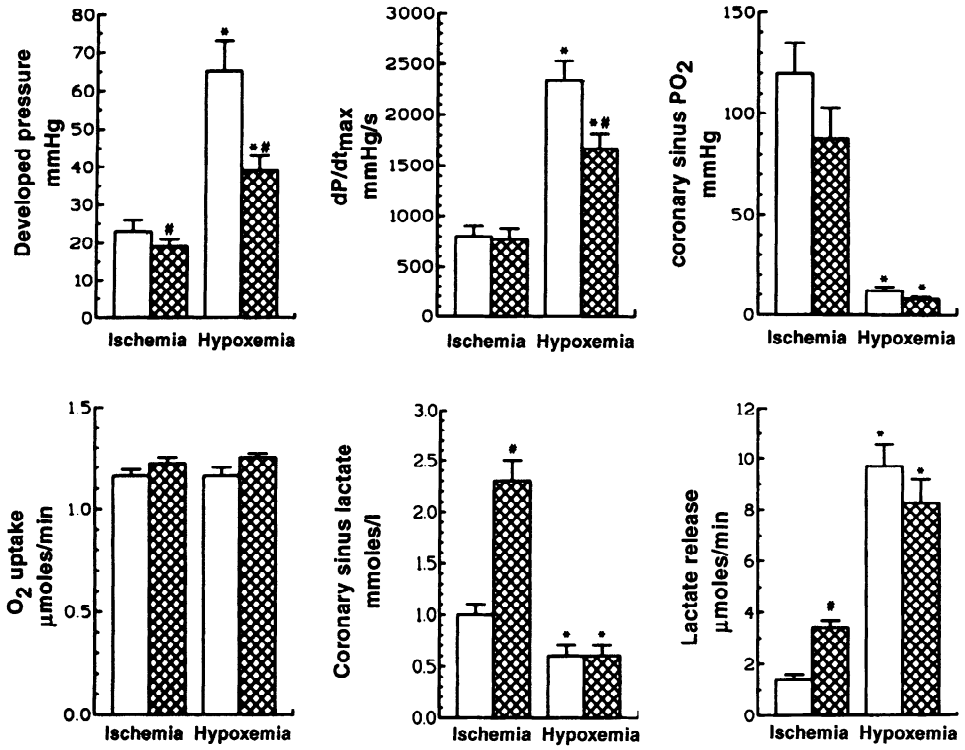

FIG. 1. Developed pressure, maximal rate of contraction (dP/dt_{max}), venous Po_2, O_2 uptake, venous lactate concentration, and lactate release during ischemia and hypoxemia in spontaneous (open bars) and paced (crosshatched bars) hearts ($n = 6$ for each group). # Significantly different from respective value in spontaneous hearts, $P < 0.0005$ (paired Student's t-test). * Significantly different from respective value in ischemic hearts, $P < 0.0005$ (unpaired Student's t-test).

creased in H hearts ($P = 0.01$) but not in I hearts (NS). In H hearts, LVDP × HR remained constant (NS). In I hearts, LVDP × HR nearly doubled ($P = 0.005$) but remained less than that in H hearts ($P < 0.0005$). Pacing slightly increased the O_2 extraction to 86.5 ± 2.7% and 88.6 ± 1.5% in I and H hearts (NS), respectively. Therefore, Pv_{O_2} and $\dot{V}O_2$ in paced hearts were similar to those measured in spontaneous hearts. Pacing increased both venous [lactate] and J_{lac} ($P = 0.002$) during I but not during H. As an overall result of the above changes, J_{ATP} increased by 3.3 ± 0.5 µmol/min during I ($P = 0.001$), whereas it decreased slightly (1.6 ± 1.5 µmol/min; NS) during H.

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

DISCUSSION

Critique of the experimental model. Reliability of $\dot{Q}O_2$ ($CF \times Pa_{O_2}$) 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 O_2 content was controlled by the described microoxygenator that yields the desired Po_2 and 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.

$\dot{Q}O_2$ during baseline (14.1 µmol/min or 12.7 µmol · $min^{-1} \cdot g^{-1}$) was slightly higher than $\dot{Q}O_2$ in the heart in vivo (8.5–10.1 µmol · $min^{-1} \cdot g^{-1}$, if we assume CF of 70–85 ml · 100 $g^{-1} \cdot min^{-1}$, hemoglobin concentration of 15.5 g/dl, and 98% O_2 saturated at Pa_{O_2} of 100 Torr). Nevertheless, $\dot{V}O_2$ and O_2 extraction (see Table 1) were near normal (3.6–6.7 µmol · $min^{-1} \cdot g^{-1}$ and 66%, respectively; Ref.

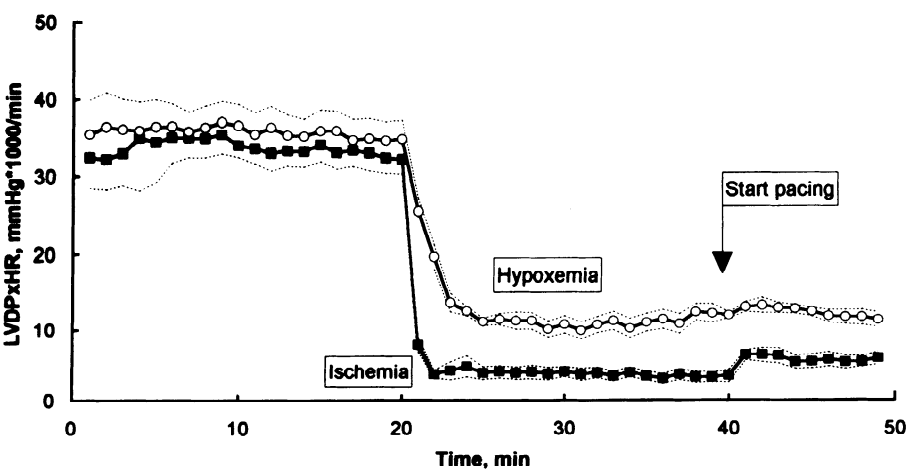

FIG. 2. Time courses of left ventricular developed pressure (LVDP) × heart rate (HR) in hearts exposed to hypoxemia or ischemia. Dashed lines are SE. Pacing upgraded LVDP × HR in ischemic ($P = 0.005$) but not in hypoxemic hearts.

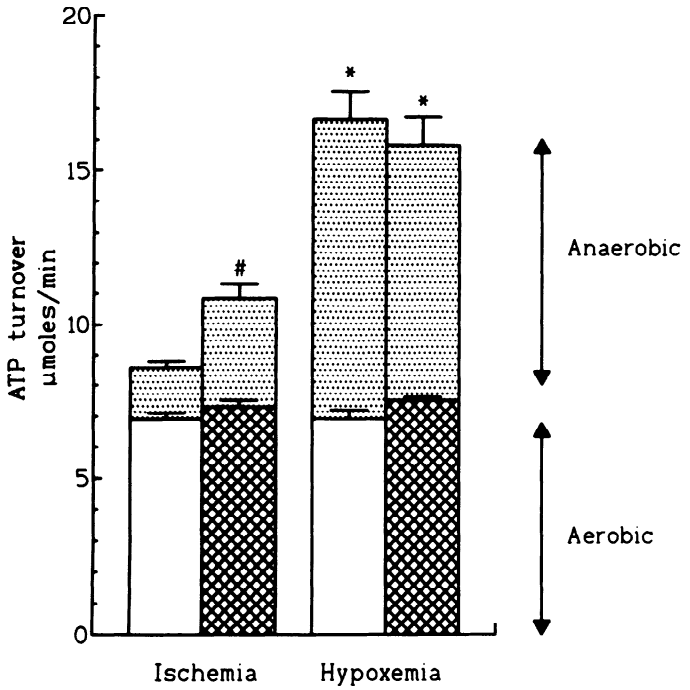

FIG. 3. Contribution of aerobic and anaerobic metabolisms to ATP turnover in our model. Open bars, spontaneous; crosshatched bars, paced. * Significantly different from respective value in ischemic hearts ($P < 0.0005$; unpaired Student's t-test). # Significantly different from respective value in spontaneous 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, $\dot{Q}O_2$ during baseline was adequate to support myocardial activity. We avoided the use of blood to minimize interferences, to retain the accuracy of $\dot{Q}O_2$ and $\dot{V}O_2$, 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°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 $\dot{Q}O_2$, H and I elicited different responses in our model. During H, hearts performed at a higher level than during I despite same $\dot{V}O_2$. However, J_{lac} was also higher, possibly contributing to increase J_{ATP} and to account for higher contractility during H (Fig. 4). In skeletal muscle exposed to 25% $\dot{Q}O_2$ reduction, the increase of J_{lac} failed to account for the observed increase of muscle contractility during H (38). Perhaps inexact matching of $\dot{Q}O_2$ 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

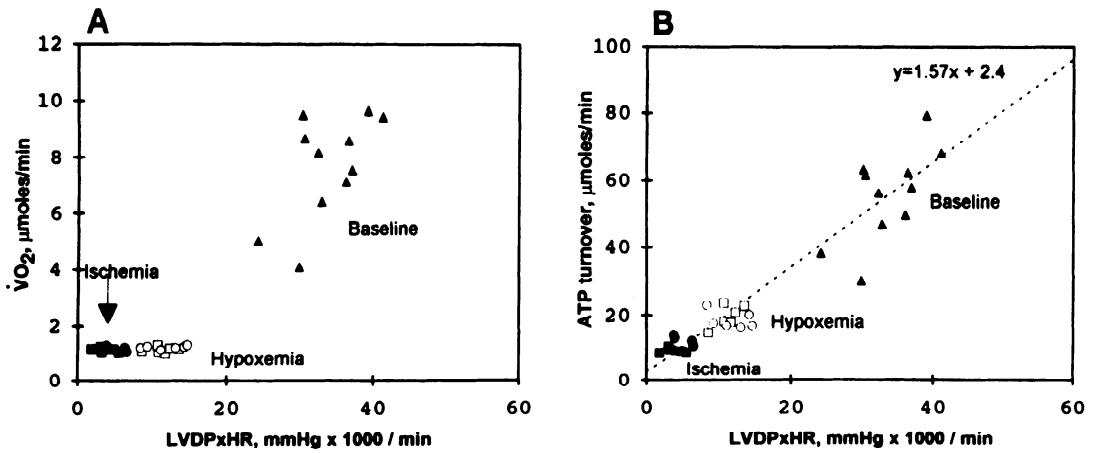

FIG. 4. Relationship between LVDP × HR and O_2 uptake ($\dot{V}O_2$; A) or ATP turnover rate (B). All data are shown. Squares and circles are spontaneous and paced hearts, respectively. Best-fit line uses all data points ($r = 0.975$; $P < 0.001$).

imply some degree of flow heterogeneity in this group. Indeed, skeletal muscle $\dot{V}O_2$ 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 isovolumic 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. hypoxemic hearts, we are not aware of other studies where the $\dot{Q}O_2$ 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 gastrocnemius 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 $\dot{V}O_2$ under all dysoxic conditions. In contrast, LVDP × HR was highly related to J_{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 $\dot{Q}O_2$ and $\dot{V}O_2$ (Fig. 1*B* 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 $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1} or 71–83 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1}) are close to the values of 10–14 $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1} (27) and 60–70 $\mu mol \cdot min^{-1} \cdot g$ 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 I than during H, 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 H^+ 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 posthypoxemic hearts. On the contrary, one of the reasons postischemic hearts recover better than posthypoxemic hearts (11, 36) may involve downregulation that prevents exhaustion of the energy reserves in ischemic but not in hypoxemic hearts, similar to observations made in rabbit skeletal muscles (20).

Conclusions. Ischemic hearts are more downregulated than hypoxemic hearts at the same $\dot{Q}O_2$. 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.

We acknowledge the help of Drs. A. Galavotti and A. Menghini (Dideco, Mirandola, Italy) in the design of the microoxygenator.

This study was supported by Consiglio Nasionale delle Ricerche Target Project Biotecnology and Biostruments (Rome) and Fondazione San Romanello del Monte Tabor (Milan, Italy).

Address for reprint requests: M. Samaja, Dept. of Biomedical Science and Technology, Scientific Institute San Raffaele, Univ. of Milan, Via Olgettina 60, I-20132 Milan, Italy.

Received 29 November 1993; accepted in final form 25 July 1994.

REFERENCES

1. **Achs, M. J., D. Garfinkel, and L. H. Opie.** Computer simulation of metabolism of glucose-perfused rat heart in a work-jump. *Am. J. Physiol.* 243 (*Regulatory Integrative Comp. Physiol.* 12): R389–R399, 1982.
2. **Arai, A. E., G. A. Pantely, C. G. Anselone, J. Bristow, and J. D. Bristow.** Active downregulation of myocardial energy requirements during prolonged moderate ischemia in swine. *Circ. Res.* 69: 1458–1469, 1991.
3. **Asimakis, G., K. Inners-McBride, and V. R. Conti.** Attenuation of postischaemic dysfunction by ischaemic preconditioning is not mediated by adenosine in the isolated rat heart. *Cardiovasc. Res.* 27: 1522–1530, 1993.
4. **Bing, O. H. L., W. W. Brooks, and J. V. Messer.** Heart muscle viability following hypoxia: protective effect of acidosis. *Science Wash. DC* 180: 1297–1298, 1973.
5. **Bladergroen, M. R., H. Takei, T. D. Christopher, R. G. Cummings, S. M. Blanchard, and J. E. Lowe.** Accelerated transmural gradients of energy compound metabolism resulting from left ventricular hypertrophy. *J. Thorac. Cardiovasc. Surg.* 100: 506–516, 1990.
6. **Bond, J. M., E. Chacon, B. Herman, and J. L. Lemasters.** Intracellular pH and Ca^{2+} homeostasis in the pH paradox of reperfusion injury to neonatal rat cardiac myocytes. *Am. J. Physiol.* 265 (*Cell Physiol.* 34): C129–C137, 1993.
7. **Bond, J. M., B. Herman, and J. J. Lemasters.** Protection by acidotic pH against anoxia/reoxygenation injury to rat neonatal cardiac myocytes. *Biochem. Biophys. Res. Commun.* 179: 798–803, 1991.
8. **Braunwald, E.** Control of myocardial oxygen consumption: physiologic and clinical considerations. *Am. J. Cardiol.* 27: 416–432, 1971.
9. **Chance, B., J. S. Leigh, J. Kent, K. McCully, S. Nioka, B. J. Clark, J. M. Maris, and T. Graham.** Multiple controls of oxidative metabolism in living tissues as studied by phosphorus magnetic resonance. *Proc. Natl. Acad. Sci. USA* 83: 9458–9462, 1986.
10. **Connett, R. J., C. R. Honig, T. E. J. Gayeski, and G. A. Brooks.** Defining hypoxia: a systems view of $\dot{V}O_2$, glycolysis, energetics, and intracellular P_{O_2}. *J. Appl. Physiol.* 68: 833–842, 1990.
11. **Corno, A. F., R. Motterlini, L. Brenna, F. Santoro, and M. Samaja.** Ischaemia/reperfusion in the posthypoxaemic re-oxygenated myocardium: haemodynamic study in the isolated perfused rat heart. *Perfusion* 8: 113–118, 1993.
12. **Corno, A. F., R. Motterlini, M. Tarantola, M. Samaja, M. Beretta, G. Dell'Antonio, F. Santoro, and A. Grossi.** Reperfusion injury of the hypoxemic heart: role of acute reoxygenation before ischemia. In: *Cardiology and Cardiac Surgery: Current Topics*, edited by A. Grossi, F. Donatelli, A. Corno, and R. Brodman. Mount Kisco, NY: Futura, 1992, p. 379–396.
13. **Cunningham, M. J., C. S. Apstein, E. O. Weinberg, W. M. Vogel, and B. H. Lorell.** Influence of glucose and insulin on the exaggerated diastolic and systolic dysfunction of hypertrophied rat hearts during hypoxia. *Circ. Res.* 66: 406–415, 1990.
14. **Currin, R. T., G. J. Gores, R. G. Thurman, and J. J. Lemasters.** Protection by acidotic pH against cell killing in perfused rat liver: evidence for pH paradox. *FASEB J.* 5: 207–210, 1991.
15. **Dennis, S. C., W. Gevers, and L. H. Opie.** Protons in ischemia: where do they come from; where do they go to? *J. Mol. Cell. Cardiol.* 23: 1077–1086, 1991.
16. **Dodd, S. L., S. K. Powers, E. Brooks, and M. P. Crawford.** Effects of reduced O_2 delivery with anemia, hypoxia, or ischemia on peak $\dot{V}O_2$ and force in skeletal muscle. *J. Appl. Physiol.* 74: 186–191, 1993.
17. **Downing, S. E.** Wall tension and myocardial dysfunction after ischemia and reperfusion. *Am. J. Physiol.* 264 (*Heart Circ. Physiol.* 33): H386–H393, 1993.
18. **Ganote, C. E., S. Armstrong, and J. M. Downey.** Adenosine and A1 selective agonists offer minimal protection against ischemic injury to isolated rat cardiomyocytes. *Cardiovasc. Res.* 27: 1670–1676, 1993.
19. **Gorman, M. W., J. K. Barclay, and H. V. Sparks.** Effects of ischemia on $\dot{V}O_2$, tension, and vascular resistance in contracting canine skeletal muscle. *J. Appl. Physiol.* 65: 1075–1081, 1988.
20. **Gutierrez, G., R. J. Pohil, J. M. Andry, R. Strong, and P. Narayana.** Bioenergetics of rabbit skeletal muscle during hypoxemia and ischemia. *J. Appl. Physiol.* 65: 608–616, 1988.
21. **Hochachka, P. W., and G. O. Matheson.** Regulating ATP turnover rates over broad dynamic work ranges in skeletal muscles. *J. Appl. Physiol.* 73: 1697–1703, 1992.
22. **Hogan, M. C., D. E. Bebout, and P. D. Wagner.** Effect of blood flow reduction on maximal O_2 uptake in canine gastrocnemius muscle in situ. *J. Appl. Physiol.* 74: 1742–1747, 1993.
23. **Hogan, M. C., S. Nioka, W. F. Brechue, and B. Chance.** A ^{31}P-NMR study of tissue respiration in working dog muscle during reduced O_2 delivery conditions. *J. Appl. Physiol.* 73: 1662–1670, 1992.
24. **Kobayashi, K., and J. R. Neely.** Control of maximum rate of glycolysis in rat cardiac muscle. *Circ. Res.* 44: 166–175, 1979.
25. **Lee, J. A., and D. G. Allen.** The effects of repeated exposure to anoxia on intracellular calcium, glycogen and lactate in isolated ferret heart muscle. *Pfluegers Arch.* 413: 83–89, 1988.
26. **Levi, E., A. Vivi, Y. Hasin, M. Tassini, G. Navon, and M. Horowitz.** Heat acclimation improves cardiac mechanics and metabolic performance during ischemia and reperfusion. *J. Appl. Physiol.* 75: 833–839, 1993.
27. **Matthews, P. M., D. J. Taylor, and G. K. Radda.** Biochemical

mechanisms of acute contracture failure in the hypoxic rat heart. *Cardiovasc. Res.* 20: 13–19, 1986.

28. **Owen, P., S. Dennis, and L. H. Opie.** Glucose flux rate regulates onset of ischemic contracture in globally underperfused rat hearts. *Circ. Res.* 66: 344–354, 1990.
29. **Peterson, D. J., N. Vejistrup, D. C. Willford, and M. C. Hogan.** Effect of a sulphonylurea on dog skeletal muscle performance during fatiguing work. *Acta Physiol. Scand.* 144: 399–400, 1992.
30. **Rau, E. E., K. E. Shine, and G. A. Langer.** Potassium exchange and mechanical performance in anoxic mammalian myocardium. *Am. J. Physiol.* 232 (*Heart Circ. Physiol.* 1): H84–H85, 1977.
31. **Rayford, C. R., E. M. Khouri, F. B. Lewis, and D. E. Gregg.** Evaluation of use of left coronary artery inflow and O_2 content of coronary sinus blood as a measure of left ventricular metabolism. *J. Appl. Physiol.* 14: 817–822, 1959.
32. **Roughton, F. J. W., and J. W. Severinghaus.** Accurate determination of O_2 dissociation curve of human above 98.7% saturation with data on O_2 solubility in unmodified human blood from 0 to 37°C. *J. Appl. Physiol.* 35: 861–869, 1973.
33. **Rovetto, M. J., W. F. Lamberton, and J. R. Neely.** Mechanisms of glycolytic inhibition in ischemic rat hearts. *Circ. Res.* 37: 742–751, 1975.
34. **Rovetto, M. J., J. T. Whitmer, and J. R. Neely.** Comparison of the effects of anoxia and whole heart ischemia on carbohydrate utilization in isolated working rat hearts. *Circ. Res.* 32: 699–711, 1973.
35. **Runnman, E. M., and J. N. Weiss.** Exogenous glucose utilization is superior to glycogenolysis at preserving cardiac function during hypoxia. *Circulation* 78, *Suppl.* 2: 251–261, 1988.
36. **Samaja, M., R. Motterlini, F. Santoro, G. Dell'Antonio, and A. F. Corno.** Oxidative injury in reoxygenated and reperfused hearts. *Free Radical Biol. Med.* 16: 255–262, 1994.
37. **Schaefer, S., G. G. Schwartz, J. R. Gober, A. K. Wong, S. A. Camacho, B. Massie, and M. W. Weiner.** Relationship between myocardial metabolites and contractile abnormalities during graded regional ischemia. *J. Clin. Invest.* 85: 706–713, 1990.
38. **Stainsby, W. N., W. F. Brechue, D. M. O'Drobinak, and J. K. Barclay.** Effects of ischemic and hypoxic hypoxia on $\dot{V}O_2$ and lactic acid output during tetanic contractions. *J. Appl. Physiol.* 68: 574–579, 1990.
39. **Takeshita, A., A. L. Mark, F. M. Abboud, P. G. Schmid, D. D. Heistad, and U. J. Johannsen.** Phentolamine and isoproterenol: comparison of effects on vascular resistance and oxygen uptake in skeletal muscle during hypotension. *J. Pharmacol. Exp. Ther.* 199: 353–359, 1976.
40. **Tani, M., and J. R. Neely.** Vascular washout reduces Ca^{2+} overload and improves function of reperfused ischemic hearts. *Am. J. Physiol.* 258 (*Heart Circ. Physiol.* 27): H354–H361, 1990.
41. **Walker, D. M., and D. M. Yellon.** Ischaemic preconditioning: from mechanisms to exploitation. *Cardiovasc. Res.* 26: 734–739, 1992.
42. **Williamson, J. R.** Glycolytic control mechanisms. II. Kinetics of intermediate changes during the aerobic-anoxic transition in perfused rat heart. *J. Biol. Chem.* 241: 5026–5036, 1966.
43. **Zahler, R., E. Barrett, S. Majumdar, R. Greene, and J. C. Gore.** Lactic acidosis: effect of treatment on intracellular pH and energetics in living rat heart. *Am. J. Physiol.* 262 (*Heart Circ. Physiol.* 31): H1572–H1578, 1992.
44. **Zhou, H. Z., D. Malhotra, and J. I. Shapiro.** Contractile dysfunction during metabolic acidosis: role of impaired energy metabolism. *Am. J. Physiol.* 261 (*Heart Circ. Physiol.* 30): H1481–H1486, 1991.
45. **Zweier, J. L., and W. E. Jacobus.** Substrate-induced alterations of high energy phosphate metabolism and contractile function in the perfused heart. *J. Biol. Chem.* 262: 8015–8021, 1987.