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MYOCARDIAL ADAPTATION TO ACUTE OXYGEN SHORTAGE

A Kinetic Analysis

Giampiero Merati,[1] Sonia Allibardi,[2] Giovanna Marrazza,[3] Marco Mascini,[3] and Michele Samaja[1]

[1]Dipartimento di Scienze e Tecnologie Biomediche, Università di Milano
[2]Istituto Scientifico San Raffaele, Milano
[3]Dipartimento di Sanità Pubblica, Firenze
Italy

1. INTRODUCTION

In this study, we examined the bioenergetic mechanisms underlying myocardial adaptation to O_2-limited perfusion. Shortened O_2 supply to contracting tissue results in nearly immediate metabolic and performance decline due to fast turnover rate of high-energy phosphates compared to their intracellular concentration.[1] Thus, to maintain adequate ATP production, tissue is forced to divert from aerobic to anaerobic pathways: although less efficient than aerobic ones, glycolytic ATP production under hypoxic, high-flow conditions may account for up to half of total energy requirements.[2] However, if low O_2 supply is associated with reduced flow, the heart preferentially downregulates energy demand to meet supply.[3] Whereas these processes were verified during *sustained* ischemia or hypoxia, it appears important to assess the mechanisms underlying *acute* regulation of performance. The main reason for this is the need to understand to a greater extent reperfusion injury and the generation of endogenous myocardial protection, both of which may be strictly linked to bioenergetic processes.

In the past, several studies of metabolic and biochemical processes were accelerated by kinetic analysis of data, where the role of single components of a reaction or chain of reactions is assessed free of the effects of products. By analogy, we tested the hypothesis that the kinetic approach is useful to understand the mechanism of heart adaptation to O_2 supply limitation. Although the heart response to O_2 shortage is complicated by several overlapping phenomena (changes of lactate production, O_2 consumption, glycolysis activity, coronary reactivity and others), we believe that kinetic analysis may provide clues both to identify those paths that, although plausible, are not synchronous with the ob-

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served phenomena, and to give a deeper insight into relevance and importance of various paths of regulation of contractile systems under conditions of O_2 limitation.

Comparing the response to low-flow ischemia (LFI) and to hypoxemia (Hyp) at the same degree of O_2 deprivation provided a tool to characterize the role of free radicals in determining reperfusion injury,[4] of lactate in downregulating myocardial function [2,3] and of coronary flow in preserving the ATP pool.[5] In this study, we report the time course of myocardial adaptation to LFI or Hyp at the same degree of O_2 deprivation. We show that metabolic pathways, and especially the changes in glycogen and lactate metabolisms, play a primary role in developing acute myocardial response to stress.

2. MATERIALS AND METHODS

Ad libitum fed Sprague-Dawley male rats (250–280 g, n=13/group) were anesthetized by i.p. heparinized sodium thiopental (100 mg/kg body weight), hearts were excised and perfused (Langendorff mode) with Krebs-Henseleit buffer (2.0 mM free Ca^{++}, 11 mM glucose, pH 7.4, 37°C). The medium was equilibrated at the desired PO_2 (670 or 67 mmHg) and PCO_2 (43 mmHg) in membrane oxygenators. The volume of the intraventricular balloon was adjusted to achieve end-diastolic pressure (EDP) ~10 mmHg and was kept constant throughout. Measurements also included heart rate (HR), left ventricle developed pressure (LVDP), coronary perfusion pressure (CPP), venous PO_2 (cannula in the pulmonary artery and Clark-type electrode) and venous [lactate] (n=4/group, lactate oxidase-based electrochemical biosensors, Mascini and Marrazza, personal communication). To account for different flows, this parameter is expressed as net lactate release (J_{Lac}=[lactate]·flow). Hearts (n=4/group) were finally freeze-clamped for tissue glycogen determination after digestion with amyloglucosidase.[6]

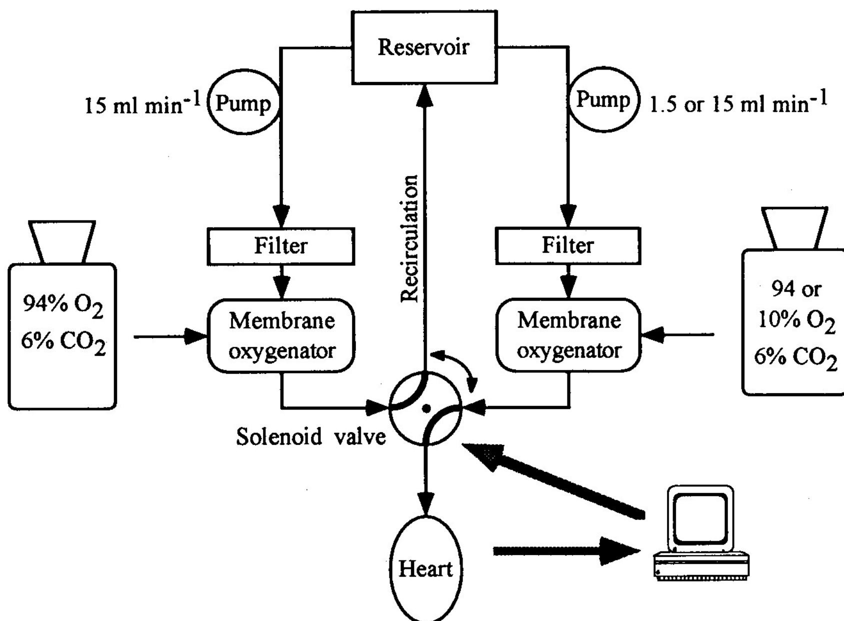

Figure 1. Scheme of the apparatus used in this study.

To synchronize the rapid change of one condition with fast data acquisition, two circuits were devised (Fig.1), each consisting of a Minipuls 2 roller pump (Gilson, France), a filter (8 μm pore size, 47 mm diameter, Nuclepore Corp., Pleasanton, CA) and an oxygenator (Dideco, Mirandola, Italy). One of the circuits was normally set for baseline perfusion (Table 1) whereas the other was set for either LFI or Hyp. The flow of the circuits was diverted either to the aortic cannula (dead volume=0.35 ml) or through recirculation under the control of a computer-activated solenoid electric valve (Sirai, Pioltello, Italy). A back pressure of 80–100 mmHg was applied to recirculation to prevent undesired adjustments of flow secondary to changes of perfusion pressure and resistance.

Data were acquired and the solenoid valve was operated by LabView 3.0 software and NB-MIO-16 Multifunction I/O Board (National Instruments, Austin, Texas) running on an Apple Macintosh Quadra 700 (Cupertino, California). We employed one output (solenoid valve) and three input channels (two pressure transducers and PO_2 electrode). The application acquires default and calibration values, controls valve switching, monitors myocardial performance, performs automatic background calculations and stores data in worksheets. Data were normally sampled at 30 s intervals, but the sampling rate was 5 s for 2 min in the correspondence of valve switching.

3. RESULTS

The described system was suitable to monitor the desired kinetics. The response time for all parameters except PO_2 was <1 ms and ~1.4 s for LFI and Hyp, respectively. The response time for PO_2 was ~17 s due to the intrinsic characteristics of the electrode. The reader is cautioned of this problem when dealing with the PO_2-time curves.

As expected, myocardial performance decreased by a larger extent during LFI than during Hyp.[4] Figure 2 shows typical patterns of contraction at different times after the onset of O_2 shortage. Whereas the immediate ($t \leq 20$ s) responses to LFI and Hyp were essentially similar, performance declined steadily during LFI, but remained sustained for several minutes in Hyp hearts (Fig.3, $p<0.0001$). It appears that at the onset of LFI the decline of LVDP approached an exponential decay of the type $y=0.035^x$ (Fig.4). Whereas EDP increased steadily in Hyp hearts *vs* decrease in LFI hearts (Fig.3, $p<0.0001$), HR remained essentially constant for several minutes after onset of LFI and Hyp (p=NS, not shown). Figure 3 also shows that at the onset of LFI, CPP fell within the time required for the first sampling, i.e., ≤5 s, whereas it remained essentially stable at the onset of Hyp. Whereas during LFI J_{Lac} reached a steady value within 120 s, during Hyp it overshooted up to 7 μmoles/min ~120 s after beginning of O_2 shortage and then stabilized at 4.4±0.4 μmoles/min ($p<0.0001$ *vs* LFI). The adaptation of PO_2 was faster at the onset of Hyp

Table 1. Experimental protocol

Parameter	Baseline	O_2 shortage	
		Low-flow ischemia	Hypoxemia
Time duration, min	20	10	10
Flow, ml/min	15	1.5	15
PO_2, mmHg	670	670	67
O_2 supply, μmoles/min/heart	14.1	1.41	1.41

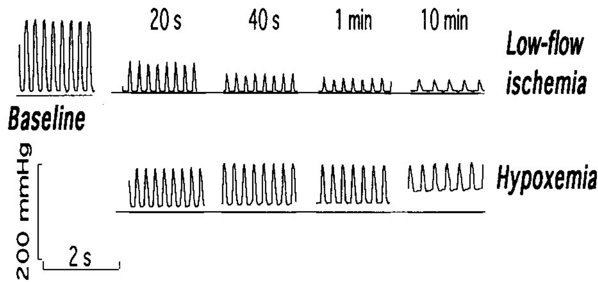

Figure 2. Typical examples of ventricular pressures during baseline and at selected times after the onset of either low-flow ischemia or hypoxemia.

($t_{50\%}$=36±12 s) than of LFI ($t_{50\%}$=85±30 s, $p<0.05$ since t=20 s). Tissue glycogen content was 107±19, 51±9 and 21±5 μmoles/g dry weight at the end of baseline, LFI and Hyp, respectively.

4. DISCUSSION

Even when matched for O_2 supply, LFI and Hyp elicit different steady-state responses because of a chain of events originating from flow: different flows induce different lactate washout rates and hence intracellular [lactate] levels, thereby causing different degrees of depression of glycolysis and hence different ATP production and performance.[2,3] The kinetics of adaptation to LFI and Hyp were essentially similar for t≤20 s suggesting that the factors that acutely regulate myocardial performance are related to O_2 availability, in agreement with previous studies in working dog gastrocnemius.[7] For t20 s, however, the adaptations to LFI and Hyp are different indicating that flow-related factors overtake control of myocardial performance. Explaining such differences may help to identify the mechanisms underlying myocardial response to O_2 shortage.

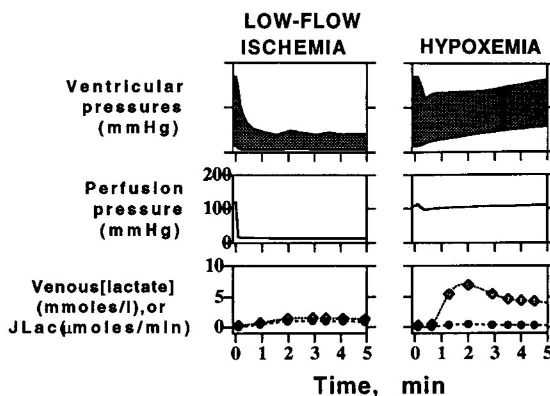

Figure 3. Kinetic adaptation of systolic and end-diastolic pressures (top panels), perfusion pressure (central panels), and venous [lactate] (circles) and net lactate release (diamonds). Error bars omitted for clarity.

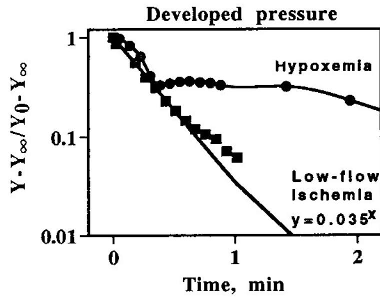

Figure 4. The adaptation of developed pressure to low-flow ischemia approximates an exponential equation.

The features of the employed model and comparative analysis of the observed kinetics help to exclude some paths of regulation. For example, control by neurohormonal factors is ruled out in isolated perfused hearts. Changes of intracellular Ca^{++} transients do not provide satisfactory explanation as they occur on a time scale longer than that observed here [8] and therefore appear to be a consequence, not the cause, of the new heart metabolic status. The changes in coronary perfusion inhomogeneities and the garden hose effect,[9] although likely to occur in this model, do not appear primarily involved in short-term myocardial regulation as they depend on CPP changes, which are not synchronous with the observed ventricular pressures changes. Adaptations of aerobic metabolism are also unlikely as the changes of PO_2 were slower than those of performance. Although this should be taken with care as the response time of the PO_2 electrode may be misleading, it was already shown that the contribution of aerobic metabolism is essentially similar during LFI and Hyp.[3]

If venous [lactate] is considered a reliable index of cell [lactate], then the higher [lactate] during LFI reflects low washout. Lactate-induced acidosis inhibits glycolysis and depresses contractility [10,11] thus explaining depressed performance in LFI hearts. Down-regulation is blunted in Hyp hearts due to high flow that increases washout of lactate. The glycolytic source of the extra-ATP required to maintain sustained performance in Hyp hearts is indicated by the higher J_{Lac} observed during sustained Hyp. It was calculated that under the selected experimental conditions, glycolytic ATP production may account for up to half of the total energy requested by Hyp hearts.[3]

The J_{Lac} peak observed at the onset of Hyp appeared to be synchronous with the phase of sustained myocardial activity. It is thus tempting to speculate that the activity outbreak observed in Hyp, but not in LFI hearts is due to transient increase of anaerobic ATP production. Indeed, J_{Lac} bursts indicate O_2-limited ATP production.[12] In dog gracilis muscles subjected to rest-work transition, the burst of glycolysis can occur in times as short as 5 s, and glycolysis can be activated to its maximal rate within 30 s indicating that glycolysis may function effectively in support of mitochondrial oxidative phosphorylation.[13] Computer simulation studies have predicted that the early J_{Lac} peak that occurs in hearts subjected to work-jump is due to rapid activation of phosphofructokinase and phosphorylase *b*.[14]

Despite the high glucose concentration in the medium that should have saturated the glucose transport system,[15] our hearts used relevant amounts of glycogen when exposed to Hyp. In contrast, tissue glycogen decreased by a lesser extent in hearts exposed to LFI. The fast utilization rate of glycogen is supported by the acceleration of glycogenolysis when fatty acids are omitted from the perfusate.[16] Our data are consistent with the com-

puter simulation prediction that the acute increase of energy demand is first met by glycogen breakdown followed by increased glucose uptake from the perfusate.[14]

In conclusion, kinetic analysis of the phenomena that occur during acute exposure to LFI or Hyp may allow to assess in detail relevance and sequence of some of the metabolic events that originate functional changes (lactate and glycogen). Noticeably, this role was documented in the past by theoretical computer-assisted extrapolations only.[14] We believe that kinetic analysis of properly selected protocols represents an approach that allows understanding microcirculation and the effects of morphological and metabolic recruitments secondary to external stresses.

5. ACKNOWLEDGMENTS

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