EFFECTS OF ENERGY DEMAND IN ISCHEMIC AND IN HYPOXEMIC ISOLATED RAT HEARTS

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INTRODUCTION

While myocardial ischemia is characterized by low coronary flow rate (CFR) at high arterial PO_2 (P_aO_2), during hypoxemia P_aO_2 is low at high CFR. Although both situations may potentially lead to dysoxia, defined as a condition with unbalanced O_2 supply/demand ratio (Connett *et al.*, 1990), the washout of membrane-diffusible catabolites such as lactate is depressed during ischemia but not during hypoxemia because of the different CFR's that determine different washouts of intracellular lactate. It is therefore tempting to speculate that, when ischemia and hypoxemia are matched for the O_2 supply, hypoxemia becomes equivalent to an ischemic condition with enhanced washout of lactate. This hypothesis provides a good opportunity to evaluate the role of O_2 and lactate in dysoxic contractile systems.

The isolated Langendorff-perfused rat heart is particularly suitable to study the effects of hypoxemia and ischemia because CFR and P_aO_2 can be regulated by a pump and a membrane oxygenator to yield selected O_2 supplies:

$$O_2 \text{ supply} = CFR \times P_aO_2 \times \alpha \qquad (1)$$

where α, which represents the O_2 solubility coefficient, remains constant in aqueous buffers. Furthermore, isolated hearts are accessible to several physiological and metabolic measurements. Finally, use of blood in the perfusing medium was avoided at the expense of dealing with unphysiological conditions, but with clear advantages of accuracy and precision in measuring the O_2 content. The purposes of this study were: 1) Determining whether hypoxemia and ischemia at the same O_2 supply elicit the same responses, and hence if O_2 is critical regulator of myocardial function and metabolism in dysoxia; 2) Defining the effect of increased energy demand (electrical stimulation) in hypoxemic and ischemic hearts to assess if these hearts have a reserve of energy.

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MATERIALS AND METHODS

General

We perfused isolated rat hearts with oxygenated buffer by a Langendorff technique monitoring myocardial function, O_2 uptake (VO_2) and lactate production rate (J_{Lac}). Hearts were stabilized for 20 min at CFR=15 ml/min and P_aO_2=670 mmHg. The volume of the intraventricular balloon was set to yield end-diastolic pressure (EDP)=7.0±0.5 mmHg and was kept constant throughout. Under these conditions, the O_2 supply was (see eq.1):

$$\text{Baseline: } 15 \times 670 \times \alpha = 14.1 \text{ } \mu\text{moles } O_2/\text{min} \qquad (2)$$

where α is 1.4×10^{-6} moles/L/mmHg (Roughton and Severinghaus, 1973). At t=0 min, the O_2 supply was shortened reducing either CFR (ischemic group, n=6) or P_aO_2 (hypoxemic group, n=6) to 10% of the baseline. The O_2 supplies under these conditions were, respectively:

$$\text{Ischemia: } 1.5 \times 670 \times \alpha = 1.41 \text{ } \mu\text{moles } O_2/\text{min} \qquad (3)$$

$$\text{Hypoxemia: } 15 \times 67 \times \alpha = 1.41 \text{ } \mu\text{moles } O_2/\text{min} \qquad (4)$$

Hearts were initially allowed to adjust their heart rate (HR), but at t=20 min HR was set to 300 min^{-1} for 10 min in all groups. Measurements were taken at the end of the various phases at stable myocardial function.

Apparatus

The perfusing buffer (115.6 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 0.5 mM EDTA, 1.2 mM Na_2SO_4, 28.5 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 16.6 mM glucose, pH 7.4 at 37°C) was equilibrated in Sylastic membrane oxygenators (Dideco, Italy) at 37°C with gases containing either 94/6/0 or 0/6/94 $O_2/CO_2/N_2$ to yield P_aO_2=670 or 67 mmHg at constant P_aCO_2 (43 mmHg). A roller pump delivered the buffer at either 15 or 1.5 ml/min to a filter (8 μm pore size, 47 mm diameter, Nuclepore Corp., Pleasanton, CA), a preheater and the aortic cannula.

Hearts from male Sprague Dawley rats (250-280 g), anesthetized by i.p. heparinized sodium thiopental (10 mg/100 g b.w.), were mounted on the system and immersed in the buffer kept at 37°C. The venous return was collected by the pulmonary artery and a saline-filled Latex balloon was introduced into the left ventricle. A square wave stimulator (Harvard, South Natick, MA) with 5 ms pulse duration and 10 V pulse amplitude was connected to electrodes placed on the aortic cannula and on the apex of the ventricle.

Measurements, calculations and statistics

A pressure transducer (Harvard Apparatus mod.52-9966, Natick, MA) connected to the balloon provided EDP and the developed pressure (LVDP) by means of a dedicated LabVIEW®2 system (National Instruments, Austin, TX) running on Macintosh Quadra 700 computer. The venous return was analyzed for PO_2 (YSI mod.5300 Oxygen Monitor, Yellow Springs Inc., OH) and lactate (Sigma Diagnostic, St.Louis, MO). Data are expressed as mean±SEM. The Student's t-test for unpaired and paired observations was used to compare hypoxemic and ischemic hearts and to evaluate the effects of pacing, respectively. The significance level was set to p=0.05 (two-tailed).

RESULTS

HR decreased in ischemic ($p=0.001$) but not in hypoxemic hearts (Fig.1). Hypoxemic hearts underwent diastolic contracture ($p<0.0005$) but their LVDP was greater than in ischemic hearts ($p=0.001$). Pacing did not affect EDP and decreased LVDP ($p=0.01$) in hypoxemic hearts.

Although P_vO_2 was higher in ischemic than hypoxemic hearts (Fig.2, $p<0.0005$), VO_2 was the same in both groups. Since P_vO_2 was not changed by pacing, VO_2 remained constant. Venous [lactate] was not affected by pacing in hypoxemic hearts, but increased in ischemic hearts (Fig.3, $p=0.002$). The lactate production rate (J_{Lac}) was calculated from venous [lactate] and CFR, and was higher in hypoxemic than in ischemic hearts ($p<0.0005$). Pacing, however, increased J_{Lac} in ischemic hearts only ($p=0.002$).

The myocardial contractile work was expressed as LVDP×HR (Fig.4) and was lower in ischemic than in hypoxemic hearts ($p<0.0005$). Pacing increased LVDP×HR in ischemic hearts ($p=0.005$) only without influencing hypoxemic hearts. The turnover of ATP (J_{ATP}) was calculated using steady-state stoichiometry $6.42\times VO_2+1.25\times J_{Lac}$ (Paul, 1980) assuming no mitochondrial uncoupling. As for LVDP×HR, J_{ATP} was higher in hypoxemic hearts ($p<0.0005$) and pacing increased J_{ATP} in ischemic hearts only ($p=0.001$) without affecting hypoxemic hearts.

DISCUSSION

Myocardial function was more depressed during ischemia than during hypoxemia. However, ischemic hearts upgraded their performance when stimulated, while this ability was blunted in hypoxemic hearts. Despite different P_vO_2 in the two groups, VO_2 was the same and was unaffected by pacing. Both venous [lactate] and J_{Lac} increased in ischemic hearts upon pacing but was constant in hypoxemic hearts.

Thus, when matched for the O_2 supply, hypoxemia and ischemia elicited different responses in our model consistently with recently reported data (Stainsby *et al.*, 1990; Dodd *et al.*, 1993). In another study (Hogan *et al.*, 1992), however, working dog gastrocnemius muscles *in situ* were exposed to either hypoxemia or ischemia applying an O_2 reduction of ~50%, but no bioenergetic differences were observed at three levels of stimulation. The discrepancy between these results is likely due to major procedural differences and to the more severe energy imbalance in our hearts.

One the main differences between hypoxemia and ischemia is the washout of diffusible catabolites. Thus, it may be inferred that this feature, and not the O_2 supply *per se*, determined the observed differences between ischemic and hypoxemic hearts. Since VO_2 was the same in both groups and was not varied by pacing, O_2 was not an adequate reserve of energy in this model. The different P_vO_2 values in ischemic and hypoxemic hearts reflect the low perfusion pressure in ischemic hearts secondary to their lower CFR. Such condition may have diminished the number of open capillaries per unit tissue volume increasing the artero-venous shunt and decreasing the tissue ability to extract O_2 (Hogan *et al.*, 1993).

The greater performance of hypoxemic hearts with respect to matched ischemic hearts, and the ability of ischemic hearts to increase their performance when stimulated appear linked to the anaerobic glycolysis capacity. Both venous [lactate] and J_{Lac} increased upon stimulation in ischemic hearts, but remained constant in hypoxemic hearts. Further, J_{Lac} was higher in hypoxemic than in ischemic hearts, irrespectively of pacing, suggesting that during hypoxemia anaerobic glycolysis was working at or near maximum, irrespectively of the actual demand of energy. This observation is consistent with the

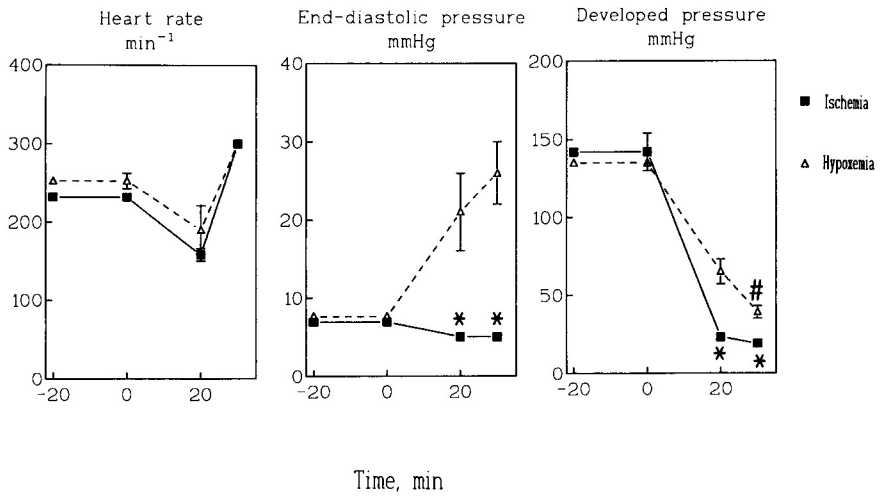

Figure 1. Heart rate, end-diastolic pressure and developed pressure in ischemic (■) and hypoxemic (Δ) hearts (n=6 for each) during baseline (t=0 min), dysoxia (t=20 min) and in dysoxic paced hearts (t=30 min). *=significant difference between hypoxemic and ischemic hearts, unpaired Student's t-test; #=significant difference between paced and spontaneous hearts, paired Student's t-test.

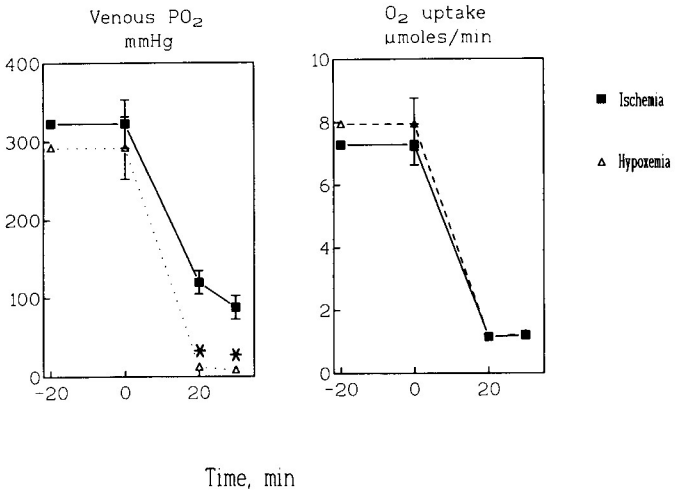

Figure 2. Metabolism of O_2 (venous PO_2 and O_2 uptake) during baseline (t=0 min), dysoxia (t=20 min) and in dysoxic paced hearts (t=30 min). Other information in Fig.1.

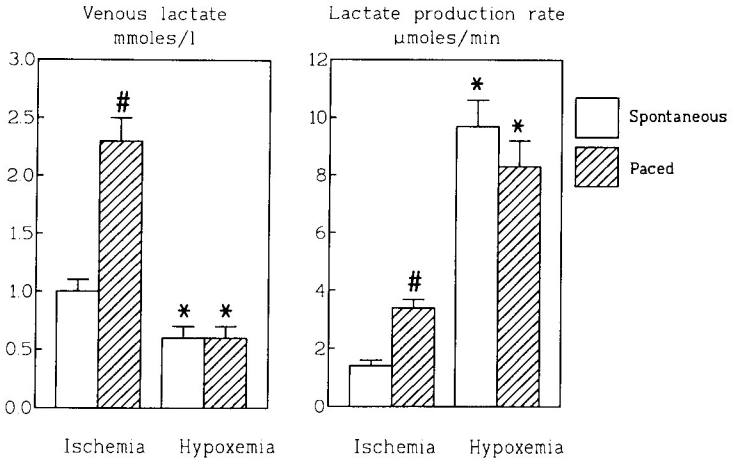

Figure 3. Metabolism of lactate (venous [lactate] and lactate production rate in ischemic and hypoxemic hearts, with and without pacing. Other information in Fig.1.

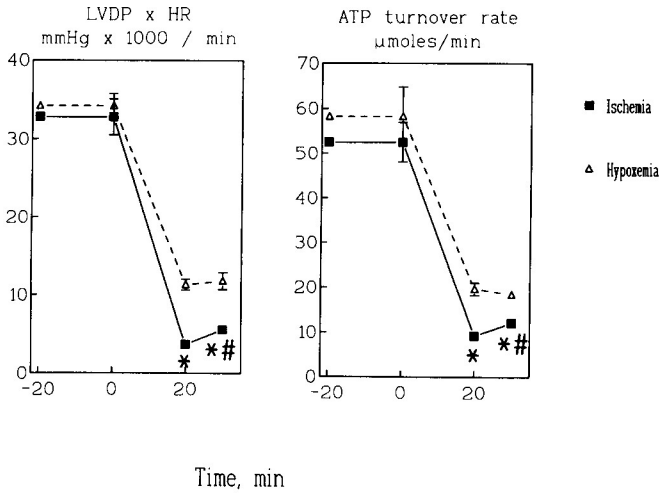

Figure 4. Myocardial contractile work (LVDP×HR) and ATP turnover rate (J_{ATP}) during baseline (t=0 min), dysoxia (t=20 min) and in dysoxic paced hearts (t=30 min). Other information in Fig.1.

lower glycolytic rate in ischemic than anoxic working rat hearts (Rovetto *et al.*, 1973; Kobayashi and Neely, 1979). However, these data also show that the depression of anaerobic glycolysis in ischemic hearts can be relieved following the increased demand of energy, while this mechanism does not operate during hypoxemia.

Venous [lactate] was higher in ischemic than in hypoxemic hearts reflecting impaired washout in ischemia, consistently with earlier observations (Tani and Neely, 1990). Lactate-induced acidosis is known to inhibit glycolysis (Kobayashi and Neely, 1979; Zhou *et al.*, 1991; Rovetto *et al.*, 1975). An important consequence of the associated functional depression is enhanced post-ischemic metabolic and functional recovery (Currin *et al.*, 1991; Bing *et al.*, 1973; Schaefer *et al.*, 1990). The protective effect of lactate accumulation during ischemia is consistent with our previous reports that hearts recovered from hypoxemia are more injured than those recovered from ischemia, when the two conditions are matched for O_2 supply, duration of the insult and temperature (Corno *et al.*, 1993; Samaja *et al.*, 1994).

In conclusion, ischemic hearts are downregulated. In contrast, hypoxemic hearts are not. Most likely, this process is modulated by lactate: low CFR during ischemia induces high intracellular lactate that depresses glycolysis and myocardial function; high CFR during hypoxemia prevents lactate accumulation releasing the inhibition. The former feature may be considered advantageous in terms of myocardial protection because it prevents energy wasting and allows better recovery.

SUMMARY

Aim of this study was to assess the role of O_2, lactate and energy demand in the regulation of myocardial work during severe dysoxia. For this purpose, we measured function and metabolism in isolated Langendorff-perfused rat hearts exposed to either ischemia or hypoxemia (matched for the O_2 supply, 10% of baseline) with/out electrical stimulation. When hearts could adjust their HR, hypoxemia demanded more energy than ischemia ($p<0.05$) despite same O_2 supply. Venous PO_2 was 12±2 or 139±20 mmHg ($p<0.0001$), respectively, but VO_2 was the same. After 10 min at HR=300 min^{-1}, myocardial performance increased in ischemic but not in hypoxemic hearts. P_vO_2 and VO_2 were not affected by pacing. In contrast, both venous [lactate] and lactate production rate increased, but in ischemic hearts only. We conclude that ischemic hearts were downregulated while hypoxemic hearts were not. Likely, depressed washout of lactate during ischemia could offset the effects of O_2 in severely dysoxic hearts. Anaerobic glycolysis provided the energy necessary to meet increased energy demand in ischemic hearts, but could not exploit this action in hypoxemic hearts probably because in these hearts it was already working near maximum.

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