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Microcalorimetric study of myocardial tissue metabolism in heart failure after experimental myocardial infarction in rats

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Abstract

Using microcalorimetry and measuring oxygen consumption rate, we have studied metabolic activity in myocardial tissue from rats suffering from congestive heart failure (CHF) after ligation of the left coronary artery followed by myocardial infarction, comprising 30–40% of the endocardial circumference, known to be large enough to induce heart failure and neuroendocrine counter-regulation.

The heat production in the preserved part of the left ventricle, harbouring the myocardial infarction, was 0.94 ± 0.05 mW g^{-1} ($n = 10$) and not different from the controls i.e 0.94 ± 0.05 mW g^{-1}, ($n = 23$). The oxygen consumption rate was 1.36 ± 0.20 nmol O_2 s^{-1} g^{-1} in CHF myocardium and 1.55 ± 0.24 nmol O_2 s^{-1} g^{-1} in the control rats, ($n = 20$), n.s. The calculated contribution from aerobic metabolism to total heat production was $71 \pm 9\%$ in the CHF rats and $80 \pm 6\%$ in the control group, n.s.

In tissue samples taken from the region of the border of and into the macroscopically damaged area of the myocardial infarction ($n = 5$), the value for heat production was 1.15 ± 0.13 mW g^{-1} and oxygen uptake 1.79 ± 0.23 nmol O_2 s^{-1} g^{-1}.

These results show that the contribution from aerobic metabolism to total energy expenditure in preserved left ventricular tissue is not higher than normal and suggest that the effects of left coronary occlusion are not limited to the generation of a myocardial infarction, but may also have a substantial impact on the ability of myocardial cells to increase metabolic activity in macro- and microscopically preserved regions of the left ventricle. In addition, the data demonstrate persistent metabolic activity within the region of ischemically damaged myocardium not different from that found in undamaged myocardium.

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1. Introduction

Congestive heart failure (CHF) due to ischemic heart disease is followed by enhanced neuroendocrine activity, including increased plasma levels of noradrenalin and adrenalin, mediating inotropic and chronotropic myocardial effects [1]. The metabolic response of the failing myocardial cells to this mode of stimulation is, however, less well known. Enzymatic studies of human myocardial cells have indicated a lack of general adaptation to chronic ischemia in terms of increased oxidative capacity. Thus the activity of citrate synthase as well as the content of coenzyme Q10 and of myoglobin was less than expected in human ischemic heart cells [2]. This suggests that the ability to increase mitochondrial oxidative capacity, oxygen transport and storage capacity can be impaired at the cell level and thereby the ability to adapt to the increased demand on myocardial performance in CHF due to ischemic heart disease.

Myocardial oxygen consumption studies have been proposed as an index integrating anatomic and functional characteristics of energy-producing and energy-requiring myocellular processes [3]. Oxygen consumption models will, however, not include total cellular metabolic activity from aerobic as well as anaerobic processes. In order to take into account these considerations we have used microcalorimetry [4] to study the metabolic activity and oxygen consumption of myocardial tissue from rats with congestive heart failure after a myocardial infarction, induced by ligation of the left coronary artery.

2. Materials and methods

2.1. Animals and preparation processes

Male rats, about 200 g b.w. were used for controls and in the CHF experiments. They were kept in separate cages with a regular day–night regimen and with free access to food and drinking water.

The study was approved by the Local Ethical Committees of the Universities of Lund and Gothenburg.

In one group of rats the left coronary artery was ligated, followed by a myocardial infarction comprising 30–40% of the endocardial circumference of the left ventricle. This procedure has been demonstrated to induce a state of heart failure [5] accompanied by increased levels of catecholamines and atrial natriuretic peptide [6]. Consequently, signs of congestive heart failure were verified at sacrifice 6–8 weeks after the operation through pleural and abdominal effusions and general organ congestion.

Separate groups of rats were kept under similar conditions, except for surgery, and were used as controls.

In order to avoid any inhibiting influence of sedatives on metabolic processes, the animals were sacrificed by decapitation. The heart was immediately prepared and a tissue slice was cut from the apical region into the left ventricle of the heart where there were no macroscopical signs of myocardial infarction.

From the hearts of five CHF rats, another slice was prepared from the region bordering and within the definite area of macroscopical myocardial infarction.

Until measurement the prepared tissue slice was carefully handled and kept at 37°C in Krebs–Ringer solution (values in mol dm^{-3}): 0.12 NaCl, 0.015 $NaHCO_3$, 0.05 KCl, 0.015 $CaCl_2$, 0.012 Na_2HPO_4 supplemented with 0.00825 glucose and insulin (Actrapid, Novo Nordisk, Denmark) at a concentration of 0.1 unit cm^{-3} buffer.

In addition to preparation for calorimetric measurements, tissue specimens from the macroscopically preserved and damaged areas of the heart were immersed into formalin solution for histological evaluation after haematoxylin–eosin and van Gison staining. The preserved apical tissue was normal while severe fibrosis was verified in the damaged tissue. In the macroscopically damaged region, an infiltration of hypertrophied myocardial cells was present.

2.2. Microcalorimetric measurements

The heat production rate of the myocardial tissue sample was measured with a microcalorimeter of the thermopile heat-conduction type [4], arranged as twin instrument with one of the units containing the reactive system and the other containing the reference ampoule with water. Measurements were performed at 37°C. A 3.0 or 0.7 ml flow-through vessel for liquid perfusion was used. Good baseline stability was obtained during 12 h, established with the vessel charged with water. The voltage output was amplified with a Keithley 150 B microvoltmeter, 10 μV range; calibration was carried out electrically. The calorimetric vessel was thermostatted in four consecutive heat-exchange positions before reaching the thermopile zone after 15–20 min. During the experiment the tissue slice was kept static on a thin stainless steel needle inside the steel ampoule. The perfusion medium was introduced very close to the muscle sample by means of a peristaltic pump (LKB Perpex, Sweden) in order to maintain a constant pH during the measurement procedure; the perfusion rate was 5 cm^3 h^{-1}. Carbogen (95% O_2 + 5% CO_2) was bubbled through the perfusion medium outside the calorimeter. Calorimetric recording lasted several hours. During the registration period the pH in the effluent was measured intermittently by means of a radiometer type G 297/G7 capillary electrode; a change in pH level was noted, decreasing from 7.8 to 7.3.

Heat production rate, as calculated from the power–time curves, refers to 2 h after the start of the calorimetric experiment, and is expressed in units of mW g^{-1} tissue wet weight. The coefficient of variation for the procedure is estimated to be 4.2% [7].

2.3. Oxygen-consumption measurements

For oxygen consumption studies, a similar tissue specimen of 5 mg, from the same myocardial slice, was put in a 1 mL measurement chamber of a Clark oxygen electrode

(Hansatech, UK) where the rate of oxygen consumption could be continuously monitored at 37°C, expressed as nmol O_2 uptake $s^{-1} \times g^{-1}$ wet tissue.

Estimation of the oxygen-dependent heat-energy production of the myocardial tissue was based on the assumption that glucose was used as substrate, the only substrate available in the buffer medium. Thereby the contribution from aerobic metabolic processes to total heat production was calculated from the product of oxygen consumption (nmol $s^{-1} g^{-1}$) and the enthalpy change for glucose ($\Delta H = -2931$ kJ (mol glucose)$^{-1}$), where ΔH is the thermodynamic quantity for the combustion of glucose to carbon dioxide and water [8].

2.4. Statistics

Data are expressed as mean ± SEM (standard error of the mean). Differences between means were calculated by Student's *t*-test or by a rank order test. Correlations between variables were by the method of least squares.

3. Results

Data on heat production rate (P) and oxygen consumption rate (V_{O_2}) in the *macroscopically preserved region* of myocardial tissue from the CHF rats ($n = 10$) and from the corresponding myocardial areas in control rats ($n = 23$) are summarized in Fig. 1.

In CHF rats the mean (± SEM) value for P was 0.94 ± 0.05 mW g^{-1}, not different from controls (0.94 ± 0.05 mW g^{-1}; $n = 23$).

The oxygen consumption rate in CHF myocardium was 1.36 ± 0.20 nmol O_2 s^{-1} g^{-1} and 1.55 ± 0.14 nmol O_2 s^{-1} g^{-1} in the control rats ($n = 20$), n.s. vs. the CHF group.

The contribution from aerobic metabolism to total heat production, as calculated from the rate of oxygen consumption and glucose metabolism, corresponded to $80 \pm 6\%$ of total heat production in the control group and to $71 \pm 9\%$ in the CHF rats (n.s.).

In the control group ($n = 20$) there was a significant correlation between the values for heat production and oxygen consumption, $r = 0.49$, $p < 0.05$. For the controls and the CHF group together, the correlation coefficient was 0.38, $p < 0.05$.

In tissue samples, taken from the region of the *border of and into the macroscopically damaged area* of the myocardial infarction ($n = 5$), the P value was 1.15 ± 0.13 mW g^{-1} (range 0.82–1.50). Oxygen uptake rate was specifically studied in these slices of myocardial tissue, thus taken from the margin of and into the area of myocardial infarction, and compared to the oxygen uptake rate in the myocardial slice prepared from the preserved apical region of the same rat ($n = 5$). In the damaged area, V_{O_2} was 1.79 ± 0.23 nmol O_2 s^{-1} g^{-1} (range 1.12–2.33) as compared to 1.06 ± 0.17 nmol O_2 s^{-1} g^{-1} (range 0.61–1.67) in the preserved apical region (n.s.).

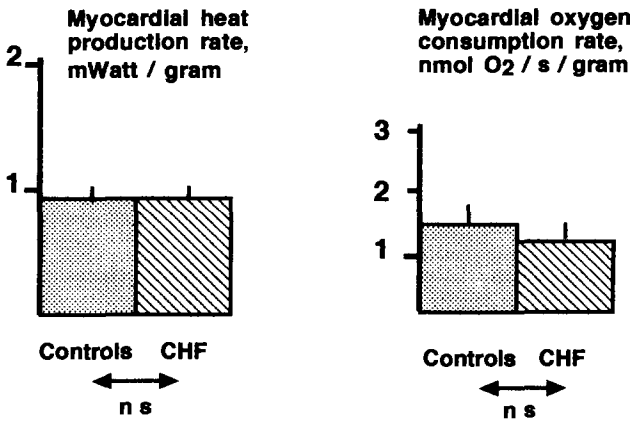

Fig. 1. Myocardial heat production rate and oxygen consumption rate in tissue slices cut from the apical and left ventricle region of the heart from untreated rats, controls ($n = 23$ for heat production data and $n = 20$ for oxygen consumption data) and from the corresponding region of the heart with macroscopically preserved myocardium from rats with congestive heart failure after left coronary ligature and myocardial infarction, CHF ($n = 10$).

The calculated value for the contribution of aerobic metabolism to heat production in the damaged tissue region was 76%.

4. Discussion

The main finding in the present study was the demonstration that the calculated relative contribution of aerobic metabolism to the total expenditure of energy in rat myocardial tissue is about 75% and that this figure is of the same magnitude in rats suffering from CHF due to myocardial infarction as it is in controls. Furthermore, total heat production was not increased in the preserved part of the myocardium from the rats with heart failure, indicating that energy expenditure by this tissue is not increased. This is opposite to what we expected since the heart in these animals can be expected to be exposed to increased levels of catecholamines [6] to meet the increased demands on myocardial performance after such large ischemic lesion. In addition, the present study also showed that the energy expenditure in the damaged area of the left ventricle does not seem to be decreased compared to that of preserved myocardium.

In a recent investigation, the effect of excess of thyroid hormones on myocardial heat production and oxygen consumption was studied [9]. Triiodothyronine administration daily during two weeks induced an increase of myocardial tissue heat production by 63% and of oxygen consumption by about 77%. These data demonstrate the potential of myocardial tissue to increase its metabolic activity. Notably, this increase was paralleled by enhanced oxygen consumption, indicating a major role of aerobic metabolism in myocardial tissue, corresponding to about 87% of total heat production in the hyperthyreoid state. Decreasing ATP content of the myocardium under these

conditions [9] suggests that the maximum capacity for energy expenditure was reached. The finding in the present study that the rate of oxygen consumption was not increased in the preserved part of the myocardium, otherwise harbouring a myocardial infarction, might suggest that oxygen supply is a major limiting factor for further increase of energy expenditure by the failing rat heart in ischemic heart disease. This interpretation would be in accordance with earlier demonstrations of a close relationship between myocardial oxygen consumption and systolic pressure volume area [3,10].

The idea that myocardial cells can adapt to increasing work demand would also be in accordance with the findings of recent studies on oxidative enzymes in human myocardial cells, showing increasing activities of lactate dehydrogenase [11], citrate synthase [12], myoglobin and coenzyme Q 10 content [12, 13] from the atria, through the right to the left ventricle, i.e. along different parts of the heart with increasing demands on work performance. However, according to the present data there was no increase of the myocardial cell metabolic activity in the macro- and microscopically intact part of the left ventricle in rats with heart failure after myocardial infarction. These findings indicate that the preserved part of the left ventricle is unable to respond to the stimulation with increased metabolic activity by, e.g., enhanced sympathetic nervous system activity, demonstrated to accompany CHF [5,6]. These results are in agreement with suggestions from previous studies that the ability to enhance oxidative capacity, oxygen transport and storage at the cell level is impaired in ischemic heart disease [2]. The pathophysiological mechanism for this incapacity is unclear although impaired enzymatic activity has been shown [2]. In addition, decreased oxygen delivery might contribute. Furthermore, the area close to and within the infarcted region showed metabolic activity of the same magnitude as in myocardium from control rats and in macroscopically preserved areas of the left ventricle from rats with myocardial infarction.

Microscopic study of these slices from damaged myocardium showed a fibrous tissue content but also of some hypertrophied myocardial cells in between. Obviously, this tissue, bordering and containing infarcted myocardium, still has a continuous demand on substantial oxygen and fuel supply. It has been suggested that an augmented basal cellular metabolism, devoted to post-ischemic reparative processes, would result in increased oxygen utilization [3].

In conclusion, the present experiments indicate that the consequences of an occlusion of the proximal part of the left coronary artery in rats are not limited to the damage of a large area of the left ventricular wall with impaired inotropic performance, but will also have substantial impact on the metabolic activity of myocardial cells in other parts of the left ventricle. It seems as if unability to increase oxygen uptake can be of major importance for this incapability. Furthermore, the finding that the border of the infarcted area has a continuous demand on oxygen and fuel supply, should be of interest with regard to the possibility of using various drugs and procedures to modulate the metabolic conditions and survival for myocardial cells in this region. Although these animal data have to be interpreted cautiously, they might suggest the possible inability of myocardial cells to increase their metabolic activity in the case of congestive heart failure in patients who had a myocardial infarction.

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