Myocardial force production and energy turnover in anoxia

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Summary. ATP turnover of isolated rabbit papillary muscles, contracting isometrically at 20 °C, was determined in oxygen and during 40 min of exposure to nitrogen (anoxia). Stimulus frequency was 0.2 hertz (Hz) in oxygen and 0.2 or 1.0 Hz in nitrogen. In oxygen, ATP turnover was determined from oxygen consumption using a P/O_2 ratio of 6.3. The time-dependent rate of ATP turnover in nitrogen was found from the production of lactate, and the changes in adenine nucleotides and phosphocreatine, measured in rapidly frozen preparations at different time-points during the anoxic period. A P/lactate ratio of 1.5 was used. In muscles stimulated at 0.2 Hz, twitch force dropped during the anoxic period to 33% while force production of muscles stimulated at 1.0 Hz stopped completely. However, in the latter muscles, resting force rose to 19% of the twitch force in oxygen. The rate of ATP hydrolysis in anoxia depended strongly on stimulus frequency, indicating that it is not solely determined by the glycolytic capacity. In the 0.2 Hz-stimulated muscles the decrease in energy turnover occurred in parallel with the drop in force. However, the rise in resting force in muscles stimulated at 1.0 Hz occurred when ATP turnover was close to zero. It was concluded that anoxia hardly affects the energy required for twitch force production, but that the rise of resting force measured when twitch force had disappeared occurred when the rates of cross-bridge cycling and calcium turnover were very low.
Key words. Energy turnover; anoxia; twitch force; passive force.

Introduction

Oxidative phosphorylation is the preferred pathway of ATP formation in the heart. In the normal myocardium, functioning in the steady state, ATP formation meets the energy demand. Cellular energy demand is mainly determined by the contractile activity of the myocytes. When no oxygen is available, ATP formation is insufficient to maintain cellular function at the aerobic level, and the demand for energy is no longer met. When heart muscle, contracting isometrically, becomes anoxic the limited ATP supply causes the force to drop. To assess the energetic cost of force production (i.e. the economics of contraction) in anoxia it is necessary to know whether the drop in force is proportional to that in force-related energy turnover. Under the non steady-state conditions occurring in anoxia one might suppose that cellular energy turnover is no longer determined by the contractile activity, but by the rate at which glycolysis and changes of the energy store (adenine nucleotides and creatine phosphate) can supply ATP. Thus in that case energy turnover in anoxia would be independent of the demand.

We studied energy turnover in isolated rabbit papillary muscle contracting isometrically at 20 °C in oxygen and in nitrogen. The experiments were designed to find out whether energy turnover in anoxia is indeed independent of demand. Energy demand was varied by stimulating the muscles at different frequencies. We furthermore compared force and ATP turnover in aerobic and anaerobic papillary muscles stimulated at 0.2 and 1.0 Hz to see how anoxia affects the economy of force production. Energy turnover was estimated from the rate of ATP hydrolysis estimated from oxygen consumption (using a P/O_2 ratio of 6.3), lactate production (assuming a P/lactate ratio of 1.5), and the changes in adenine nucleotides and phosphocreatine (PCr).

Methods

The methods used for the present study have been described in detail previously [6], therefore a short summary will suffice here.

Preparation. Rabbits are killed by a blow on the neck and their hearts are rapidly excised. Papillary muscles are selected from the right ventricle and mounted in the oxygen or in the lactate chamber described below. All experiments are done at 20 °C; the composition of the saline is: 128 mM NaCl; 4.7 mM KCl; 1.0 mM $MgCl_2$; 1.4 mM NaH_2PO_4; 20.0 mM $NaHCO_3$; 2.5 mM $CaCl_2$, and 11.1 mM glucose. When saturated with 95% O_2 and 5% CO_2 the pH of the solution is 7.2.

Oxygen chamber. The oxygen chamber shown in figure 1 A consists of a closed chamber with a volume of 219 µl, in which the saline circulates rapidly. Oxygen is measured with a 2-mm diameter polarographic electrode. Oxygen concentrations are calculated from the measured P_{O_2} and the solubility of oxygen in 126 mM NaCl at 20 °C (29.9 ml O_2/l). The proximal end of the muscle is fixed at the bottom of the chamber while the tendon end is tied to a 0.2-mm diameter stainless steel wire that leaves the chamber via a glass capillary and is suspended from a force transducer. The muscle is stimulated by a single period of a sine wave of 200 Hz which is applied via the stainless steel inlet and the bottom of the chamber.

Lactate chamber. The lactate chamber shown in figure 1 B consists of an open acrylic plastic reservoir, containing 300 µl solution, and a removable plastic muscle holder. The proximal end of the muscle is fixed to the lower end of the holder while the tendon end is tied to a stainless steel wire suspended from a force transducer. By changing the solution, and the composition of the gas mixture, from O_2/CO_2 to N_2/CO_2, the muscles can be made anoxic. When rapid freezing is required the clamp

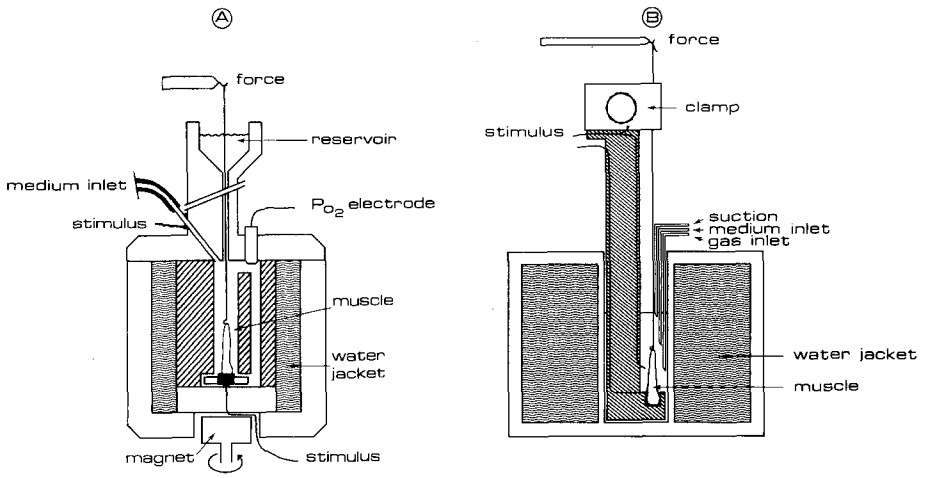

Figure 1. *A* Glass chamber to measure oxygen uptake of papillary muscle. The preparation is fixed to the bottom of the chamber and to a stainless steel wire suspended from a force transducer. Changes in P_{O_2} of the circulating fluid are followed with a polarographic electrode. The inlet is used to refresh the solution between measurements. *B* Chamber used to study the changes in force, lactate production, adenine nucleotides, and phosphocreatine when the muscles are made anoxic. Anoxia is achieved by (1) stopping flow of oxygenated saline through the chamber, (2) rapidly exchanging the oxygenated solution in the chamber with a solution saturated with nitrogen, and (3) using nitrogen instead of oxygen at the gas inlet.

of the muscle holder is closed and the muscle with the entire holder is submerged into liquid Freon 22, which is cooled by liquid nitrogen.

Assays. Freeze-dried muscles are ground and extracted in a precooled Potter homogenizer tube containing 0.6 M perchloric acid. Following neutralization (pH 6.5) with KOH the extracts are centrifuged for 20 min at 0 °C. Lactate is determined in muscle tissue and chamber fluid by enzymatic analysis[5]. Adenine nucleotides and creatine compounds are determined using a modification[3] of the method described by Sellevold et al.[9]. To determine glycogen a fraction of the acid extract was neutralized to pH 4.8 with 1.8 M $KHCO_3$. Glycogen was hydrolyzed enzymatically and the glucose formed was determined as described by Krebs et al.[4].

Results

ATP turnover in oxygen

With fresh oxygenated saline in the oxygen chamber, oxygen tension decreases continuously. The disappearance of oxygen occurs even when no muscle is mounted in the chamber. Most likely the P_{O_2} electrode itself and the stainless steel spinner (fig. 1 A) are responsible for that. By determining the difference in rate of the oxygen decrease with and without a muscle in the chamber, the rate of oxygen uptake of the resting muscle can be determined. It is possible also to determine resting metabolism by measuring oxygen uptake in the absence and in the presence of 2 mM potassium cyanide. From these experiments we found the oxygen uptake of the resting muscle to be 1.4 $\mu mol \cdot min^{-1} \cdot gdw^{-1}$. This value corresponds to an ATP turnover of 8.8 $\mu mol \cdot min^{-1} \cdot gdw^{-1}$. When the muscle is stimulated the rate of oxygen disappearance from the chamber increases. When the recording is corrected for the rate measured when the muscle does not contract, tracings such as that shown in figure 2 are obtained. Oxygen uptake increases gradually from the onset of force production onwards to attain a steady rate later during the twitch train. From this rate the contraction-related ATP turnover was calculated for different stimulus frequencies as is shown in figure 3. To obtain the total ATP turnover the value for quiescent muscles has been added to the data, assuming that contraction does not affect basal metabolism.

Figure 3 shows that a proportional increase of the contraction-related ATP turnover occurs up to a stimulus frequency of 0.5 Hz. The non-linear nature of this relationship can be explained by the fact that the muscle core becomes anoxic at higher rates of oxygen uptake[6]. Therefore no reliable aerobic value for oxygen uptake can be obtained for muscles stimulated at 0.5 Hz or

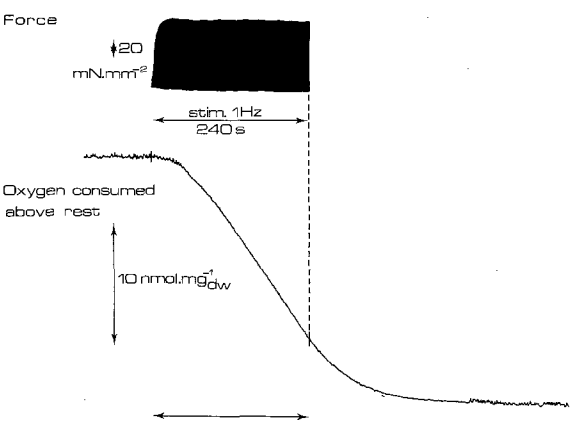

Figure 2. Force per cross sectional area (top), and force related oxygen consumption (bottom).

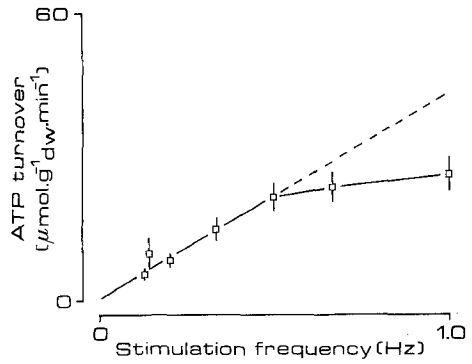

Figure 3. The rate of ATP turnover as a function of stimulus frequency. The loss of proportionality above a frequency of 0.5 Hz is related to anoxia in the center of the preparation.

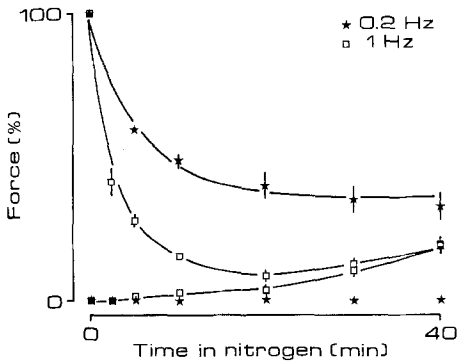

Figure 4. Changes in active and passive force during 40 min of anoxia of muscles stimulated at 0.2 Hz and 1.0 Hz. Resting force increased in the 1.0 Hz stimulated muscles.

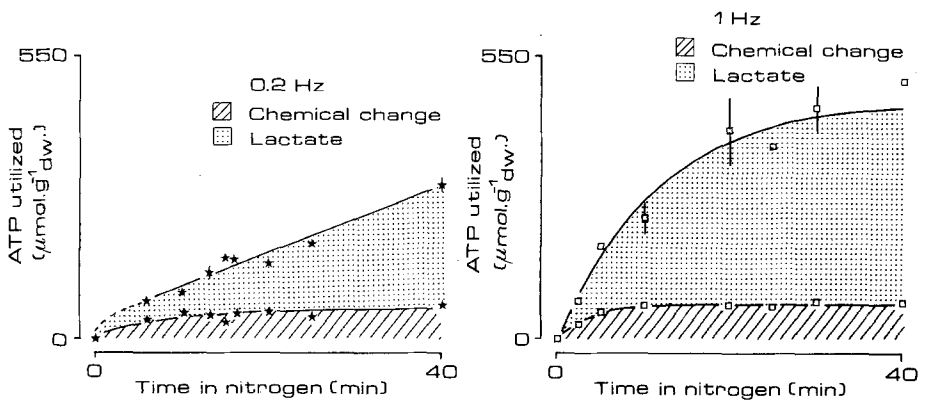

Figure 5. Contribution of the changes in intracellular ATP, AMP, IMP and PCr (chemical change) and of glycolysis to the ATP hydrolyzed during 40 min of anoxia of muscles stimulated at 0.2 Hz and 1.0 Hz.

higher. An estimate for the aerobic ATP turnover at these frequencies can be determined by extrapolation (fig. 3).

Force and ATP turnover in anoxia

Before making the muscles anoxic for a period of 40 min in the lactate chamber (cf Methods) they are stimulated in oxygen at 0.2 Hz. Anoxia causes a rapid fall in force. However, the drop in force over the 40-min period depends on whether stimulation continues at 0.2 Hz or is changed to 1.0 Hz at the onset of anoxia. In the muscles which continue to contract at 0.2 Hz during the anoxic period (fig. 4), force drops towards the end of the anoxic period to about 33% of the control value in oxygen. When 1.0 Hz is used for stimulation, twitch force falls steeply and in almost all muscles disappears altogether well before the end of the anoxic period. Resting force rises in the muscles stimulated at 1.0 Hz to 19% of the twitch force produced in oxygen (fig. 4).

The average change in force found in the two groups of muscles during the anoxic period (fig. 4) may be compared with the changes in energy turnover during the same period (fig. 6). Energy turnover in anoxia was calculated from 1) the chemical change (obtained from the decrease in PCr and ATP and the increase in AMP and IMP[2]) and 2) the amount of lactate formed (P/lactate ratio of 1.5). Both components and the sum thereof are presented as a function of time (fig. 5) for muscles stimulated at 0.2 Hz and 1.0 Hz. The sums of the two components representing the amount of ATP hydrolyzed as a function of time in anoxia for the two stimulation frequencies were fitted with a single exponential, and the time derivative thereof was taken as the rate of ATP hydrolysis, which is presented for both groups of muscles in figure 6.

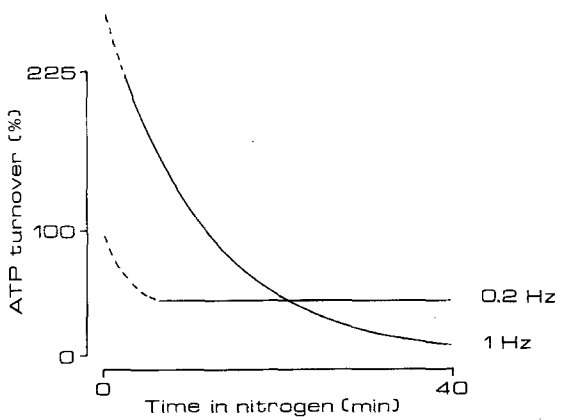

Figure 6. ATP turnover during 40 min of anoxia (0.2 and 1.0 Hz). The values are expressed as percentages of the value of well-oxygenated muscles stimulated at 0.2 Hz.

Glycogen was determined in control muscles (n = 6) and in muscles stimulated at 1.0 Hz for 80 (n = 5) min in anoxia. The glycogen content in the former group of preparations was 128.5 ± 1.76 μmol · gdw^{-1}; no glycogen was found in the other group. The amount of lactate produced by these muscles during 40 min of anoxia was 272.6 ± 17.1 μmol · gdw^{-1}.

Discussion

Determination of energy turnover

In this study, energy turnover was assumed to be proportional to the rate of ATP hydrolysis. This latter quantity cannot be measured directly in isolated papillary muscles. The rate of ATP hydrolysis under aerobic conditions was estimated from the rate of oxygen consumption. This approach can be followed because in the aerobic steady state no lactate is formed [6] and ATP hydrolysis equals ATP formation. The ratio relating the rates of ATP turnover and oxygen consumption was taken to be 6.3, assuming glucose to be the substrate.

To determine ATP turnover under anaerobic conditions is more complex. Apart from cytosolic ATP there are other sources which provide ATP for the energy-requiring processes; one is the breakdown of glucose and/or glycogen to lactate, the other is the formation of ATP from phosphocreatine (PCr) and from ADP. Depending on the nature of the substrate (glucose or glycogen) the P/lactate ratio equals 1 or 1.5. The ATP formed from ADP and PCr can be estimated from changes in cellular AMP, IMP and PCr content [2]. It has to be pointed out that cellular energy turnover will be proportional to ATP turnover provided the free energy change of ATP hydrolysis remains constant. However, during ischemia this will not be the case; the value will decrease when inorganic phosphate and ADP accumulate and ATP falls [1]. Thus in anoxia ATP turnover may be an overestimate of the energy turnover.

When the preparations are well oxygenated and stimulated at 0.2 Hz, they were able to maintain a steady state for hours [3]. We therefore assumed that these preparations used exogenous glucose as the substrate for oxidation. Glycogen, in these muscles under control conditions, i.e. after 2–8 h of stimulation at 0.2 Hz in oxygen, was 128.5 μmol · gdw^{-1}. When the muscles were made anoxic and were stimulated during the anoxic period for 80 min at 1.0 Hz, glycogen appeared to be depleted. The total amount of lactate formed corresponded closely to the amount of glycogen found in the muscle under control conditions. This suggests that in the muscles stimulated at 1.0 Hz, most if not all lactate was formed from glycogen. Between 40 and 80 min of anoxia no lactate was formed in muscles stimulated at 1.0 Hz. These observations imply that in muscles stimulated at 1.0 Hz glucose entry is already inhibited early during anoxia.

We do not know why, in these preparations, glucose cannot be used by the myocytes. The underlying mechanism may be related to that responsible for the finding that glucose utilization in ischemic heart is depressed as compared to well perfused aerobic and anoxic preparations [8]. It was suggested that lactate accumulation played a role but that a change in intracellular pH was not involved [7]. We have not yet determined whether glucose can also not be used by muscles stimulated at 0.2 Hz during anoxia. However, in view of the conclusion drawn from the 1.0 Hz-stimulated muscles we will assume during the following discussion that glycogen was also the substrate during anoxia in the 0.2 Hz-stimulated muscles.

Energy turnover in anoxia

From figure 6 it is quite clear that ATP turnover rapidly decreases when muscles stimulated at 0.2 Hz are made anoxic. However, when the stimulus frequency was changed from 0.2 to 1.0 Hz at the same time, the rate of ATP hydrolysis attained initial values well above that found in oxygen at 0.2 Hz. This demonstrates that in anoxia the rate of ATP turnover is not a fixed quantity reflecting the maximum glycolytic capacity. The rate at which glycolysis and changes of the energy stores can supply ATP to the cellular ATPases appears to be quite sensitive to the energy demand.

In contracting cardiac muscle three processes require most of the energy, i.e. basal metabolism, actomyosin interaction, and calcium uptake by the sarcoplasmic reticulum. On the basis of the present study we cannot distinguish the latter two energetically, but it is possible to separate basal ATP turnover from contraction-related ATP turnover, assuming that basal metabolism is unaltered by contraction. Basal metabolism as estimated from the oxygen uptake of quiescent papillary muscles [6] is 8.8 μmol ATP · gdw^{-1} · min^{-1}. Papillary muscles contracting at 0.2 Hz had an ATP turnover of 17.4 μmol · gdw^{-1} · min^{-1}. Thus at this frequency contraction-related ATP turnover equalled 8.6 μmol ATP · gdw^{-1} · min^{-1}.

During 40 min of anoxia, quiescent muscles produce lactate at a constant rate of 1.2 μmol · min^{-1} · gdw^{-1} [3]. Using the P/lactate ratio of 1.5 – in contrast to unity as assumed earlier [3] – this yields an ATP turnover of 1.8 μmol · gdw^{-1} · min^{-1}. When the muscle contracts at 0.2 Hz glycolysis provides ATP at a rate of 6.0 μmol · gdw^{-1} · min^{-1}. Since towards the end of the anoxic period little change occurs in the energy stored in the muscles, the contraction-related ATP turnover is about 4.2 μmol · gdw^{-1} · min^{-1}.

Force produced by the muscles stimulated at a frequency of 0.2 Hz falls during anoxia, rapidly at first, more slowly later on. At the end of the 40-min period, the peak force developed is on average 33% of that found under aerobic conditions. Contraction-related ATP turnover is 49% at the corresponding time. This seems to suggest that force production in anoxia may be somewhat less economical than under aerobic conditions. However, the decrease in

the free energy change of ATP hydrolysis under these circumstances (about 20%) should be taken into account also. This would minimize the difference between the two percentages, suggesting that the economics of force production may in fact be quite similar.

In the muscles stimulated at 1.0 Hz, resting force rose towards the end of the anoxic period to 19% of the peak force produced in oxygen at 0.2 Hz stimulation. To compare the energetic cost of the production of resting force with that of twitch force the use of mean force instead of developed force is more convenient. If we take twitch duration at 0.2 Hz to be 2 s, average force at 0.2 Hz is about 20% of peak force. The ATP turnover at the end of the 40-min period in muscles stimulated at 1.0 Hz is very low, 1.3% of that in the oxygenated muscles stimulated at 0.2 Hz. This indicates that in the anoxic 1.0 Hz-stimulated muscles the rise of passive force to 19% of the twitch force produced in oxygen does indeed require little energy, which suggests that cross-bridge cycling and calcium cycling occur at a very low rate.

In conclusion this study shows that, in anoxia, when ATP formation is limited the energy demand is still an important determinant of the rate of ATP hydrolysis. Furthermore, it is found that the economics of force production does not change very much when twitch force drops to a low level in anoxia. However, the increase in resting force is not accompanied by an increase in ATP turnover and occurs when the rate of ATP hydrolysis is very low. This suggests that under these conditions force production is related to the formation of rigor bridges.

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Adenosine is a sensitive oxygen sensor in the heart

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Summary. Cardiac adenosine is formed both by an oxygen-sensitive (AMP→adenosine) and by an oxygen-insensitive (S-adenosylhomocysteine → adenosine) pathway. The phasic adenosine release during β-adrenergic stimulation with isoproterenol is closely linked to coronary venous P_{O_2} (isolated heart) and can be almost fully prevented when diastolic aortic pressure is maintained constant (heart in situ). During pressure autoregulation the transmural gradient of free adenosine is only increased when the autoregulatory reserve is exhausted. The critical P_{O_2} below which adenosine formation is enhanced was found to be 3 mm Hg (isolated cardiomyocytes). Collectively, these data indicate that the formation of adenosine is not primarily coupled to the energy expenditure of the heart but to the supply/demand ratio for oxygen.

Key words. Coronary blood flow; energy metabolism; hypoxia, P_{O_2}; β-adrenergic stimulation; autoregulation; S-adenosylhomocysteine.

Regulation of coronary flow is in essence the regulation of cardiac energy metabolism. Taking a purely regulatory point of view, two principal alternatives by which coronary flow may be adjusted to cardiac metabolic demand are conceivable:

A) Any mismatch between oxygen supply and oxygen demand is linked to the production of vasodilatory metabolites. Such a P_{O_2}-dependent metabolite may be adenosine, a degradative product of ATP which is thought to increase coronary flow and to bring the oxygen supply/demand ratio back to a new equilibrium[3]. Essential features of this model are:

1) the vasodilator concentration inversely reflects tissue oxygen tension and 2) at least a minor degree of hypoxia is required. This local vascular control is not perfect so