Heat Produced by Rabbit Papillary Muscle During Anoxia and Reoxygenation

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Resting heat rate was measured in superfused rabbit papillary muscles at 20°C during 40 minutes of anoxia and subsequent reoxygenation. To reveal the nature of the reactions underlying energy output under such conditions, the data obtained were compared with values predicted from data on chemical change. Before and after the anoxic period, muscles were stimulated at 0.2 Hz, during which time the contraction-related heat rate was measured. During anoxia, muscles were kept at rest or stimulated at 1 Hz. Stimulation was switched off intermittently to determine resting heat rate. Before anoxia, resting heat rate was 8.7±1.1 (mean±SEM) mW·g dry wt⁻¹. During anoxia, it decreased to 38% and 50% of the preanoxic level in resting and stimulated muscles, respectively (P<.05). In resting muscles, heat rate increased with reoxygenation in approximately 10 to 15 minutes to 1.3 times the preanoxic level, whereas this was 3.7 times in stimulated muscles. Resting heat rate returned within 65 (resting muscles) or 150 (stimulated muscles) minutes to the baseline. The ratio of force- and contraction-related heat rate, ie, the economy of contraction, was not different before and after anoxia. We estimated that the heat produced by muscles during anoxia was not different from the heat to be expected from the hydrolysis of creatine phosphate, the breakdown of nucleotides, and the formation of lactate. The overshoot in resting heat during reoxygenation of resting muscles could be accounted for by the resynthesis of the energy store. The much larger overshoot in resting heat of stimulated muscles was due to the contracture. The finding that the economy of contraction was not altered by anoxia and reoxygenation suggests that both sarcoplasmic reticulum Ca²⁺-ATPase and myofibrillar ATPase are depressed by anoxia and that the enhancement of cytosolic calcium transients with reoxygenation, reported in other studies on papillary muscle, results from reduced binding of calcium rather than from enhanced release. (Circ Res. 1993;73:1177-1187.)

KEY WORDS • heat rate • stunning • anoxia • reoxygenation • heart

The leading event in the cascade of cellular responses in heart muscles after anoxia or reoxygenation is energetic in nature. However, rather few direct measurements have been reported on changes in energy output of the myocardium with anoxia and reoxygenation. In the present study, we measured the effects of anoxia and reoxygenation on heat production of isolated isometric cardiac muscle. We used a flow-through chamber, similar to the one described by Daut and Elzinga¹ for trabeculas, built to accommodate papillary muscles. The latter preparation was preferred to be continuous with earlier work²-⁴ reporting data from that preparation on isometric force production, nucleotide composition, phosphocreatine (PCr), and lactate during anoxia and reoxygenation. This information was needed for the present work to explain the changes in heat output found with anoxia and reoxygenation.

The following specific questions were pursued: (1) What is the time course of energy output during anoxia, and to what extent can it be explained by the reactions known? (2) What is the time course of energy output during reoxygenation, and do reactions other than oxidation contribute in a major way? In the experiments addressing these questions, we studied the muscles under stimulated and unstimulated conditions.

For whole hearts,⁵,⁶ as well as for isolated cardiac muscle,⁷,⁸ it has been shown that in well-oxygenated steady states the heat produced originates from substrate oxidation. During anoxia, however, oxidation of substrate does not contribute to heat production; ATP hydrolysis continues for some time at the expense of PCr and nucleotide stores, whereas ATP can still be formed by the breakdown of glycogen or glucose to lactate. These reactions also produce heat. In addition, changes of ionic gradients over the membranes, as well as other processes that are not as well defined, may contribute to heat production by anoxic myocardium.

Holubarsch et al⁹ studied heat output of anoxic myocardium. They showed that the heat output during the rise of passive force in anoxia was less than predicted on the basis of active force development. In their study, no attempt was made to explain the heat produced on the basis of the underlying cellular reactions. Furthermore, scanty information was presented about the time course of myocardial energy output during anoxia.

When oxygen is readmitted, part of the heat produced results, no doubt, from substrate oxidation. However, it is not known whether or not this accounts for all the heat produced; restitution of the depleted energy stores as well as other recovery processes may contrib-
ute. A study of the heat produced during reoxygenation is also of interest because anoxia may affect the mechanical and energetic properties of reoxygenated myocardium. This is particularly the case in stunned myocardium, a state characterized by prolonged mechanical depression and often a loss of nucleotides with enhanced cytosolic calcium transients.\(^{11-13}\)

It has been shown that in stunned whole hearts oxygen consumption is not diminished in proportion with the depressed mechanical state.\(^{14-16}\) Laster et al\(^{16}\) have concluded that the relative increased energy turnover is not due to an increase in basal metabolism but is related to abnormalities in contraction or electromechanical coupling. This view is shared by Ogoshi et al,\(^{15}\) who studied the relation between the systolic pressure-volume area and oxygen consumption in stunning. These authors have concluded that the relative enhanced energy turnover follows from an increased cost of contractility. Unfortunately, these studies provide only limited information about the time course of basal and contraction-related oxygen consumption. The time course of energetic processes, however, is important because the conclusions may change with time after the onset of reperfusion.

Materials and Methods

Experiments were performed on papillary muscles from the right ventricle of New Zealand White rabbits weighing 2.5 to 4.2 kg. The animals were anesthetized with 9.6 mg · kg\(^{-1}\) fluanisone and 0.3 mg · kg\(^{-1}\) fentanyl citrate (Hynnorm) and injected with 150 IU heparin. The hearts were rapidly excised and exanguinated in warm saline containing (mmol/L) NaCl, 128; KCl, 4.7; MgCl\(_2\), 1.0; CaCl\(_2\), 1.4; NaHCO\(_3\), 20; NaH\(_2\)PO\(_4\), 0.4; and glucose, 11.1 (pH 7.2, gassed with 95% O\(_2\)-5% CO\(_2\)). Papillary muscles (diameter, 0.7 to 1.1 mm) were dissected from the right ventricle at room temperature and transferred to the heat chamber for mounting; Mast and Elzinga\(^8\) showed that, when such preparations are stimulated at 0.2 Hz in oxygenated saline, they do not develop an anoxic core. All experiments were done at 20°C.

Heat Chamber

The experimental setup (Fig 1) was a modification of that used by Daut and Elzinga\(^1\) to measure the resting heat rate of trabeculae. Temperature difference was measured in a Perspex tube (inner diameter, 2 mm; outer diameter, 2.2 mm) glued into a stainless-steel block. The Perspex tube was connected, on either side, by means of 2-mm channels to chambers in the stainless-steel block (see Fig 1). In one of these chambers, the preparation was mounted (see below); the other contained a port through which a force transducer could be inserted. The stainless-steel block was mounted on top of a cylindrical aluminum block and covered with an aluminum lid during the experiment. The aluminum block was isolated from the environment by a double-walled tank. The space between the walls was filled with polystyrene. The temperature of the system was controlled by water circulating through the aluminum block and lid.

The Perspex tube was perfused at a constant rate by a suction pump. The perfusate could be changed by switching a four-port valve located on the upstream side of the recording chamber. Temperature difference between the upstream and downstream side of the muscle was measured with 16 pairs of chromel-constantan thermocouples (sensitivity, 22.67 μV · K\(^{-1}\) per thermocouple) mounted 18 mm apart. The thermocouples were embedded in the wall of the Perspex tube and had no direct contact with the perfusing solution. Voltage output of the thermocouples was amplified, filtered, and recorded on a chart recorder or sampled by computer. The system was calibrated by replacing the muscle by a thermistor and dissipating a known amount of energy (compare with Daut and Elzinga\(^1\)).
mounting the preparation

mounting of the preparation takes typically approximately 1.5 hours. Depending on the variables to be measured, two different mounting procedures were used.

resting heat rate and contraction-related heat rate. the preparation was tied at both ends to a thin platinum wire acting as a stimulating electrode. the platinum wires entered the chamber through fine holes on either side of the tube and were attached to micrometer screws. by turning the micrometer screws simultaneously, the preparation was moved along the perspex tube into the measuring chamber.

paired force and heat rate measurements. the preparation was tied at its base to a stimulating electrode. at its tendon end, the preparation was tied to a thin platinum wire extending to the force transducer. the preparation was moved into the measuring chamber as described previously, and the platinum wire was hooked to the force transducer.

protocols

when the preparation was mounted, the chamber (with the muscle in it; fig 2, left) was perfused at 12 mL·h⁻¹ with oxygenated saline.

muscles were stimulated at 0.2 Hz with 4-millisecond block pulses at approximately 20% above threshold. the heat chamber was closed, and the system was left to stabilize. by turning the micrometer screws, the muscle was stretched to a length just below that at which no increase in heat rate could be recorded if stretched further. when the heat rate signal was stable, stimulation was stopped, and the voltage output of the thermocouples during stimulation of the muscle and at rest was determined. the voltage output of the resting preparations was determined by moving the preparation 3 to 4 mm outside the downstream thermocouple (fig 2, right). this procedure was not possible in the experiments in which heat and force were measured simultaneously (see above). in all the other experiments, this procedure was repeated several times during the experiment to detect possible changes in the reference potential. the stability of the reference potential was tested in experiments without a preparation in the chamber, and conditions were created such that no systematic changes in the reference potential would occur for more than 8 hours. in spite of these conditions, small unexpected deviations of the reference potential were sometimes found when there was a preparation present in the chamber. in those cases, we drew the baseline through the corresponding values of the reference potential only.

muscles were made anoxic by perfusing the muscles with saline gassed with 95% N₂−5% CO₂ to which 3 mmol/L NaCN was added. anoxia was terminated by switching back to the oxygenated perfusate. the following experiments were performed:

stimulated muscles. eight muscles were stimulated at 1 Hz during anoxia. after anoxia, five of the muscles were stimulated at 0.2 Hz throughout reoxygenation. in three muscles, stimulation was switched off for the first 30 minutes of reoxygenation; thereafter, it was stimulated at 0.2 Hz.

resting muscles. five muscles were not stimulated during anoxia. two of the muscles were stimulated at 0.2 Hz immediately at the onset of reperfusion. in three muscles, stimulation remained off during the first 30 minutes of reperfusion.

when muscles were stimulated during anoxia or reperfusion, the stimulation was switched off intermittently for approximately 2 minutes to determine the resting heat rate and contraction-related heat rate. perfusion was continued until the resting heat rate and contraction-related heat rate were stable (1% to 2% difference) for 15 to 30 minutes.

paired observations of force and heat. in four experiments, three of which were successful, heat rate and force were measured simultaneously. muscles stimulated at 0.2 Hz, 20% above threshold intensity, were stretched until the force recorded was maximal. when
Comparison of Parameters Measured Before Anoxia and at the End of the Reoxygenation Period

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<tr>
<th></th>
<th>Before Anoxia</th>
<th>After Anoxia</th>
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<tbody>
<tr>
<td>Resting heat rate</td>
<td>8.7±1.1 (13)</td>
<td>8.2±0.9* (13)</td>
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<tr>
<td>Contraction-related heat rate</td>
<td>11.2±1.5 (4)</td>
<td>6.3±1.2* (13)</td>
</tr>
<tr>
<td>Force</td>
<td>39.6±6.5 (4)</td>
<td>29.5±7.4* (3)</td>
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<tr>
<td>Force/heart rate</td>
<td>3.7 (3)</td>
<td>4.6* (3)</td>
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Values are mean±SEM. The number of experiments is given in parentheses.

*P=NS and 1P=.052 vs values before anoxia.

The force and heat rate signal were stable, muscles were made anoxic for 40 minutes and stimulated at 1 Hz. Subsequently, the preparations were reoxygenated while stimulated at 0.2 Hz until the force and heat rate were stable (less than 2% change) over 15 to 30 minutes. At the end of all experiments, muscles were moved into the mounting chamber; the diameter was measured; and after cutting off the dead tissue, the muscles were air-dried for 24 to 36 hours. The dry weight of the tissue was then determined.

Analysis and Statistics

Heat rate is expressed in milliwatts per gram dry weight. Force is expressed in millinewtons per square millimeter. Results are expressed as mean±SEM. Statistical analysis was performed with Student’s t test for paired or unpaired observations. Values were considered to be significant at P<.05.

Results

To determine the stability of the rate of heat production by quiescent papillary muscles in this new experimental setup, we monitored resting heat rate in three experiments over a period of 6 hours. Changes were less than 5% during that time and did not vary in any consistent pattern. Thus, the preparations showed a stable behavior similar to that reported by Daut and Elzinga for rat cardiac trabeculae at 37°C. The magnitude of resting heat rate, as determined from 13 other preparations, was 8.7±1.1 mW·g dry wt⁻¹ (see the Table). This value is not very different from that obtained for resting heat rate of the same preparation in studies on thermopiles at corresponding temperatures. For instance, Gibbs reported a value of 1.83 mW·g wet wt⁻¹, whereas Mast and Elzinga found 1.78 mW·g wet wt⁻¹ (wet weight to dry weight ratio, 4.55).

In oxygenated heart muscle, heat produced in the resting state corresponds to the energy liberated by the oxidation of substrate to CO₂ and water. In anoxia, this reaction does not, per definition, contribute to the heat produced. From the recording of a muscle kept at rest during anoxia (Fig 3, top), it can be seen that resting heat rate diminishes, steeply at first and very slowly thereafter, but does not stop during the anoxic period. In Fig 4, the average behavior during the anoxic period (ie, from 0 to 40 minutes) on resting muscles can be seen. Fig 4 also presents average data obtained during reoxygenation and from stimulated muscles to be discussed below.

Fig 4 shows a comparison of the resting heat rate during anoxia produced by quiescent muscles and by muscles stimulated at 1 Hz. Early in anoxia, a rapid drop occurred in both groups; thereafter, heat production continued rather steadily for the remainder of the anoxic period. The final anoxic value for resting heat rate differed between muscles kept at rest or stimulated at 1 Hz during anoxia (P<.05). The levels were 38% and 50%, respectively, of that found before anoxia. A similar (50%) decrease in resting heat rate was measured on thermopiles by Holubarsch et al in muscles stimulated at 0.2 Hz. To obtain insight into the nature of the heat-producing reactions in anoxia (see “Discussion”), we estimated all the resting heat produced during that period for the five muscles not stimulated over 40 minutes of anoxia. It was 9.7±0.5 J·g dry wt⁻¹. In contrast to the resting heat, total heat output in muscles stimulated at 1 Hz during the anoxic phase rose steeply at the beginning of anoxia (Fig 3, bottom) because of the concomitant increase in stimulus frequency from 0.2 to 1 Hz.

On reoxygenation, resting heat rate increased in all muscles to a level above the preanoxic value and subsequently returned gradually to the control level (Figs 4 and 5). This overshoot in resting heat was much larger in muscles stimulated at 1 Hz during the anoxic period (Figs 3, 4, 5A, and 5B) than in muscles not stimulated during anoxia (Figs 3, 4, 5C, and 5D).

During reoxygenation, all muscles were stimulated at 0.2 Hz. To see whether contractile activity during reoxygenation influenced the overshoot in resting heat rate during reoxygenation, we also measured resting heat rate in muscles when stimulation was switched off for the first 30 minutes of reoxygenation. The data obtained are shown in Fig 5, where heat rate is expressed relative to the preanoxic value. Comparison of Fig 5A and Fig 5B reveals that there was no effect of stimulation during the first half hour of reoxygenation on heat rate in muscles stimulated at 1 Hz during the anoxic period. For muscles kept at rest during anoxia (Fig 5C and 5D), however, it could not be excluded that the peak of the overshoot of the resting heat rate occurred somewhat earlier in the three muscles that were not stimulated for the first 30 minutes of reoxygenation (Fig 5D compared with Fig 5C).

For all muscles, the amount of excess heat, ie, the area underneath the heat rate curve above the preanoxic resting heat rate level, was determined. The values for the experiments shown in Fig 5A and 5B covered the same range, as did the values for the experiments shown in Fig 5C and 5D. These comparisons strengthened the idea that contraction during the reoxygenation phase had little, if any, effect on the excess resting heat occurring with reoxygenation. This allowed us to combine the data of Fig 5A with 5B and that of Fig 5C with 5D to construct the average reoxygenation curves (Fig 4).

These averaged data show that, in muscles not stimulated during anoxia, resting heat rate on reoxygenation increases after 15 minutes to 1.34 times the preanoxic level. Subsequently, it returns to the preanoxic value in approximately 1 hour. In muscles stimulated at 1 Hz during anoxia, resting heat rate increases on reoxygenation to 3.7 times the preanoxic value. This maximum is also reached approximately 15 minutes after the onset, but in this case, it takes approximately 150 minutes before the preanoxic level is reattained. The
Fig 3. Top, Recording shows resting heat rate of rabbit papillary muscle during 40 minutes of anoxia and after reoxygenation. During the control period, contraction-related (0.2 Hz) heat rate is also shown. At the beginning of anoxia, stimulation is switched off; it remains off at the start of reoxygenation. The broken line is the reference potential. Bottom, Recording shows resting heat rate and contraction-related heat rate during a representative experiment. A shows resting heat rate and contraction-related (0.2-Hz stimulation) heat rate at the start of the experiment. When the stimulus frequency is increased to 1 Hz and anoxia is started, there is a rapid increase in heat rate. B shows an increase in resting heat rate during reoxygenation. The muscle was stimulated at 0.2 Hz during reoxygenation. Note that when stimulation was stopped 5 minutes after anoxia, a drop in potential was hardly visible, making measurement of contraction-related heat rate impossible. C and D show the resting heat rate measured 90 and 270 minutes after the start of the experiment with the muscle moved out of the measuring chamber. The resting heat rate was still relatively high after 50 minutes of reoxygenation (90 minutes, C) but returned to the preanoxic level by the end of the experiment (D). The broken line representing the reference potential in this experiment was based on measurements at 0, 90, 120, 180, and 270 minutes. The reference potential at 90 came out somewhat above the line, but all others were, in that respect, similar to the measurement at 270 minutes (see "Materials and Methods").

total amount of heat produced under the overshoot was for muscles kept unstimulated during the anoxic period (3.5±1.2 J · g dry wt⁻¹) and for muscles stimulated at 1 Hz (51 J · g dry wt⁻¹).

In a previous study describing the changes in force production during anoxia and reoxygenation (no heat data), we found that, in rabbit papillary muscles stimulated at 1 Hz during 40 minutes of anoxia at 20°C, passive force increased toward the end of the anoxic period, whereas active force development by electrical stimulation stopped altogether. We present reanalyzed force data from our study in Fig 6A and 6B. Fig 6A, presenting the force data of muscles stimulated at 1 Hz during anoxia, reveals that in these muscles reoxygenation caused an initial rapid drop of passive force. In our earlier study, we therefore concluded that the increase in passive force during the anoxic phase was due to rigor, whereas the subsequent increase and gradual decrease in passive force thereafter reflected a rise and fall in cytosolic calcium. Muscles kept at rest during the 40 minutes of anoxia did not show any change in passive force during anoxia or reoxygenation (Fig 6B). When the time course of change in passive force during reoxygenation (Fig 6A) is compared (Fig 6C) with that of the heat overshoot during reoxygenation (compare with Fig 4), the similarity in time course of the two variables is striking.

One muscle stimulated at 1 Hz during anoxia, to investigate whether or not the energy cost of active force production during reoxygenation differs from the preanoxic value, we compared contraction-related heat rate during reoxygenation with active force development. The force data were obtained from the same study as used for Fig 6. The result of this analysis is
Resting Heat Produced in Anoxia

The total amount of heat produced by the muscles kept at rest during 40 minutes of anoxia was $9.7 \pm 0.5$ J · g dry wt $^{-1}$. In muscles stimulated during anoxia, this integrated value was not determined, because resting heat was only measured at intervals. However, the data presented in Fig 4 indicate that the total amount of resting heat these muscles produced was approximately $50/38 \times 9.7 = 12.8$ J · g dry wt $^{-1}$.

In rabbit papillary muscle at 20°C, ATP turnover in resting anoxic muscles was 21% of the value in oxygen.$^3$ Oxidative ATP formation could no longer occur in anoxia, but the energy required to drive basal metabolism was still derived from ATP. The chemical reactions involved in the hydrolysis and formation of ATP together with their respective molar enthalpies are given in the Appendix. Using these molar enthalpy values, it is possible to calculate the heat produced by these reactions provided that the extent of each of them is known. In an earlier study,$^3$ we measured the extent of these reactions over 40 minutes. The amount of heat from these reactions in anoxic quiescent muscle was predicted to be $10.5 \pm 1.4$ J · g dry wt $^{-1}$, which is not significantly different from the measured value. This implies that the heat produced in resting muscles during anoxia can be accounted for by the reactions coupled to hydrolysis and formation of ATP.

It is not possible to do the same analysis for the resting heat produced by muscles stimulated at 1 Hz during the anoxic period because the changes in nucleotides and the formation of lactate as reported earlier$^3$ hold for all ATP-hydrolyzing processes, including activation and contraction. Fig 4 shows that resting heat rate is higher in stimulated than in unstimulated muscles. We found in earlier studies that at the end of anoxia lactate formation has stopped in 1-Hz-stimulated muscles,$^4$ whereas the overall ATPase activity is, if anything, lower than in quiescent muscles.$^2$ This may indicate that in 1-Hz-stimulated muscles, some other process, which does not hydrolyze ATP, contributes to the heat produced. Dissipation of ion gradients could be such a process.

**Overshoot in Resting Heat During Reoxygenation**

When anoxic muscles are reoxygenated after 40 minutes of anoxia, oxidative metabolism starts immediately. This is illustrated by the steep drop in passive force at the onset of reoxygenation in muscles stimulated at 1 Hz during anoxia (Fig 6A), reflecting the detachment of rigor bonds because of the formation of ATP. Also, the finding that, after approximately 12.5 minutes, PCr levels are almost fully restored$^2$ provides further evidence.

At least two processes requiring the ATP formed after the onset of reoxygenation should be considered in an explanation of the heat overshoot: (1) restoration of nucleotides and PCr to the preanoxic levels and (2) ATP hydrolysis due to contracture. In addition, restoration of dissipated ion gradients and other less-well-defined processes that have suffered from the lack of ATP during anoxia may play a role.

**Muscles Kept at Rest During Anoxia**

When muscles kept at rest during 40 minutes of anoxia were reoxygenated, developed force recovered fully and rapidly (Fig 6B); no increase in passive force occurred.$^3$ This indicates that during reoxygenation the ionic gradients are not disturbed to such a degree that cytosolic calcium rises to contractile levels and...
FIG 5. Graphs showing resting heat rate, relative to the preanoxic value, in rabbit papillary muscle after 40 minutes of anoxia. Each symbol represents a different muscle studied. A, Muscles (n=5) were stimulated at 1 Hz during anoxia and at 0.2 Hz during reoxygenation. B, Muscles (n=3) were stimulated at 1 Hz during anoxia; stimulation was off during the first 30 minutes of reoxygenation; thereafter, muscles were stimulated at 0.2 Hz. C, Muscles (n=2) were not stimulated during anoxia but were stimulated at 0.2 Hz during reoxygenation. D, Muscles (n=3) were not stimulated during anoxia and the first 30 minutes of reoxygenation; thereafter, muscles were stimulated at 0.2 Hz.

thus that no ATP hydrolysis (and thus formation) is needed to support such a contracture.

The heat produced to restore nucleotides and PCr to the preanoxic levels by oxidative phosphorylation contains two components: endothermic and exothermic. An endothermic effect is to be expected from the restoration of the pool of energetic compounds, whereas the oxidation of glucose is an exothermic reaction yielding 470 kJ·mol⁻¹ of oxygen. The calculation of the endothermic component requires information about the difference in the levels of the various compounds involved in control muscles, fully reoxygenated muscles, and preparations kept anoxic for 40 minutes. These data are available from earlier studies.² ³ The equations given in the Appendix (except Equation 5) can be used; it is assumed that no glycolysis formation from lactate occurs during the period considered. Calculation of the exothermic component requires, apart from the value of the molar enthalpy change, information² ³ about the amount of ATP needed to restore nucleotides and PCr to the preanoxic levels and to bring about glycosy formation from glucose (approximately 20% of the glycogen is resynthesized). Following this approach, we calculated an overshoot in the heat produced of 4.1±0.6 J·g dry wt⁻¹. This value is not significantly different from the measured one (3.5±1.2 J·g dry wt⁻¹), which suggests that for muscles kept at rest during anoxia the overshoot in heat produced during reoxygenation is due to the restoration of nucleotide and PCr levels.

Muscles Stimulated at 1 Hz During Anoxia

Reoxygenation of muscles stimulated at 1 Hz during 40 minutes of anoxia caused an elevation of resting heat rate for up to 150 minutes, yielding 51 J·g dry wt⁻¹ in excess of the normal resting heat (Fig 4). This is more than 10 times that produced by quiescent muscles in anoxia during the same phase. Consequently, restoration of nucleotide and PCr levels can explain only the smaller part thereof. Applying the same calculation as above for the resting muscles but using the appropriate biochemical data,² ³ we estimated that recovery of the metabolite levels produced 3.32 J·g dry wt⁻¹ in these
muscles. The value is lower than that for muscles kept at rest during anoxia because nucleotides are lost in these muscles.

Fig 6C shows that the time course of the changes in passive force and of the heat overshoot are approximately the same, suggesting that heat rate and passive force are causally related. To find out if the excess heat can be explained quantitatively by the increased passive force, we estimated the energy cost of force production from normal twitch contractions. Such an estimate requires assumptions about how the force energy ratio during a twitch compares with the force energy ratio during a contracture.

Experiments from isolated papillary muscle19 and whole ventricle20 indicate that energy turnover related to contraction is fully determined by processes occurring.
before peak force or peak isovolumic pressure. This suggests the following approach: to divide the stress integral underneath the passive force curve (Fig 6C) by the stress integral underneath the rising phase of the twitch, rather than underneath the full twitch, and to multiply that ratio by the heat produced by a twitch contraction. Following that approach, a value of 53.6 J·g dry wt⁻¹ was obtained. This estimate, in combination with the similarity in time course of passive force and resting heat rate (Fig 6C), suggests that most of the excess heat is force related.

In whole hearts at 37°C, Ohgoshi et al.¹⁵ measured no increase in resting oxygen consumption in potassium-arrested hearts 60 minutes after 15 minutes of normothermic global ischemia. Laster et al.¹⁶ measured no increase in resting oxygen consumption in potassium-arrested hearts 10 minutes after 20 minutes of ischemia. At this point in time, resting heat rate was at its highest in our preparations.

Assuming that no contracture developed in these whole-heart studies and thus that the severity of the ischemic period would be more or less comparable to that of our resting muscles, these experimental results of Ohgoshi et al.¹⁵ and Laster et al.¹⁶ are not necessarily in disagreement with the present findings. The Q₁₀ of oxidation is 2.4.²¹ This implies that oxidation-driven recovery processes occur almost six times faster at body temperature. Thus, at body temperature the extra energy turnover to restore the nucleotides and PCR to their preanoxic levels is already complete within 10 minutes.

The decay following the overshoot in resting heat rate during reoxygenation is reminiscent of the decay in heat production of isolated heart muscle on thermopiles during the first 2 to 4 hours after cardiectomy.²² The magnitude of the resting heat rate determined in the present study was comparable with the resting heat rate measured on thermopiles after this decay (see "Results"). The reason that resting heat decays on the thermopile during the first hours after cardiectomy is not known. The present experiments show that, when muscle is reoxygenated after a period of anoxia, a large overshoot that returns to the baseline over a period of 2.5 hours may occur (Fig 5). When a heart is taken out to isolate a papillary muscle, the heart becomes transiently ischemic. This suggests that this transient period of ischemia underlies the decay in resting heat rate after cardiectomy.

**Contraction-Related Heat Rate During Reoxygenation**

In our experiments, the ratio of contraction-related heat rate and force during the reoxygenation phase is similar to that before anoxia. This finding differs from that of Laster et al.¹⁶ and Ohgoshi et al.¹⁵ They claim that, in whole heart, stunning may cause energetic abnormalities in contraction-related processes. Ohgoshi et al, studying relations of myocardial oxygen consumption in excised cross-circulated dog heart, found an increase in pressure-volume-area-independent oxygen consumption and, since resting metabolism was not different from control, concluded that the energy cost of activation was increased. This difference with our re-
Fig 8. Graph showing paired (●) and unpaired (○, △, and ◇) heat rate and force data of Fig 7 plotted against one another. The drawn line is obtained when the fall in contraction-related heat rate found at the onset of reoxygenation and its subsequent increase with time are the results of changes in myofibrillar ATPase only. The dashed line indicates that these changes in heat rate are due to changes in myofibrillar ATPase as well as sarcoplasmic reticulum ATPase. The data favor the latter explanation.

Results may suggest that the time course and cascade of cellular responses in cardiac myocytes after anoxia and reoxygenation is different at body temperature. This is not unexpected in view of the complexity of these processes and the variety of Q10 values involved.

Although we are unable to explain this difference in more detail, the present findings at 20°C are of interest in their own right because it has been shown for isolated papillary muscle at similar temperatures11,12 that the amplitudes of the cytosolic calcium transients are considerably increased during reoxygenation. A possible explanation of this phenomenon is that calcium release, and consequently calcium uptake, by the sarcoplasmic reticulum is increased. However, the same finding can be interpreted as evidence that the binding of calcium to the contractile system is severely depressed.

Although we did not measure activation heat, which reflects the ATP hydrolysis required for calcium uptake by the sarcoplasmic reticulum, our energetics findings presented in Fig 7 favor the latter explanation. It is generally accepted that activation heat is, under normal circumstances, responsible for approximately 30% of the contraction-related heat production. By taking the data of Fig 7 and plotting percent heat rate recovery against the percent recovery of force, the relation presented in Fig 8 is obtained. The solid line drawn in Fig 8 is obtained when, during the whole reoxygenation period, contraction-related calcium uptake equals the preanoxic value. In that case, the changes in heat rate and force during the reoxygenation period (Fig 7) are fully due to changes in myofibrillar ATPase. All observed data, paired and unpaired, are clearly below the solid line. Therefore, it is concluded that the lower heat rate found at the onset of reoxygenation and the subsequent increase cannot be explained solely by changes in myofibrillar ATPase; the sarcoplasmic reticulum ATPase contributes as well. This conclusion strongly supports the view that the increased cytosolic calcium transients found during reoxygenation are not due to an increase in calcium cycling but to a decreased calcium binding. The dashed line in Fig 8 is obtained when it is assumed that during the reoxygenation period the myofibrillar ATPase and the sarcoplasmic reticulum ATPase vary in proportion with the force produced. Although a relative difference in position of the paired and unpaired data cannot be excluded, the data in Fig 8 scatter around the dashed line. This demonstrates graphically that the economy of contraction is not different before and after anoxia. Consequently, it is likely that calcium cycling is in fact depressed during reoxygenation. This strengthens the above view that the increased calcium transients during reoxygenation11,12 result from a decrease in calcium binding.

Appendix

From the lactate formed and the change in nucleotides reported previously,3 the heat produced by quiescent muscles during 40 minutes of anoxia was calculated from the following reactions11:

1. ATP → ADP + Pi, −48 kJ · mol⁻¹
2. PCr → Cr + Pi, −34 kJ · mol⁻¹
3. 2ADP → ATP + AMP, −2.4 kJ · mol⁻¹
4. AMP → IMP + NH₃, −2.2 kJ · mol⁻¹
5. 0.5 glycosyl-lactate → H⁺, −95 kJ · mol⁻¹

where Pi is inorganic phosphate and Cr is creatine.

The heat (h) produced by the individual reactions was calculated from the following: h = nᵢΔHᵢ, where nᵢ is the number of moles of the reaction i, and ΔHᵢ is the enthalpy change per mole of reaction.

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