Po₂-modulated performance of cardiac muscle

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FREZZA, W. A., AND O. H. L. BING. Po₂-modulated performance of cardiac muscle. Am. J. Physiol. 231(5): 1620-1624. 1976.—An inverse linear relationship between normalized tension development (T/mm²) and muscle cross-sectional area (range 0.32-1.68 mm²) is seen in fully oxygenated rat papillary and columnar carneaæ muscles studied while contracting isometrically at the apex of the length-tension curve. The data demonstrate progressively poorer performance with thicker preparations, presumably due to core hypoxia. However, when iodoacetate 10⁻⁴ M (glycolytic blockade) is added to fully oxygenated muscle preparations, no significant change in performance is seen even with the thickest preparations, suggesting that no portion of mechanical activity is supported by anaerobic glycolysis. With progressive lowering of the muscle bath Po₂, the relative contributions of aerobic and glycolytic activity to mechanical performance are demonstrated. Viewed from the Hill model of oxygen and lactic acid distribution, the data suggest the presence of a hypoxic core appear contrary to the evidence that indicates the absence of tension supported by glycolytic activity. A possible solution to this apparent contradiction is presented. The findings of these experiments emphasize limitations of isolated muscle studies and help define the relationship between oxygenation and mechanical activity of cardiac muscle.

Methods

The hearts of freshly decapitated rats were rapidly removed and placed in Krebs-Henseleit solution (8) bubbled through with 95% O₂ and 5% CO₂. The composition of this solution, in millimoles per liter, was: NaCl, 118.5; KCl, 4.69; CaCl₂, 2.52; MgSO₄, 1.16; KH₂PO₄, 1.16; NaHCO₃, 24.88; and glucose, 5.50. Left ventricular papillary and columnar carneaæ muscles were carefully dissected free and mounted between two spring clips in an isolated muscle chamber in which the solution was maintained at 28°C by a Lauda type K2 controlled-temperature circulating pump.

The upper clip was attached by a thin gold chain to a rigid magnesium lever arm, above which a micromanometer stop was placed so that the resting length of the muscle could be carefully adjusted. The lower spring clip was connected to a Statham G'?B-0.75-350 force transducer by a 0.015-inch diameter tungsten wire that passed through a mercury seal on the bottom of the chamber.

The Po₂ of the solution varied between 550 and 600 mmHg, the pH was 7.40, and the Pco₂ was 40 mmHg. The latter two measurements remained constant throughout the experiments. The muscles were stimulated 12 times per minute by a Grass model S-88 stimulator delivering 10-msec square wave pulses through parallel platinum electrodes at voltages that were 10% above the minimum required to produce a maximum mechanical response. After a 30-min equilibration period, the muscles were stretched to the apices of their length-tension curves. After an additional 15-min period, the preparations were subjected to hypoxia by reducing and equilibrating the bath Po₂ in three successive 15-min steps. This was accomplished by changing the oxygen content in the circulating gas mixture from 95% to 75%, 50, and then to 25%. The CO₂ was held constant, while N₂ was substituted for O₂. Bath Po₂, Pco₂, and pH were monitored with an Instrumentation Laboratory, Inc. model 113 gas analyzer. In experiments where glycolysis was blocked by addition of iodoacetate, 10⁻⁴ M, another 15 min were allowed to elapse prior to the institution of hypoxia. Muscle preparations in which mechanical performance was not completely stable prior to hypoxia were discarded. The in vitro length of each muscle was measured at the apex of its length-tension curve with a Gaertner Scientific catheterometer-telescope combination. At the end of each experiment, the muscle between the spring clips was blotted and weighed. Cross-sectional area was calculated based on the assumption of cylindrical uniformity and a specific gravity of 1.00. Absolute values for isometric tension (T) were calculated, and changes during hypoxia are expressed as a percentage of prehypoxia control values.

Although valuable insights into the intrinsic mechanical properties of muscle have been achieved with use of the isolated muscle preparation, questions have been raised as to whether these preparations are uniformly oxygenated. This concern has been emphasized by the presence of an inverse relationship between normalized performance and muscle cross-sectional area (2, 5), which has led previous investigators to suspect the presence of hypoxia in the core in thicker preparations. For these reasons, the thinnest muscles are selected and experiments carried out at relatively low temperatures and stimulation rates. In an effort to further assess the question of hypoxia in isolated muscle studies, the relationship between mechanical performance and muscle thickness was studied in preparations with graded hypoxia and glycolytic blockade.
RESULTS

Relationship between muscle cross-sectional area and developed tension. Experiments were carried out using 86 rat left ventricular papillary and trabecular muscles to determine the relationship between cross-sectional area (A) and normalized peak isometric tension development (T/mm²). Data from both papillary and trabecular muscles were pooled because of insignificant differences in performance (10, and Bing, unpublished observations). The muscle cross-sectional areas were collected in bins of 0.1 mm² and plotted against the corresponding mean T/mm² of the group (Fig. 1). For example, preparations with a cross-sectional area of 0.9–1.0 mm² were grouped and the average T/mm², standard error of the mean, and number of muscles in that group plotted at the average cross-sectional area. An inverse relationship is observed, with thicker preparations producing less T/mm² than thinner ones. This relationship has also been observed by others (2, 5). Linear regression analysis of the raw data yields T/mm² (g) = 9.20 – 3.20 A, with a correlation coefficient of r = 0.6, reflecting a relatively large variation in the data.

Effect of hypoxia and glycolytic blockade. When 10⁻⁴ M iodoacetic acid was added under oxygenated conditions, no significant changes in T/mm² were seen in 11 preparations with cross-sectional areas varying from 0.72 to 1.76 mm² (Fig. 2). This observation is consistent with a previous study (1) and suggests that in isolated muscle preparations oxygenated with 95% O₂, no significant portion of isometric tension development is supported by anaerobic glycolysis.

Reduction in bath PO₂ for 12 preparations was associated with a linear decline in T/mm². The magnitude of this decline was, in general, inversely proportional to the cross-sectional area of the preparation (Fig. 3). Thus, thinner muscles tended to develop more T/mm² than thicker muscles but were affected to a greater extent by changes in PO₂. When these data were expressed as a percentage of prehypoxia control, however, the curves appeared superimposable. Average percentages at intervals of 50 mmHg PO₂ are depicted in Fig. 4. The same procedure for iodoacetate-treated muscles yields a curve that does not deviate significantly from control until the bath PO₂ is reduced below 450 mmHg. At this point, preparations with glycolytic blockade undergo a precipitous fall in T/mm². The anaerobic contribution to tension development, then, can be assessed by calculating the difference between the two curves at any given PO₂, as indicated in Table 1.

DISCUSSION

Hill, in 1929 (6), first solved the diffusion equations of oxygen and lactic acid through tissue, which consumes the former and, in the absence of oxygen, produces the latter. Basic to his derivations is the assumption that the oxygen consumption of each cell is constant and
independent of the local Po$_2$. This assumption appears to be valid for skeletal muscle (7) and has become generally accepted for cardiac muscle as well.

A profile for oxygen and lactic acid distribution within a cylindrical muscle is depicted diagrammatically in Fig. 5 (top). As the surface oxygen concentration falls below a critical concentration, defined as the minimum surface concentration necessary to oxygenate the center of a muscle, an anaerobic core appears that produces lactic acid. Lactic acid diffuses outward and is metabolized when it reaches the oxygenated zone.

Hill defines the critical radius as the radius of the largest muscle that will have an oxygenated center. This value has become an important parameter in determining the largest preparations that can be used for isolated muscle studies without the appearance of artifact due to a hypoxic core. A number of estimates have been made for its value in mammalian cardiac muscle (4, 5, 9, 10). It is generally assumed that muscle preparations studied are smaller than the critical radius, oxygenated throughout, and develop tension uniformly. This is the rationale for the normalization of experimental data for cross-sectional area. Preparations that are forced to contract anaerobically will presumably draw on glycogen stores until these are depleted or consume glucose in the bath solution, but will develop only a fraction of the force of an aerobic muscle preparation.

As a first approximation, for muscles larger than the critical radius a tension-density profile can be constructed that consists of two zones: an anaerobic core whose function is supported by glycolysis, and which only produces a fraction of the tension present under aerobic conditions, and a surrounding aerobic sleeve that uniformly consumes oxygen diffusing inward from the surface and contracts homogeneously with greater force than the anaerobic core. One can represent the tension developed from a tension-density profile (Fig. 5, bottom) by integration of the aerobic and anaerobic cross-sectional areas of the muscle preparation.

When normalized tension is plotted against muscle cross-sectional area, as carried out in Fig. 1, a plateau of constant T/mm$^2$ is predicted for muscles smaller than the critical radius. We have not observed, nor have previous studies reported, a plateau or zone of constant tension development over the range of muscle cross-sectional areas we have studied. These observations suggest that all of the muscles studied are larger than the critical radius and must contain anaerobic cores. The reduction in T/mm$^2$ in larger muscles might then be attributed to the anaerobic core of these seemingly non-physiological preparations. If glycolysis is blocked, a large fall in tension would be predicted in thicker muscles, with a less prominent fall in tension in thinner preparations. We have found, however, that under oxygenated conditions isometric tension does not change significantly after addition of iodoacetate to muscles of any cross-sectional area (Fig. 2). A paradox seems to exist between the necessity of predicting an anaerobic core for larger muscles and data suggesting the absence of tension supported by anaerobic glycolysis.

![Figure 4](http://ajplegacy.physiology.org/Downloaded/)

**FIG. 4.** Data from Fig. 3 are averaged and expressed as a percent of prehypoxia control. Note that data points diverge at Po$_2$ < 450 mmHg. Aerobic and glycolytic contributions to mechanical performance have been calculated and are shown in Table 1. Brackets indicate mean ± SE.

![Figure 5](http://ajplegacy.physiology.org/Downloaded/)

**FIG. 5.** Top: diagram of distribution of oxygen and lactic acid in a cylinder of muscle (5). Bottom: diagram of a corresponding tension-density profile for this muscle.

### TABLE 1. Assessment of aerobic and anaerobic contribution to tension development

<table>
<thead>
<tr>
<th>Po$_2$, mmHg</th>
<th>Developed tension, g/mm$^2$</th>
<th>Aerobic contribution, %</th>
<th>Anaerobic contribution, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>550</td>
<td>4.08 ± 0.73</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>3.85 ± 0.65</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>450</td>
<td>3.68 ± 0.62</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>3.48 ± 0.58</td>
<td>86.0</td>
<td>14.0</td>
</tr>
<tr>
<td>350</td>
<td>3.28 ± 0.54</td>
<td>67.6</td>
<td>32.4</td>
</tr>
<tr>
<td>300</td>
<td>3.07 ± 0.50</td>
<td>52.6</td>
<td>47.4</td>
</tr>
<tr>
<td>250</td>
<td>2.81 ± 0.47</td>
<td>41.9</td>
<td>58.1</td>
</tr>
<tr>
<td>200</td>
<td>2.38 ± 0.46</td>
<td>30.9</td>
<td>69.1</td>
</tr>
</tbody>
</table>

Values are means ± SE.
PO_2 AND CARDIAC MUSCLE

While a number of interpretations might explain these observations, an attractive and simple solution is to consider that cellular oxygen consumption varies with local PO_2 and is not constant, as in Hill’s original simplifying assumption. A similar suggestion has been offered by Whalen et al. (11). Oxygen concentration at any point within the muscle is related through a time-independent diffusion equation to oxygen consumption, the muscle radius, and the diffusion length. If we assume that oxygen consumption varies with local PO_2, the distribution of oxygen within the muscle would be the same as Hill predicted at all points where a saturating concentration is reached, but between the point at which the oxygen concentration first falls below a saturation value and the center of the muscle the local oxygen concentration will be higher than in the Hill model, as less would be consumed in transit. Thus, oxygen would penetrate further than previously expected, and glycolysis would not play a major role in supporting tension until the local PO_2 fell below a level necessary to sustain oxidative metabolism. This three-zone profile contains a core supported by anaerobic glycolysis and an outer sleeve of homogeneous tension development, as in the two-zone model, but further includes an intermediate zone of graded response where tension density is proportional to local PO_2.

Tension-density profiles for two muscles of differing thickness at varying PO_2 are graphically depicted in Fig. 6. In the control state there is no anaerobic core, which is consistent with the observations made in the case of blocked glycolysis, yet an inverse relationship between T/mm^2 and cross-sectional area is still predicted because the thicker muscle would have a larger zone of reduced performance.

The concept of PO_2-modulated oxygen consumption at the cellular level in no way contradicts studies of isolated mitochondria which demonstrate that oxygen consumption is constant to less than 1 mmHg PO_2 (3). The relationship between PO_2 and oxygen consumption in the intact cell is a function not only of mitochondrial enzyme kinetics but also the kinetics of oxygen distribution within each cell. There must be a PO_2 gradient within each muscle cell, and the cell surface PO_2 at which the mitochondria become oxygen-deprived may be much higher than the PO_2 at the mitochondria. The amount of this difference will depend on the morphology of the cell, the distribution of mitochondria within the cell, and the diffusion constant for oxygen in the cytoplasm.

In terms of studies using isolated muscle preparations, this experiment emphasizes that the practice of normalizing data from preparations of varying thickness for cross-sectional area is not valid. The most meaningful data are obtained when the performance of preparations of similar cross-sectional area is compared. Nonetheless, we should consider that the differences in performance of even these properly selected preparations may also relate to differences in diffusion of oxygen or oxygen consumption, in addition to other intrinsic properties of the muscle.

If cardiac muscle does have the ability to alter its function in accord with the availability of oxygen, several implications are suggested. Local abnormalities of myocardial contraction associated with coronary artery disease are frequently not accompanied by symptoms or metabolic evidence of ischemia. These symptoms can be evoked, however, by further reducing coronary flow or increasing the demand of the myocardium. Thus, myocardial ischemia may conceivably exist in two forms. In the first form both the function and requirements of the tissue are reduced so that there is no imbalance between supply and demand. In the second, the muscle is forced to function despite an inadequacy of flow, and demand exceeds supply, causing the muscle to rely on anaerobic metabolism.

This concept of PO_2-regulated performance may explain the common finding of myocardial dyskinesis in the absence of scar and clinical or metabolic evidence of ischemia. Further, it is of interest to speculate that oxygen-modulated performance may serve a protective function by preventing injury to local areas of ischemic myocardium whose contraction is not essential for total cardiac function.

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