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INTRACELLULAR O_2 GRADIENTS IN CARDIAC MYOCYTES. LACK OF A ROLE FOR MYOGLOBIN IN FACILITATION OF INTRACELLULAR O_2 DIFFUSION

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The P_{50} for oxygenation of myoglobin in intact cells was very high relative to that for isolated myoglobin, and was changed by addition of agents that altered respiratory rate. The P_{50} for cytochrome a oxidation in cells was very high relative to that for isolated mitochondria, but was unaffected by oxidation of myoglobin to metmyoglobin. These results demonstrate the existence of a substantial intracellular O_2 gradient in myocytes and indicate that myoglobin does not have a significant role in facilitation of O_2 diffusion to mitochondria.

INTRODUCTION

Oxygen delivery to mitochondria is of critical importance in maintenance of energy-dependent functions in heart cells. Both the high concentration of myoglobin (Mb) in these cells and the ability of Mb to increase total O_2 flux through solution by providing another O_2-diffusing species (MbO_2) have led to the postulate that it facilitates intracellular O_2 diffusion (1). However, experiments to test this have been inconclusive, and in some cases contradictory (2,3).

In recent studies we observed that a substantial intracellular O_2 gradient exists in isolated rat liver cells (4,5), and Tamura et al. (6) found that a substantial O_2 gradient exists between Mb and the mitochondrial inner membrane in perfused rat heart. These results tend to underscore the apparent desirability of facilitation of O_2 diffusion, yet do not establish that Mb has this role.

In the current studies, we have used dual-wavelength spectroscopy to measure the O_2 dependence of Mb and cytochrome a in isolated rat cardiac

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myocytes. The results show that a substantial O_2 gradient exists from the extracellular space to the space occupied by Mb as well as between Mb and cytochrome oxidase. In addition, oxidation of Mb to metmyoglobin does not alter the O_2 dependence of cytochrome a and therefore provides clear evidence that Mb does not have a significant role in facilitation of intracellular O_2 diffusion under these conditions.

METHODS AND MATERIALS

Isolated myocytes were obtained from male white rats (Charles Rivers, 250-350g, fed ad libitum) essentially by the method of Kao et al. (7). Cells were 80-92% viable as judged by trypan blue (0.2%) exclusion. Approximately 90% of the viable cells were rod-shaped. Cells were quiescent and tolerated physiological levels of Ca^{2+}. Myoglobin content was 6.6-7.1 nmol/10^6 cells. Incubations were performed in modified Krebs-Henseleit buffer composed in mM of NaCl, 102; KCl, 4.2; KH_2PO_4, 0.87; $MgSO_4$, 1; $NaHCO_3$, 20.9; Hepes, 11; creatine, 20; taurine, 30; glucose, 10; and plasma levels of 20 amino acids (8), and adjusted to pH 7.4.

Dual-wavelength spectroscopy was performed with a Aminco DW2A spectrophotometer equipped with a 3.9 cm light-path incubation vessel as previously described (9). Cytochrome a oxidation was measured at 605-630 nm and myoglobin oxygenation was measured at 581-590 nm. Cells (10^5/ml) were maintained in suspension by gentle magnetic stirring under an atmosphere of prepurified argon. An O_2 electrode (Clark-type, Yellow Springs Instruments, Yellow Springs, Ohio) was inserted through the cover of the vessel to provide a measure of solution O_2 concentration and was calibrated by additions of known volumes of O_2 saturated H_2O. O_2-dependence curves were obtained by addition of O_2-saturated modified Krebs-Henseleit buffer. Complete oxidation and oxygenation were obtained by changing the gaseous phase to 100% O_2. Incubation temperatures were 28-30°. Analysis of the time course of spectral change under these conditions relative to spectral changes associated with steady-state changes in gaseous pO_2 indicated that measurements were obtained under near-steady-state conditions.

RESULTS

The P_{50} value for oxygenation of Mb in control cells was 8.9 μM (Fig. 1). This value is much higher than that for isolated Mb [approx. 2.2 μM at 30° (10)] and suggests that a respiration-dependent gradient of O_2 concentration exists in these cells. Addition of 1.1 μM antimycin A, a concentration that gives 60-80% inhibition of O_2 consumption, resulted in a shift of the P_{50} (Fig. 1) to 2.8 μM, a value comparable to that for the isolated protein. Addition of 1 μM FCCP, a concentration that stimulates respiration 2.2 fold, resulted in a shift of the P_{50} (Fig. 1) to 21 μM. These results demonstrate that the intracellular oxygenation of Mb is

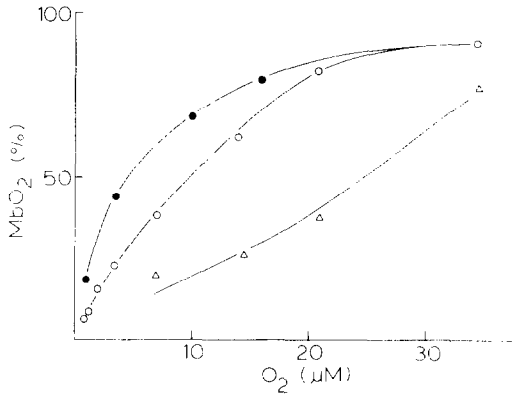

Figure 1. Oxygenation of myoglobin (581-590 nm) in isolated myocytes. Cells (10^5/ml) were incubated in Hepes-supplemented modified Krebs-Henseleit buffer, pH 7.4, under a steady stream of pre-purified argon. After cells became anaerobic, O_2 was introduced by injection of O_2-saturated buffer to give the concentrations indicated. Complete oxygenation was obtained by introduction of 100% O_2 in the gas phase. Control and antimycin A data are averages from 6 preparations. Control cells, ○; plus 1.1 μM antimycin A, ●; plus 1 μM FCCP, △.

dependent upon the O_2 consumption rate of the cells, apparently due to the establishment of an intracellular O_2 gradient.

Existence of such a gradient is a precondition to the function of myoglobin in facilitation of intracellular O_2 diffusion (1). If it has this function, the magnitude of the gradient must be enhanced by elimination of functional Mb. To test this, we examined the effect of oxidation of Mb upon the O_2 dependence of cytochrome a oxidation. Measurement of O_2 dependence of cytochrome a can be obtained directly in both absence and presence of Mb, since MbO_2 gives only a small spectral interference at 605-630 nm (6).

As previously found with isolated liver cells (5), half-maximal oxidation of cytochrome a in heart cells (Fig. 2) occurred at a much higher O_2 concentration than that for isolated mitochondria (6.0 μM vs 0.3 μM). This indicates that the O_2 gradient from the extracellular space into the mitochondrial inner membrane is substantially greater than the gradient to Mb and thus provides proper conditions to test for the function of Mb in facilitation of O_2 diffusion.

Oxidation of Mb to metmyoglobin was obtained by anaerobic addition of a single injection of H_2O_2 to give a final concentration of 70 μM. This treatment oxidized 90-100% of the Mb without loss of cell viability and

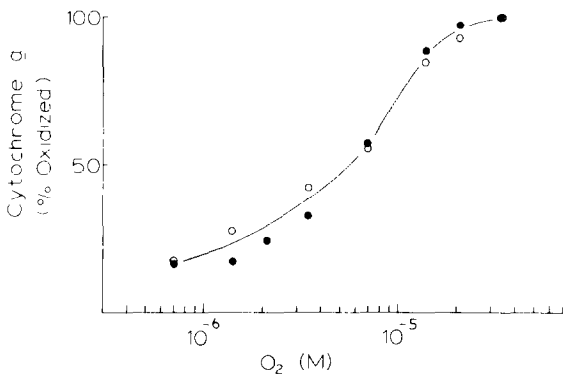

Figure 2. O_2 dependence of oxidation of cytochrome a (605-630 nm) in myocytes before (○) and after (●) oxidation of myoglobin to metmyoglobin. Incubations and O_2 additions were made as described in Fig. 1. Data are averages from 5 cell preparations for each condition.

without significant effect on cytochrome contents or respiratory rate. This treatment had no effect upon the O_2 dependence of cytochrome a oxidation (P_{50} = 5.8 μM; Fig. 2) and thus demonstrates that the presence of Mb does not alter the O_2 supply to mitochondria under these O_2-limiting conditions.

DISCUSSION

The results obtained here demonstrate that the intracellular O_2 gradient in respiring heart cells is substantial and that it occurs throughout the cell, i.e. from the extracellular space to the Mb as well as between the Mb and the mitochondrial inner membrane. The Mb, however, does not have a significant role in facilitation of O_2 supply to mitochondria under these conditions. Alternate functions (11), such as supplying a short-term beat-to-beat reservoir of O_2 must therefore be explored in greater detail.

In earlier studies on the function of Mb in the O_2 supply in skeletal muscle, Wittenberg et al. (2) found that agents, such as nitrite, that abolish the O_2-binding function of Mb result in diminished steady-state O_2 consumption by isolated bundles of muscle fibers at low pO_2. However, interpretation of these results is limited by the complexity of the experimental model. Oxygen diffusion into a tissue mass with a mean minimum cross section of 500 μm is very different than O_2 supply from

normal perfusion, in which the average diffusion distance is only about 10 μm (12). The tissue O_2 gradients in the bundles must be large, and the pattern of these gradients may be complicated by differences in diffusion coefficients between the extracellular and intracellular spaces within the bundles. The ratio of such diffusion coefficients has been estimated to be between 10 and 10^3 (13,14). In addition, there is no evidence provided that the bundles were free from hemoglobin, which might also contribute to the oxygenation characteristics. Subsequent experiments with fluorocarbon-perfused dog heart (3) failed to demonstrate any effect upon the O_2 dependence of O_2 consumption due to oxidation of Mb with nitrite and therefore are in agreement with the results reported here.

The existence of large intracellular O_2 gradients in both heart and liver cells (4,5) suggests that in mammalian tissues consisting of relatively large cells and with a high O_2 consumption rate, the total tissue O_2 gradient is composed of both intracellular and extracellular components. Since the capillary pO_2 must be sufficient to maintain the intracellular gradient and supply mitochondria with adequate O_2 for maximal respiration, it indicates that a relatively high venous pO_2 must be maintained under all respiratory conditions. Thus, the sigmoidal hemoglobin dissociation curve ensures that O_2 is delivered at a sufficiently high O_2 concentration for use by the cell. A linear dissociation curve at low O_2 concentrations would result in a larger fraction of O_2 bound in the erythrocyte at concentrations which would not be sufficient to maintain the intracellular O_2 gradient and support normal respiration.

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REFERENCES

1. Wittenberg, J.B. (1970) Physiol. Rev. 50, 559-636.
2. Wittenberg, B.A., Wittenberg, J.B., and Caldwell, P.R.B. (1975) J. Biol. Chem. 250, 9038-9043.
3. Cole, R.P., Wittenberg, B.A. and Caldwell, P.R.B. (1978) Am. J. Physiol. 234: H567- H572.

4. Jones, D.P. and Mason, H.S. (1978) J. Biol. Chem. 253, 4874-4880.
5. Jones, D.P., Mason, H.S., Nakase, Y. and Chance, B. (1977) Fed. Proc. 36, 566.
6. Tamura, M., Oshino, N., Chance, B. and Silver, I.A. (1978) Arch. Biochem. Biophys. 191, 8-22.
7. Kao, R.L., Christman, E.W., Luh, S.L., Krauhs, J.M., Tyers, G.F.O. and Williams, E.H. (1980) Arch. Biochem. Biophys. 203, 587-599.
8. Morgan, H.E., Earl, D.C.N., Broadus, A., Wolpert, F.B., Giger, K.E. and Jefferson, L.S. (1971) J. Biol. Chem. 216, 2152-2162.
9. Jones, D.P., Thor, H., Andersson, B., and Orrenius, S. (1978) J. Biol. Chem. 253, 6031-6037.
10. Antonini, E. and Brunori, M. (1971) Hemoglobin and Myoglobin in their reactions with ligands, pp. 219-234, North Holland Publishing Company, Amsterdam.
11. Millikan, G.A. (1939) Physiol. Rev. 19, 503-523.
12. Reivich, M. (1971) In: P.L. Altman and D.S. Dittmer, eds., Respiration and Circulation. Biological Handbooks Series, pp. 424-437, Federation of American Societies for Experimental Biology, Bethesda, Md.
13. Tai, R.C. and Chang, H.-K. (1974) J. Theor. Biol. 43, 265-276.
14. Hills, B.A. (1970) Bull. Math. Biophys. 32, 219-235.