carbon monoxide. In general terms, the analyses of Haab can be applied to any tissue because they highlight the importance of the kinetics of oxygen-release for normal oxygen delivery. In this way, carbon monoxide must not be merely regarded as a poison but also as a tool that can help the physiologist and the biochemist to unravel some of the mechanisms that are involved in oxygen transport.

0014-4754/90/11-12/1157-04$1.50 + 0.20/0

Contribution of diffusion to the oxygen dependence of energy metabolism in cells

D. F. Wilson

Department of Biochemistry and Biophysics, Medical School, University of Pennsylvania, Philadelphia (Pennsylvania 19104, USA)

The oxygen dependence of mitochondrial oxidative phosphorylation extends to greater than 30 Torr, that is, well into the range of oxygen pressure in cells under normal physiological conditions [12–14]. This dependence can be most effectively discussed in terms of 'high' and 'low' ranges of oxygen concentrations.

The oxygen dependence of mitochondrial oxidative phosphorylation at oxygen pressures greater than about 10 Torr

In the 'high' oxygen pressure range (above about 10 Torr), most of the oxygen dependence is reflected in changes in the regulatory parameters for oxidative phosphorylation (cytoplasmic [ATP]/[ADP][Pi] and intramitochondrial [NAD^+]/[NADH]). In order to place the oxygen dependence of mitochondrial oxidative phosphorylation in this region of oxygen pressure in perspective, it should be remembered that the rate of ATP utilization by cells is essentially independent of oxygen pressure. Thus, in the 'high' oxygen pressure range, the response of mitochondria to a decrease in oxygen pressure to below that required to saturate the cytochrome *c* oxidase reaction is a transient decrease in the rate of respiration (rate of ATP synthesis). The decrease in respiration causes a temporary suppression of the rate of ATP synthesis, but the resultant excess of ATP utilization over ATP synthesis lowers the [ATP]/[ADP][Pi]. As the latter decreases, the respiratory rate is progressively stimulated (see for example Wilson [11]). If the oxygen pressure is held steady at the new value, the decrease in [ATP]/[ADP][Pi] continues until the rate of ATP synthesis again equals the rate of ATP utilization and a new steady state is attained. Normally, because the turnover of ATP is high and the pool small, these changes are rapid (maximally a few seconds). As noted above, the rate of ATP utilization is essentially independent of oxygen pressure, and therefore the steady state respiratory rate remains nearly constant as the oxygen pressure is lowered. Only when the oxygen concentration is too low to support the required rate of ATP synthesis even at minimal [ATP]/[ADP][Pi] does the respiratory rate decline. Thus, in the physiological range of oxygen pressure, the oxygen dependence of mitochondrial oxidative phosphorylation in cells is observed primarily in the cytoplasmic [ATP]/[ADP][Pi].

Mitochondrial respiration is also, however, dependent on the intramitochondrial [NAD^+]/[NADH] (see for example Wilson [11] and Erecinska [3]) and the latter is determined by the activity of the mitochondrial dehydrogenases. The activities of the dehydrogenases are highly regulated and, under some conditions, part of the effect of lowering the oxygen pressure is compensated for by decreasing [NAD^+]/[NADH] (reduction of the mitochondrial pyridine nucleotide pool). When the oxygen pressure is low enough that the combined changes in [ATP]/[ADP][Pi] and [NAD^+]/[NADH] can no longer stimulate respiration enough to keep the rate of ATP synthesis equal to its rate of utilization, the respiratory rate falls. This does not occur until after the conditions are reached which will, if sustained for significant lengths of time, result in severe cellular pathology.

The oxygen dependence of mitochondrial oxidative phosphorylation at low oxygen pressures

In the range of oxygen pressures where the changes in [ATP]/[ADP][Pi] and [NAD^+]/[NADH] can no longer compensate for the effects of decrease in oxygen pressure on mitochondrial respiration is, in general, below 10 Torr. In this region of oxygen pressures a decrease is accompanied by a decrease in the rate of oxygen consumption. The respiratory rate falls according to the equation for saturation kinetics:

$$v = V_{max} \times P_{O_2}/(P_{50} + P_{O_2}) \quad (1)$$

where v is the respiratory rate, V_{max} is the respiratory rate when the oxygen pressure (P_{O_2}) is saturating. Often this type of kinetic behavior is discussed in terms of an 'oxygen affinity'. In this case, however, the effective P_{50} is determined primarily by factors other than the affinity

for oxygen. Thus the measured P_{50} is dependent on the V_{max} of cytochrome *c* oxidase for those conditions, such as the level of reduction of cytochrome *c*. For cellular conditions, the P_{50} is less than 0.05 Torr [8, 15]. This value decreases even further with decreasing turnover of the oxidase, as caused for example by inhibitors of the respiratory chain, the limiting value being too low to be measured with existing methods.

Oxygen diffusion and its contribution to the oxygen dependence of cellular energetics

The oxygen dependence of cellular energy metabolism, as related to extracellular oxygen pressure, is a function not only of the oxygen dependence of mitochondrial oxidative phosphorylation, but also of the diffusion induced oxygen pressure differences which develop between the extracellular medium and the mitochondria. Evaluation of the relative contributions of the oxygen dependence of mitochondrial oxidative phosphorylation and diffusion has proven difficult due to limitations in the methods available for measuring oxygen in biological samples. In general, the experimental approach has been to continuously add oxygen to the suspensions of mitochondria or cells in order to generate 'steady states' in which the oxygen pressure was essentially constant. The oxygen measurements could then be made over longer times. Unfortunately, at the site of addition the oxygen pressure is higher than in the rest of the suspension. Regional differences in oxygen pressure in the medium are present and these cannot be adequately evaluated. An optical method for measuring oxygen has been recently developed which has both high sensitivity (10^{-3} M to 10^{-8} M) and rapid response (< 1 ms) [9, 10, 15, 16]. In this method, measurements can be made rapidly enough that continuous addition of oxygen to the medium is not necessary. Thus, there need be no differences in the oxygen pressure within the extracellular medium.

The oxygen pressure required for half-maximal rates of respiration (P_{50}) by suspensions of isolated mitochondria is different for different metabolic states of the mitochondria [9]. It is greater than 0.5 Torr when the mitochondria are well coupled and in the presence of high levels of ATP. Addition of an uncoupler of mitochondrial oxidative phosphorylation increased the respiratory rate 6–10-fold but the P_{50} decreased to less than 0.03 Torr.

In suspensions of human neuroblastoma cells, the oxygen pressure for P_{50} (approximately 0.8 Torr) was similar to that for coupled mitochondria in the presence of ATP. When uncoupler was added to the cells, the respiratory rate increased 4–5-fold but the P_{50} decreased only to 0.7 Torr [8]. The decrease in P_{50} is much smaller when cells are treated with uncoupler than is the case for suspensions of isolated mitochondria. The higher P_{50} for uncoupled cells than for uncoupled mitochondria suggests that a diffusion induced oxygen pressure difference from the extracellular medium to the mitochondria may contribute to the P_{50} in uncoupler treated cells.

The possibility that the P_{50} for uncoupler treated cells was diffusion limited was tested by decreasing the respiratory rate by addition of inhibitors of the mitochondrial respiratory chain. If the P_{50} value were a measure of the diffusion induced oxygen pressure difference between the extracellular medium and the mitochondria, it should decrease in proportion to the decrease in the respiratory rate per mitochondrion. As the respiratory rate approached zero, the P_{50} should approach the value for uncoupled mitochondria.

Decreasing cellular respiration by adding amytal, an inhibitor of the NADH dehydrogenase region of the respiratory chain, decreased the P_{50} in proportion to the decrease in respiratory rate. As the respiratory rate approached zero, the P_{50} approached a value of less than 0.05 Torr. The decrease in P_{50} was not related to the respiratory rate per se. Increasing or decreasing the respiratory rate by increasing or decreasing the concentration of cells in the suspension, maintaining the respiratory rate per cell constant, had no effect on the measured value of P_{50}. At an amytal concentration of about 1.2 mM the respiratory rate of the uncoupled cells was equal to that of normal cells. The P_{50} for oxygen by suspensions of normal cells was 0.8 Torr, while that for the uncoupled, inhibited cells was 0.15 Torr.

Diffusion of oxygen from the extracellular medium to the mitochondria limits the P_{50} for oxygen in uncoupler treated neuroblastoma cells. The oxygen pressure difference between the external medium and the mitochondria is dependent on the mitochondrial respiratory rate. This value can be as high as 0.7 Torr for maximal respiratory rates but is only 0.15 Torr in normal cells respiring at half maximal rates (P_{50} of 0.8 Torr). It appears, at least in human neuroblastoma cells, that diffusion of oxygen from the extracellular medium to the mitochondria does not contribute substantially to the oxygen pressure dependence of cellular energy metabolism. It can, however, limit the respiratory rate at low oxygen pressures when the mitochondria are maximally activated.

In the absence of direct measurements of the oxygen pressure difference between the external medium and the mitochondria, there has been much discussion of the role of oxygen diffusion in determining the oxygen dependence of cellular energy metabolism. Some authors have suggested that oxygen pressure differences of several Torr may exist between the cytosol and mitochondria [5, 6]. This suggestion which has been criticized as inconsistent with reasonable application of diffusion theory to oxygen in biological tissue [1, 2, 11, 13], with the measured oxygen dependence of monoamine oxidase (an enzyme in the outer mitochondrial membrane) activity [7, 17] and with the absence of local regions of low oxygen in the neighborhood of mitochondria [4]. Our measurements establish that, at least in the case of human neuroblastoma cells, the total oxygen pressure difference from the extracellular medium to the mitochondria is only about 0.15 Torr. The difference between the cytoplasm and the mitochon-

dria must be less than the total and is probably only a few tens of milliTorr. These values will, of course, increase with respiratory rate per cell and with the size of the cells.

Conclusion

In suspensions of normally respiring human neuroblastoma cells, respiration has an oxygen dependence similar to that of suspensions of isolated mitochondria in medium with a comparable phosphorylation state ratio. When mitochondrial oxidative phosphorylation is uncoupled, the metabolically imposed oxygen dependence is very small. The respiration of uncoupler treated cells at limiting oxygen pressures is indicative of the diffusion induced oxygen pressure difference between the extracellular medium and the mitochondria. This P_{50} is proportional to the cellular respiratory rate, with a value of 0.15 Torr for the respiratory rate of normal neuroblastoma cells. The oxygen pressure difference between the cytoplasm surrounding the mitochondria and the mitochondria is probable only a few tens of milliTorr.

Acknowledgments. This research was supported by a grant, GM-21524, from the National Institutes of Health.

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0014-4754/90/11-12/1160-03$1.50 + 0.20/0

Consequences of acute ischemia for the electrical and mechanical function of the ventricular myocardium. A brief review

A. G. Kléber

Department of Physiology, University of Bern, Bühlplatz 5, CH-3012 Bern (Switzerland)

Summary. Reduction or interruption of the blood supply to the myocardium leads to marked disturbances of electrical and mechanical function within a few seconds. Electrical dysfunction is characterized by an initial depolarization of the resting membrane, and a decrease of the amplitude, the upstroke velocity and the duration of the action potential. Both depolarization and depression of the action potential are closely associated with intracellular metabolic acidosis. After this initial phase, electrical cell-to-cell uncoupling develops, probably as a consequence of increased cytosolic free $[Ca^{++}]$.

Mechanical dysfunction is characterized by a dissociation of the initial decrease of active force development from the subsequent ischemic contracture. Active force development in acute ischemia is inhibited by the accumulation of ischemic metabolic products (H^+, inorganic phosphate (P_i), Mg^{++}) but not by a marked decrease of [ATP]. The subsequent ischemic contracture is probably initiated by release of Ca^{++} from intracellular stores. This release causes rapid consumption of ATP and the development of rigor within 1–2 minutes.

Key words. Myocardial ischemia; ischemic depolarization; cell-to-cell uncoupling; mechanical failure; ischemic contracture.