Mechanisms of Cerebral Hypoxia and Stroke

Edited by George Somjen

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FOREWORD

The articles and short communications in this volume are based on papers presented to the Symposium on Cerebral Hypoxia and Stroke held in Budapest in August of 1987. Besides participants at the meeting, three scientists who were invited but could not attend have also contributed chapters to this volume. A synopsis of the general discussion at the conference and a review chapter conclude this volume.

To the readers of this book it will not be news that stroke is a worldwide problem. Efforts to cope with this often devastating condition are worldwide also, as attested by the international membership of the conference.

It has been said of oxygen deficiency that it not only stops the machine, it also wrecks the machinery. The paramount question in stroke research is this: why can't the brain be restarted after a hypoxic episode in much the same manner as a motor car can when its gas tank is refilled after it stalled because it ran out of fuel? Participants at the Symposium had been requested in advance of the meeting to especially consider a series of specific questions in relation to this general problem. Among these specific questions were: the mechanism of synaptic blockade in hypoxic brain tissue; the transition from reversible to irreversible arrest of function; the nature of postischemic (delayed) cell death; the possible basic differences in the consequences of hypoxia and ischemia; and actual and potential approaches to the prevention and treatment of cell damage in hypoxia and stroke.

Hypoxia and ischemia may affect not only the cerebral substance itself but also the vessels that feed it. The effects on vascular endothelium and smooth muscle may be, as are those on brain cells, transient or permanent, immediate or delayed. Hemodynamics and vascular mechanisms as such were, however, not on the agenda of this symposium. Still, pathophysiologic interactions between cerebral and vascular tissue did get discussed, as the one cannot fully be understood without the other.

The chapters of this volume have been arranged so that related topics should follow one another. This is not necessarily the same sequence in which they were presented at the symposium. The final chapter reviews the results of the Symposium in the context of recent literature.

George G. Somjen

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INBORN RESISTANCE TO HYPOXIA AND THE O_2-DEPENDENCE OF METABOLISM

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SUMMARY

1. In O_2 regulating systems, mitochondrial O_2 uptake is stabilized as O_2 availability declines by means of metabolite signals that simultaneously activate glycolysis. The consequent Pasteur effect, required to counteract ion leaks, is an attempt to make up the energy deficit arising from O_2 limitation.

2. In O_2 conforming systems, the regulatory link between the ETS and glycolysis is lost. The advantage of O_2 conformity is that it avoids the Pasteur effect which is not required because ion gradients do not dissipate; the cost is reduced reactivity and an exaggerated dependence of mitochondrial respiration on O_2 availability.

3. Converting an O_2 regulating central nervous system to an O_2 conforming one would require "clamping" the phosphate potential during declining O_2 availability by a proportionate decline in cell work rates coupled with stabilization of ion gradients across membranes.

INTRODUCTION

In 1974 at a conference dedicated to C.L. Prosser on occasion of his 65th birthday, Sir Hans Krebs presented an interesting paper reviewing examples of how organisms naturally adapted for specific functions can be used to advantage in working out principles and mechanisms underlying those very functions in animals generally. This experimental strategy, termed the August Krogh principle (Krebs, 1975), is not widespread in studies of central nervous system (CNS) susceptibility to derangements in O_2 based metabolism. Hence, it may be appropriate to begin this essay with a reminder that not all animals or tissues are as sensitive to O_2 lack as is the CNS in man or in usual experimental mammalian models. From recent analyses of hypoxia tolerant systems (Hochachka, 1985, 1986a,b, 1988) we have concluded that three processes - arrest of electron transfer system (ETS) functions, arrest of glycolytic activation, and arrest of ion-specific channel functions - must be co-ordinately regulated in order to effectively protect tissues against prolonged periods of O_2 limitation. The goal of this essay is to assess our current understanding of such defense strategies and then consider if they are transferable for protecting the mammalian CNS.

O_2-DEPENDENCE OF METABOLISM

A major difference between hypoxia sensitive versus hypoxia tolerant systems is in the way they respond to declining O_2 availability. This may surprise some readers because most studies of isolated mitochondria assume that, universally, the K_m for O_2 is a fraction of a torr (0.5 μM or less) and that plots of respiration rates versus O_2 availability are far left-shifted on the *in vivo* O_2 axis. In contrast, comparative studies report two kinds of responses to varying O_2. In O_2 regulators, RMR or resting metabolic rate is regulated at a fairly stable rate down to low O_2 availability, a pattern observable in organisms, organs, tissues, and even cells. The mammalian brain typically displays an O_2 regulatory response (Jones and Traystman, 1984). Sometimes, but rarely, these kinds of systems (Gayeski et al., 1987; Fisher and Dodia, 1981) display *in vivo* O_2 saturation curves with apparent K_m values similar to mitochondria; usually the K_m values are much higher (see Maren et al., 1986, for example).

In a second kind of response, termed O_2 conformity, the mismatch between mitochondrial and *in vivo* apparent K_m values is even greater, and O_2 uptake rates fall steadily with O_2 availability; in some cases, plots of aerobic metabolic rate versus O_2 availability pass through the origin, in which case complete arrest of ETS function presumably coincides with total O_2 lack (Whalen et al., 1973). In other cases, O_2 uptake is blocked before O_2 availability falls to zero (Edelstone et al., 1984; Burggren and Randall, 1978; Mangum and Van Winkle, 1973). As with O_2 regulatory responses, O_2 conformity responses can be seen at cell to organism levels of organization. An O_2 conforming pattern also is typically seen in patients suffering acute respiratory distress syndrome (Gilston, 1985) and in the mammalian fetus (see, for example, Edelstone, 1984).

In general, then, we can conclude that in all tissues ETS function declines when O_2 availability is adequately depressed, but there are large variations in the O_2 level at which this occurs. O_2 regulating systems are in effect "left shifted" on the $[O_2]$ axis relative to O_2 conformers. The kinds of regulatory mechanisms operating in these two kinds of systems supply us with instructive insights into mechanisms of inborn resistance to hypoxia.

SIGNALS SUSTAINING STABLE RESPIRATION IN O_2 REGULATORS

Most mechanisms proposed to explain the O_2 regulatory response assume Michaelis-Menten kinetics and are basically substrate concentration-dependent models. Wilson and his colleagues (1979), for example, suggest that compensatory regulation of the phosphate potential (or of some related signal), of the redox potential, or specifically of the reduction state of cytochrome *c* are possible mechanisms for sustaining stable respiration rates in the face of varying O_2 concentration. Other workers, such as Kadenbach (1986) suggest more complex allosteric control models focussing upon cytochrome oxidases; in animal tissues this enzyme possesses many regulatory subunits (as many as 10) some of which may serve to develop tissue-specific regulatory properties and respiratory capacities. In isosteric and allosteric models, however, the same effector metabolites (especially ATP, ADP, AMP, and P_i, separately or in combination) are given key roles to play in the O_2 regulatory response. It is usually under-emphasized in all such models that the same metabolites serve in stabilizing ETS function and in activating glycolysis during O_2 limitation. This problem is reviewed elsewhere, so suffice to mention here that adenylate concentration changes involved in stabilizing ETS function with declining $[O_2]$ also lead to glycolytic activation (the Pasteur effect) via effects on key enzymes in the pathway (see Storey, 1985, 1987). Because of these controlling links,

O_2 regulators typically display large Pasteur effects (5-15 fold increases in glucose consumption rates) and come within about 50% of making up the energy deficit (Hochachka, 1985, 1987). At least one reason a Pasteur effect is required in these kinds of tissues is because the concentrations of ions such as K^+, Na^+ and Ca^{++} begin to move towards thermodynamic equilibrium; glycolytically generated ATP is presumably required for driving ATP-dependent ion pumps, such as the Na^+K^+ATPase. Often the first indication of decoupled metabolism-membrane functions is loss of intracellular K^+, which indirectly may lead to cell damage and cell death if uncontrolled for long enough (see Hochachka, 1988, for recent literature in this area).

LOSS OF SIGNALS FOR ETS-GLYCOLYSIS INTEGRATION IN O_2 CONFORMERS

While a critical property of O_2 regulators is the ETS-glycolysis regulatory link, perhaps the most distinguishing feature of O_2 conformers is the loss of these key regulatory interactions stabilizing respiration while simultaneously activating glycolysis. During hypoxia in the turtle brain, for example, initial metabolite changes are similar to those observed in O_2 regulating systems. For example, early in hypoxia, phosphocreatine (PCr) and ATP levels begin to fall, P_i levels begin to rise, and lactate production rates are fairly high. As the hypoxic episode continues, however, the similarities with O_2 regulators end: Cytochrome aa_3 becomes more reduced even while respiration rates are falling, adenylate concentrations return towards normoxic levels, and lactate production rates fall to less than 1/5 the rates observed in initial phases of O_2 lack (Lutz et al., 1984). The energy deficit obviously is not made up by anaerobic mechanisms. A similar pattern occurs in the hypoxic lungless salamander (Gatz and Piiper, 1979) and in ischemic mammalian muscle (Harris et al., 1986).

An extreme version of this response is evident in epaxial muscle of lungfish submerged for 12 hours in a state so hypoxic that the PaO_2 is 5 torr or less and the organism as a whole is relying upon anaerobic glycolysis for 50% or more of its suppressed ATP turnover rates. Under these conditions, the epaxial muscle displays no change in PCr, ATP, ADP, AMP, or P_i levels; presumably as a result there is no compensatory activation of glycolysis at all (no measurable increase in muscle lactate over the 12 hr hypoxic episode) (Dunn et al., 1983). This tissue illustrates metabolic arrest as a strategy for surviving periods of O_2 limitations particularly well (Hochachka, 1986a,b; Guppy et al., 1986). When O_2 availability falls in this kind of system, there is a total absence of the usual regulatory links between the ETS and glycolysis. O_2 uptake rates thus necessarily fall without a Pasteur effect. At least one reason why a Pasteur effect is not required in these kinds of tissues is because ion specific channel functions seem to be arrested. The usually anticipated K^+ loss, for example, does not occur in anoxic turtle brain (Lutz et al., 1984) presumably because of the suppression of ion specific channel function (Hochachka, 1988).

OXYGEN AS A SIGNAL CONTROLLING O_2 CONFORMITY

As for signals guiding the O_2 conforming response, we are hard pressed to find possible candidates. Thus, the adenylates can be ruled out as playing any key controlling functions at least in extreme cases (such as the lungfish muscle and turtle brain examples above); this is because their concentrations simply do not change enough during O_2 lack. Changes in reduction state of ETS components, while possible regulatory signals, should compensate for declining O_2 availability and stabilize respiration, but the reverse is observed. Therefore at least tentatively we can rule out such redox changes as having anything to do with mediating O_2 conformity.

PCr and P_i concentrations changes are potential regulatory signals; the direction of change, however, should favour increasing, not decreasing, respiration. That is why these too can be ruled out; they are necessarily ruled out in cases such as lungfish muscle in which their concentrations do not change during hypoxia. Lactate and H^+ concentrations could have a role in O_2 conformity as they clearly increase in most cases. Unfortunately, they increase even more in O_2 regulators under hypoxia, which show stabilized, not declining, respiration rates; thus they too can be ruled out at least tentatively as regulatory metabolites.

Such analysis in fact can rule out every metabolite thus far examined in O_2 conformers during hypoxia except O_2 itself. Thus, we propose that the main metabolite signal to which respiration of O_2 conformers is responding is O_2 per se. It appears that as O_2 concentrations drop, cellular ATPase (cell work) rates also proportionately drop so as to effectively "clamp" the phosphate and redox potentials at steady state values where energy supply and energy demand are in balance. Not only does this mechanism explain why the usual regulatory signals are now not available for stabilizing respiration and activating glycolysis in the hypoxic zone; it also explains why the O_2 dependence of O_2 uptake is so pronounced. That is, with phosphate and redox potentials "clamped", O_2 seems to serve both as a substrate per se and as an important regulatory parameter for setting the respiration rates of mitochondria.

The conclusion that O_2 plays a regulatory role in O_2 conformity is consistent with several observations already in the literature. For example, respiration in the lungless salamander is based entirely upon diffusion of O_2 across external barriers and O_2 uptake rates of mitochondria of this organism are assuredly diffusion determined. For this system, we would predict an O_2 conforming response as ambient O_2 tensions decline, as indeed is observed (see Gatz and Piiper, 1979). Secondly, in numerous O_2 conformers, $\dot{V}O_2$ is directly proportional to O_2 tensions in the arterial blood and drops to zero before arterial O_2 tensions do. This result is predictable if $\dot{V}O_2$ is tracking O_2 tensions; presumably mitochondrial respiration is simply responding to available intracellular O_2 (Burggren and Randall, 1978).

A third line of in vivo evidence indicating that O_2 per se is the metabolite signal for O_2 conformity comes from studies of animals which under some circumstances behave as O_2 regulators, while under others behave as O_2 conformers. Pseudemys scripta is an air-breathing aquatic turtle that behaves as an O_2 regulator when O_2 tensions in air decline. When in water, on the other hand, gas exchange across the lung is impossible as is the O_2 regulatory response. A residual O_2 consumption is dependent entirely upon diffusion across external barriers (the skin and cloaca). We would predict - and it is well known - that in water this aquatic turtle is a classical O_2 conformer with $\dot{V}O_2$ behaving as if it were moving down an O_2 saturation curve (see Jackson, 1968; Hochachka and Guppy, 1987).

DIVING SEALS DO NOT USE O_2 CONFORMITY TO PROTECT THE CNS AGAINST HYPOXIA

Marine mammals, such as seals, represent a mammalian example in which O_2 conformity may be a useful defense measure during prolonged breath-hold diving. In many enforced and voluntary diving situations, the amount of O_2 available in all potential storage sites is less than would be required for normoxic resting metabolic rates, not alone for the metabolic rates that may be required during active swimming or foraging. This problem, which was first comprehensively examined by Scholander (1940), could be solved by activating anaerobic metabolism, as in hypoxia-sensitive tissues of terrestrial mammals. However, during voluntary diving in marine mammals like the Weddell seal, for which recent data are available (Guppy et al., 1986), the

amount of lactate formed is less than would be expected if the energetic shortfall due to O_2 lack were made up by anaerobic glycolysis. As in many ectothermic hypoxia tolerant animals, the mystery of the missing lactate is solved not by trying to make up the energy shortfall, but by suppressing metabolism instead (Guppy et al., 1986; Hochachka and Guppy, 1987).

As far as we know, metabolic suppression during diving is focussed on peripheral organs and tissues (such as nonworking muscle, kidneys, gut, and so forth). These are hypoperfused during diving and seem to behave in a classical O_2 conforming manner: Mitochondria are arrested in a functionally protected state, glycolytic activation is avoided, and ion fluxes (ion channel functions) are suppressed (Hochachka, P.W., unpubl. data).

In striking contrast, more critically required tissues and organs, such as the brain, are preferentially perfused during diving. As far as measurements allow us to estimate, the metabolism of the seal brain during diving (like its perfusion) remains normal or at least very near to normal (see Hochachka and Somero, 1984, for literature in this area). If made globally ischemic, the Weddell seal brain activates glycolysis and sustains a 9-10 fold increase in lactate production (Hochachka, P.W., unpubl. data), which is similar to that observed during ischemic episodes in the CNS of terrestrial mammals (see Kinter et al., 1984, for example). As expected, ETS arrested brain slices leak K^+ at much higher rates than found in O_2 conforming tissues such as the liver (Hochachka, P.W., unpubl. data). Thus diving seals seem to rely almost solely upon cardiovascular adjustments to conserve O_2 for the brain; i.e., none of the three key processes seemingly requisite for protecting tissues against O_2 lack are used by seals to protect the brain during breath-hold diving.

At first glance, the above results may appear paradoxical, but closer analysis shows this is not so. The explanation for why seals do not use O_2 conformity to defend against brain hypoxia during diving is based on two conflicting demands that would then be imposed upon the tissue. On the one hand, brain O_2 conformity would require proportionate reduction in ion channel activity, while on the other, maintaining normal CNS functions (for hunting or routine behaviour during diving) would require sustained CNS channel activity. Obviously some sort of compromise is required to resolve such conflicting demands. A compromise favouring low channel activity would favour hypoxia tolerance, but would simultaneously reduce the reactivity of the system. A compromise favouring maintained or normal channel activity would mean an O_2 regulatory response, a pronounced Pasteur effect, and thus a strict dependence upon cardiovascular adaptations for preferential redistribution of O_2 to the brain during periods of O_2 limitation.

Many O_2 conformers when in metabolic and channel arrest are sluggish and nonreactive, almost as in anesthetized states (see Hochachka and Guppy, 1987; Hochachka, 1988). Whereas this strategy works well enough for animals such as aquatic turtles, particularly during overwintering submergence, it clearly is incompatible for the CNS of alert predators, such as seals and other marine mammals during diving. Could the strategy, however, be utilized for short-term protection against CNS O_2 lack during emergencies? This question, which may be of particular interest to clinical colleagues, is not fully answerable. However, it is possible to say with some certainty that to make the strategy transferable for short-term emergencies would require that the above discussed minimal provisions for O_2 conformity be met. The most important of these would seem to require (i) breaking the regulatory link between the ETS and glycolysis by "clamping" the phosphate and redox potentials at steady state values independent of O_2 concentration, and (ii) stabilizing ion gradients even in the face of reduced ATP turnover rates. The point of converting an O_2 regulating CNS to an O_2 conforming one is to allow energy demand and energy supply to remain in balance even as

metabolic rates are falling drastically. Additionally, this favourable energy balance is achieved in O_2 conformers with minimal accumulation of anaerobic end products and with minimal dissipation of glucose reserves in relatively inefficient fermentations.

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REFERENCES

Burggren, W.W., and Randall, D.J., 1978, Oxygen uptake and transport during hypoxic exposure in the sturgeon Acipenser transmontanus, Resp. Physiol., 34, 171-183.

Dunn, J.F., Hochachka, P.W., Davison, W., and Guppy, M., 1983, Metabolic adjustments to diving and recovery in the African lungfish, Am. J. Physiol., 245, R651-R657.

Edelstone, D.I., 1984, Fetal compensatory responses to reduced oxygen delivery, Seminars in Perinatology, 8, 184-191.

Edelstone, D.I., Paulone, M.E., and Holzman, I.R., 1984, Hepatic oxygenation during arterial hypoxemia in neonatal lambs, Am. J. Obstet. Gynecol., 150, 513-518.

Fisher, A.B., and Dodia, C., 1981, The lung as a model for evaluation of critical intracellular PO_2 and PCO_2, Am. J. Physiol., 241, E47-E50.

Gatz, R.N., and Piiper, J., 1979, Anaerobic energy metabolism during severe hypoxia in the lungless salamander Desmognathus fuscus (Plethodontidae), Resp. Physiol., 38, 377-384.

Gayeski, T.E.J., Connett, R.J., and Honig, C.R., 1987, Minimum intracellular PO_2 for maximum cytochrome turnover in red muscle in situ, Am. J. Physiol., H906-H915.

Gilston, A., 1985, ARDS: Another approach, Int. Crit. Care Digest, 4, 1-2.

Guppy, M., Hill, R.D., Schneider, R.C., Qvist, J., Liggins, G.C., Zapol, W.M., and Hochachka, P.W., 1986, Micro-computer assisted metabolic studies of voluntary diving of Weddell seals, Am. J. Physiol., 250, R175-R187.

Harris, K., Walker, P.M., Mickle, D.A.G., Harding, R., Gatley, R., Wilson, G.J., Kuzon, B., McKee, N., and Romaschin, A.D., 1986, Metabolic response of skeletal muscle to ischemia, Am. J. Physiol., 250, H213-H220.

Hochachka, P.W., 1985, Assessing metabolic strategies for surviving O_2 lack: role of metabolic arrest coupled with channel arrest, Mol. Physiol., 8, 331-350.

Hochachka, P.W., 1986a, Defense strategies against hypoxia and hypothermia, Science, 231, 234-241.

Hochachka, P.W., 1986b, Metabolic arrest, Intensive Care Med., 12, 127-133.

Hochachka, P.W., 1987, Metabolic suppression and oxygen availability, Can. J. Zool., in press.

Hochachka, P.W., 1988, Metabolic, channel, and pump coupled functions: constraints and compromises of coadaptation, Can. J. Zool., in press.

Hochachka, P.W., and Guppy, M., 1987, "Metabolic Arrest and the Control of Biological Time," Harvard University Press, Cambridge, Mass., pp. 1-227.

Hochachka, P.W., and Somero, G.N., 1984, "Biochemical Adaptation", Princeton University Press, Princeton, N.J., pp. 183-203.

Jackson, D.C., 1968, Metabolic depression and oxygen depletion in the diving turtle, J. Appl. Physiol., 24, 503-509.

Jones, M.D., Jr., and Traystman, R.J., 1984, Cerebral oxygenation of the fetus, newborn and adult, Seminars in Perinatology, 8, 205-216.

Kadenbach, B., 1986, Mini Review: Regulation of respiration and ATP synthesis in higher organisms: hypothesis, Bioenergetics & Biomembr., 18, 39-54.

Kinter, D., Fitzpatrick, J.H., Jr., Louie, J.A., and Gilboe, D.D., 1984, Cerebral O_2 and energy metabolism during and after 30 minutes of moderate hypoxia, Am. J. Phyisol., 247, E475-E482.

Krebs, H.A., 1975, The August Krogh Principle: For many problems there is an animal on which it can be most conveniently studied, J. Exp. Zool., V194, 221-226.

Lutz, P.L., McMahon, P., Rosenthal, M., and Sick, T.J., 1984, Relationships between aerobic and anaerobic energy production in turtle brain in situ, Am. J. Physiol., 247, R740-R744.

Mangum, C.P., and Van Winkle, W., 1973, Responses of aquatic invertebrates to declining oxygen conditions, Am. Zool., 13, 529-541.

Meren, H., Matsumura, T., Kaufman, F.C., and Thurman, R.G., 1986, Relationship between oxygen tension and oxygen uptake in the perfused rat liver, in "O_2 Transport to Tissue," I.S. Longmuir, ed., Vol. 8, pp. 467-476.

Scholander, P.F., 1940, Experimental investigations in diving animals, mammals and birds, Hvalrad. Skr., 22, 1-131.

Storey, K.B., 1985, A re-evaluation of the Pasteur effect: new mechanisms in anaerobic metabolism, Mol. Physiol., 8, 439-461.

Storey, K.B., 1987, Suspended animation, Can. J. Zool., in press.

Whalen, W.J., Buerk, D., and Thuning, C.A., 1973, Blood flow-limited oxygen consumption in resting cat skeletal muscle, Am. J. Physiol., 224, 763-768.

Wilson, D.F., Owen, C.F., and Erecinska, M., 1979, Quantitative dependence of mitochondrial oxydative phosphorylation on O_2 consumption: A mathematical model, Arch. Biochem. Biophys., 195, 494-504.