Molecular mechanisms of defense against oxygen lack

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Hochachka PW. Molecular mechanisms of defense against oxygen lack. Undersea Biomed Res 1989; 16(5):375–379.—Evidence has been accumulating over the last several years suggesting that suppression of oxidative metabolism without concomitant glycolytic activation and the maintenance of cell membrane electrochemical gradients are central and minimal provisions for protecting tissues against oxygen lack. This evidence for diving vertebrates is reviewed and evaluated. It is concluded that the model explains long-term anoxia and hypoxia tolerance of aquatic lower vertebrates. Whereas this strategy, which is dominated by metabolic suppression capacities, may also be utilized by large, long-duration divers such as Weddell seals, it is unlikely to play a significant role in smaller and faster swimming marine mammals, where the energy demands of exercise greatly exceed the energy savings achievable by switching down metabolic rates of hypoperfused tissues.

Recent analyses of hypoxia-tolerant animals have led to the conclusion that minimally three processes—arrest of oxidative metabolism and electron transfer system (ETS) functions generally, arrest of glycolytic activation (as a means of making up the energy deficit due to oxygen lack), and arrest of ion-specific channel functions (as a means of avoiding collapse of electrochemical gradients across cell membranes during periods of oxygen lack)—must be coordinately regulated to effectively protect tissues against prolonged periods of oxygen limitation (1). This paper reviews the degree to which these strategies of defense against oxygen lack are expressed in diving animals, then briefly reviews current molecular models underlying the defense. Three basic (if related) questions are considered: a) Do tissues of diving animals sustain arrested metabolism during diving? b) Do tissues of diving animals make up the energy deficit due to oxygen lack by activating anaerobic glycolysis? c) Do tissues of diving animals sustain normal electrochemical gradients across cell membranes under diving conditions or under other experimental oxygen-limiting conditions?
ARRESTING METABOLISM DURING DIVING

In lower vertebrates (turtles, frogs) the question of whether the organism becomes hypometabolic during prolonged diving can be answered in the affirmative and can be answered unequivocally. Some 20 yr ago Jackson (2) first quantified the situation in diving turtles; he showed that metabolic rates (aerobic + anaerobic) during submergence were only one sixth or so of normoxic rates at the same temperature. Later studies prolonged submergence time in anoxic waters (for periods of weeks or even months at low temperatures) and found that metabolic rates were suppressed even further [see Hochachka and Guppy (3) for literature in this area]. For these groups, therefore, no one currently doubts that many (and probably most) tissues switch down adenosine triphosphate (ATP) turnover rates drastically during oxygen limitation. The main issue remaining concerns whether the degree of metabolic arrest is similar in all tissues. According to recent work from Kelly and Storey (4), some tissues such as the liver sustain a much larger drop in metabolic rate than do others. Work from our laboratory on turtle brain indicates that at near room temperature brain metabolic rate is about one twelfth that of the normothermic rat brain (5). How much further the turtle brain ATP turnover rate may fall during submergence is not yet known, but it may be substantial, in part because of reduced electrical activity (6), which is generally taken to mean reduced synaptic transmission. In ectothermic vertebrate divers, then, metabolic suppression seems to be a standard feature of diving, with obvious function: the greater the degree of metabolic arrest the longer the organism can dive on a given amount of (on board) oxygen (1, 3, 6–8).

In marine mammals and birds the situation is not so clear. In the Weddell seal, which is by marine mammal standards a rather slow-swimming species (perhaps for that reason its diving duration is outstanding), there are two reasons to suspect that some tissues are relatively hypometabolic during diving. First, because the seal’s lungs are collapsed and do not serve in gas exchange during deep diving it is possible to obtain good estimates of change in available oxygen during diving; such estimates show that during diving episodes of less than about 25 min aerobic metabolic rates are lower than they are when the animal is at rest on the sea ice or at isolated breathing holes in the ice (7). Castellini and Kooymans (these proceedings) review direct field measurements (which include dive + interdive periods) showing that the metabolic rates of Weddell seals foraging at sea are no greater than those measured during nonactive periods at the breathing hole, and that during long diving periods the metabolic rate is depressed even further. Since these field measurements include interdive periods, when the animals are in states of tachycardia and are probably distinctly hypermetabolic, we consider it is fair to conclude that during the diving periods per se these animals are probably hypometabolic. In faster swimming animals (or in species that are faster and smaller), the energetic costs of swimming presumably greatly exceed the energetic savings that could be made by switching off some tissues; besides, in such species diving duration is a modest fraction of that achievable by Weddell seals (often only a few minutes vs. over an hour capacity in the Weddell seal). For such reason, we would not expect to find that these species rely on hypometabolism as a routine hypoxia defense strategy, an expectation consistent with those observations that are already available (Castellini and Kooymans, these proceedings) (7).
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The second reason for expecting that species such as the Weddell seal are hypometabolic relates to the amounts of lactate that are found after diving. Scholander (9) observed some half century ago that the amount of lactate found after simulated laboratory diving in harbor seals is less than would be anticipated if the animal made up the energy deficit due to oxygen lack with anaerobic glycolysis. Our field measurements of voluntarily diving Weddell seals confirm a similar situation. The mystery of the missing lactate is easily resolvable on the assumption that the seal’s average ATP turnover rate during diving is depressed substantially (3, 7). This brings us to the second major question, namely, do tissues of diving animals sustain a normal Pasteur effect?

PASTEUR EFFECT IN TISSUES OF DIVING ANIMALS

When hypoxia-sensitive vertebrate tissues become severely oxygen limited, they typically attempt to make up the energy deficit due to oxygen lack by activating anaerobic glycolysis, a regulatory metabolic response termed the Pasteur effect after its discovery in yeast in 1861. The size of the Pasteur effect varies, but usually is large enough to represent a 5-to 15-fold increase in glucose flux to lactate (3, 10). In totally ischemic Weddell seal liver this does not occur, and the amount of lactate generated is only a minute fraction of that which would be found in a similarly treated hypoxia-sensitive tissue (10). Tissues such as Weddell seal liver are said to display a reversed Pasteur effect which is part of their regulatory switch-off strategy. Mechanisms underlying these metabolic arrest strategies are not fully understood, but in turtles and hypoxia-tolerant fishes, a regulated, partially inhibited steady state seems to require covalent modification of key enzymes in glycolysis, such as pyruvate kinases and phosphofructokinases (11). Another key regulatory requirement is that ATP turnover is suppressed with minimal change in the concentration of phosphate metabolites such as ATP or in the phosphorylation potential. This means that the declining demands for ATP are so closely matched by ATP synthesis rates that the two processes in effect clamp cell adenylate concentrations. Elsewhere we emphasize that the nature of coupling of energy demand to energy supply (loose vs. tight) is in fact a highly adaptable characteristic (12, 13). It is nevertheless so repeatedly observed in hypoxia-tolerant tissues that we consider it to lie at the very heart of mechanisms allowing survival without oxygen (13).

PROTECTING ELECTROCHEMICAL GRADIENTS DURING HYPOXIA

It is widely believed (on relatively small amounts of evidence) that a major demand for ATP under all cellular conditions arises from the need to sustain activities of ion-specific pumps or ATPases, such as Na⁺ K⁺ ATPases and Ca⁺⁺ ATPase (14, 15). Thus it is not surprising that in hypoxia-sensitive tissues, such as the rat brain, attempts are made to make up the energy deficit due to oxygen lack; large glycolytic rates are therefore sustained at least during initial stages of oxygen limitation, and ion pump-linked ATPases are under these conditions momentarily sustained by glycolysis (16). However, the required ATP synthesis rates cannot be long sustained and as a consequence such cells rapidly efflux K⁺ ions, the first measurable event after the metabolic changes.
At least in cardiac cells, good evidence suggests that the breakdown in membrane-regulated ion homeostasis is coupled to metabolism via a high conductance ATP-dependent K⁺ channel (17). According to this model, the sequence of events can be summarized as follows: energy demand-energy supply uncoupling, falling ATP concentrations to a critical threshold set by the K⁺ channel affinity for ATP, K⁺ efflux, and finally an autocatalytic self-destruct cascade involving Ca²⁺ at high concentrations as an intracellular toxin (1, 5, 10, 18). An alternative model assumes that the first lesion in this kind of cell involves an initial release of Ca²⁺ from intracellular stores, followed by a Ca²⁺ activation of Ca²⁺-dependent K⁺ channels; this would then presumably set the stage for the same set of events leading to cell damage or cell death (1, 18).

In hypoxia-tolerant cells these events most assuredly do not take place; indeed, they obviously could not, for otherwise such cells would obviously die in the absence of oxygen. Probably the best available data are for turtle brain under conditions of oxygen lack, where there is no evidence for excessive K⁺ loss (6). Earlier we predicted that the cell membranes of such tissues would necessarily be channel arrested (1, 16). Recent direct measures show that turtle nerve axons are in fact substantially less leaky to ions (display lower functional ion channel densities) than are homologous cells in rat brain (C. Doll, personal communication). However, this lower, apparent background leakiness or permeability is considered to be a regulatory phenomenon rather than to represent simple differences in total channel abundance; for example, estimates of densities of at least two kinds of ion-specific channels indicate similar abundance in turtle and rat brain cells (5). What this means is that turtle brain cells are less leaky not because they have fewer channels but because they are better regulated under oxygen-limiting conditions. A key insight into how this might occur comes from experiments with iodoacetate as a glycolytic block. Even though the turtle brain is considered to sustain a reduced ATP turnover rate during oxygen lack (8), what energy turnover remains is glycolytically driven. As emphasized above, regulation remains so tight in this tissue under anoxia that ATP concentrations and phosphorylation potentials remain within the normal range. Iodoacetate inhibition of glycolysis would predictably lead to a perturbation of the adenylates, an energy demand-energy supply uncoupling, and a consequent drop in ATP concentrations. I hypothesize that it is this perturbation in energy metabolism that leads to an activation of ATP-dependent K⁺ channels and thus explains the secondary effect of iodoacetate poisoning: a massive K⁺ ion efflux fully analogous to that observed in the rat brain when its ATP concentrations fall due to oxygen limitation.

If this model is correct, it implies that maintaining metabolism-membrane functions properly integrated lies at the heart of hypoxia-tolerance. Achieving such a balance in turn relies on an exceptionally tight regulation of energy demand and energy supply: rates of ATP utilization must be so closely coupled with rates of ATP synthesis that the two processes in effect clamp the absolute concentrations of the adenylates and other high energy phosphate metabolites as well as clamp the phosphorylation potential. We reason that such close regulation of ATP turnover rate as the tissue is becoming hypometabolic is possible if—and only if—at least one regulatory signal switches down both ATP utilization rates and ATP synthesis rates simultaneously. What that signal might be, or whether the signal even exists, are questions for which there are, at least for the time being, no answers.
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REFERENCES
