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Defense Strategies Against Hypoxia and Hypothermia

P. W. HOCHACHKA

Because aerobic metabolic rates decrease in hypoxia-sensitive cells under oxygen-limiting conditions, the demand for glucose or glycogen for anaerobic glycolysis may rise drastically as a means of making up for the energetic shortfall. However, ion and electrical potentials typically cannot be sustained because of energy insufficiency and high membrane permeabilities; therefore metabolic and membrane functions in effect become decoupled. In hypoxia-tolerant animals, these problems are resolved through a number of biochemical and physiological mechanisms; of these (i) metabolic arrest and (ii) stabilized membrane functions are the most effective strategies for extending tolerance to hypoxia. Metabolic arrest is achieved by means of a reversed or negative Pasteur effect (reduced or unchanging glycolytic flux at reduced O_2 availability); and coupling of metabolic and membrane function is achievable, in spite of the lower energy turnover rates, by maintaining membranes of low permeability (probably via reduced densities of ion-specific channels). The possibility of combining metabolic arrest with channel arrest has been recognized as an intervention strategy. To date, the success of this strategy has been minimal, mainly because depression of metabolism through cold is the usual arrest mechanism used, and hypothermia in itself perturbs controlled cell function in most endotherms.

ALTHOUGH SOME DEGREE OF HYPOXIA AND HYPOTHERMIA can be sustained by all animals, both conditions ultimately are incompatible with survival of most mammalian tissues. Some ectothermic animals, however, are capable of surviving for long periods without O_2, and some mammals (notably hibernators) can tolerate—in fact, can take advantage of—hypothermia. When we investigated the means by which these abilities are achieved at the cellular level, we found that the most serious perturbations of hypoxia and of hypothermia arise from an imbalance between (i) the extent of depression of adenosine triphosphate (ATP) synthesis rates and (ii) the depression of processes requiring membrane-based ATP. When metabolic and membrane functions are decoupled, the cells (tissues or organisms) necessarily become sensitive to hypoxia, to hypothermia, or to both conditions. When, however, the two rate processes are matched despite O_2 limitations or low temperature, then an impressive tolerance to hypoxia, to hypothermia, or to both conditions is achievable. The situation of simultaneous resistance to lack of O_2 and to low temperature arises because some of the fundamental mechanisms used by hypoxia-tolerant animals to protect tissues and organs against hypoxia are the same as, or at least remarkably similar to, those used by cold-tolerant organisms such as hibernators to sustain prolonged hypothermia. In this article, similarities and differences in mechanisms of adaptation to hypoxia and hypothermia are analyzed, with emphasis on opportunities, wherever possible, for novel intervention strategies.

How Animals Survive Oxygen Lack

Careful analysis of animals profoundly resistant to hypoxia indicate two broad adaptive categories. In one category, typified by species adapted to high altitude and by patients suffering from chronic hypoxia (*1*), metabolic mechanisms are directed toward sustained oxidative function despite potentially long-term O_2 limita-

P. W. Hochachka is in the Department of Zoology, University of British Columbia, Vancouver, Canada V6T 2A9.

tion, with little or no extension in tissue anaerobic capacities. In contrast, many invertebrates and lower vertebrates under hypoxic stress direct metabolic strategies toward sustained anaerobic function; many of these species have such effective mechanisms of protection against hypoxia that they are often referred to as good animal anaerobes or as facultative anaerobes (*2, 3*). In this adaptive response, the two most fundamental metabolic problems are (i) conservation of fermentable substrate, and (ii) avoidance of self-pollution by production of undesirable end products. The first problem arises from the energetic inefficiency of anaerobic metabolism, because the yield of ATP per mole of substrate fermented is always modest compared with that in oxidative metabolism. For this reason in most animal tissues, glycogen (glucose) utilization rates vary inversely with O_2 availability [the so-called Pasteur and Crabtree effects (*4, 5*)]; this means that if demands for ATP remain unchanged during O_2 lack, carbohydrate consumption rates necessarily have to rise drastically.

Potentially large depletions of glycogen from tissue stores are minimized in facultative anaerobes by one of the following mechanisms: (i) storing more glycogen, (ii) utilizing more efficient fermentation pathways, or (iii) depressing ATP turnover rates (that is, energy demand) during O_2-limiting periods. All three mechanisms are demonstrably useful by the criterion of anoxic survival time (*2*); however, the first two mechanisms in principle could not extend hypoxia tolerance by more than a factor of 3 or 4. In contrast, metabolic arrest processes can increase anoxia tolerance in *Mytilus* by a factor of 20, in diving turtles by a factor of 60, and in brine shrimp embryos by several orders of magnitude. This strategy is expressed at its limit in estivating brine shrimp embryos, since their maximum anoxia tolerance coincides with entrance into a fully arrested or ametabolic state [when metabolism as we know it approaches a standstill (*3*)]. Even from the less extreme cases of good animal anaerobes, however, two instructive insights arise: (i) reversing the classical Pasteur effect so as to allow ATP turnover rates to drop precipitously during anoxia appears to constitute the most effective strategy for solving the problem of substrate conservation, and (ii) this metabolic strategy may, in principle at least, be universally applicable, whereas other potentially protective mechanisms, such as energetically improved fermentation pathways, are phylogenetically restricted (*3*) and therefore, even in theory, could not represent realizable strategies of hypoxia adaptation in species lacking the appropriate enzyme pathways (most mammals, including man). Thus, our current estimates of substrate-sparing advantages of metabolic arrest mechanisms for surviving O_2 limitations may actually be on the conservative side, assuming that factors such as accumulations of end products do not become limiting.

Analysis of the problem of end products is more difficult because it requires assessing the relative effects of organic (usually anionic) end products as opposed to those of H^+ itself; the metabolic sites of H^+ production, the pathways for later proton deposition, and the H^+ stoichiometry of different fermentation pathways have to be considered (*6*). In addition, any metabolic effects of net change in strong ion difference (*7*) must be evaluated. Despite these complications, it is now evident that the potentially perturbing direct or indirect effects of metabolic end products are minimized in good animal anaerobes by only a few mechanisms. These include (i) utilizing fermentation pathways that allow more ATP to be turned over per mole of H^+ generated than in classical glycolysis, (ii) tolerating proton production by improved tissue-buffering capacity, (iii) minimizing end-product accumulation by recycling end products for further metabolism or excretion, (iv) utilizing H^+-consuming reaction pathways, and (v) depressing metabolic rates during anoxia (*2, 5*).

Whereas any or all of the first four mechanisms are obviously advantageous, potentially yielding a severalfold improvement in anoxia tolerance, it is evident that by depressing demands for ATP during anoxia (the fifth mechanism), an organism not only reduces the depletion rates of glycogen (glucose) in inefficient fermentations but also automatically reduces rates of formation of anaerobic end products, including H^+. In anoxic goldfish or lungfish, for example, the rate of proton production is reduced by a factor of 5 as a result of metabolic depression, whereas in the turtle it is reduced by nearly two orders of magnitude (*2, 3*).

The picture emerging from such analyses of phylogenetically diverse groups of animals is that several processes contribute to the hypoxia tolerance of good anaerobes, but of these, metabolic arrest mechanisms yield by far the most effective protection against O_2 lack. Of the known protective strategies, only metabolic arrest resolves the problems of substrate conservation during anoxia and of end-product formation. That is why we concluded some years ago (*2, 3, 6*) that, for extended survival without oxygen, anaerobic life-support systems (whether considered at the level of organism, organ, or cell) must be able to switch down or even switch off metabolically.

Metabolic Arrest As an Intervention Strategy

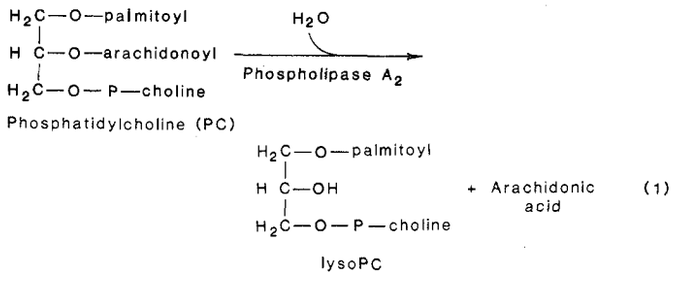
Earlier "arrest"-type concepts of protection against hypoxia are evident in the scientific literature on hibernation (*8*) and in the clinical literature on cardiac arrest, stroke, acute renal failure, and liver aschemia (*9*). It is widely accepted, for example, that ischemic myocardial damage is a function of work load or metabolic rate. Several intervention procedures all have the aim of minimizing tissue damage by reducing myocardial energy requirements during ischemia and, to a lesser or greater degree, all such maneuvers can be shown to be helpful (*10*) and therefore consistent with the above interpretative hypothesis. However, the most convincing test of our metabolic arrest hypothesis probably is provided by recent studies in which ischemic rat kidney preparations were used as models of acute renal failure (*11*). During sudden ischemic renal failure, reductions in renal blood flow occur, with consequent reduction in delivery of O_2 and substrates to the tissue. Since the main ATP-requiring processes of the kidney involve membrane-coupled ion translocations, our hypothesis leads to an explicit, testable prediction; namely, that experimentally reducing the demands for ATP by ion pumps should yield a proportionate increase in tolerance to O_2 lack. In mammalian kidney, the medullary thick ascending limb (mTAL) of Henle's loop is the most hypoxia-sensitive segment of the nephron, and during perfusion of the isolated organ, extensive, essentially irreversible, damage to mTAL cells occurs in 90 minutes. However, this hypoxia sensitivity can be greatly reduced by perfusion with ouabain, a specific inhibitor of Na^+- and K^+-dependent adenosinetriphosphatase (Na^+,K^+-ATPase) or by reducing ion-pumping work by preventing glomerular filtration. Conversely, polyene antibiotics increase membrane permeability, increase the energy requirements of ion transport, and consequently increase the hypoxia sensitivity of mTAL tubules; yet again the hypoxia-induced damage is preventable if reabsorptive transport is inhibited by ouabain. Impressive protection can be achieved even against KCN-induced lesions by simultaneous ouabain inhibition of Na^+,K^+-ATPase (*11*).

These studies effectively represent empirical attempts to establish in a mammalian organ the hypoxia tolerance that is characteristic of hypoxia-adapted lower vertebrates, and the maneuvers satisfyingly support the general predictions of our hypothesis. Yet it is important to emphasize that metabolically arrested mammalian systems are only able to extend hypoxic survival times from minutes to hours

(depending on tissue type), whereas good animal anaerobes are able to sustain anoxia for days, weeks, and even months in some cases (*3, 5*). Such order-of-magnitude differences in hypoxia tolerance between mammalian preparations and facultatively anaerobic animals means that something is still missing in our analyses of strategies for protecting tissues against O_2 lack. It is proposed that the missing element is to be found at the interface between cell membrane functions and cell metabolism—the two processes are in effect decoupled in hypoxia-sensitive cells during O_2 lack—but to put this concept in perspective it is necessary to consider membrane functions in anoxia in mammals and in ectothermic anaerobes.

Decoupled Metabolism-Membrane Functions in Anoxia

The breakdown of cell membrane functions in anoxia can perhaps be best illustrated by considering the brain, one of the most hypoxia-sensitive organs in the mammalian body, for which events during various kinds of energy perturbations are well charted [see (*12, 13*) for reviews]. In complete cerebral ischemia, for example, as occurs in cardiac arrest, the electroencephalograph (EEG) becomes isoelectric within 15 to 25 seconds. This electrically silent period precedes a massive outflux of K^+ from the neurons and a flux of Na^+ into the neurons, attributable to energy insufficiency and thus to the failure of membrane ion pumps (at regional cerebral blood flow of about 10 percent of normal). When the potassium concentration $[K^+]$ in the extracellular fluid (ECF) drops to about 12 to 13 m*M*, changes in membrane potential apparently become large enough to activate (or open) voltage-dependent Ca^{2+} channels and develop a largely uncontrollable influx of Ca^{2+} (*12, 13*), a cation which at abnormally high cytosolic concentration acts as a cellular toxin (*10, 12–18*). Although high cytosolic $[Ca^{2+}]$ may disrupt various intracellular functions (*17*), its activations of phospholipases A_1, A_2, and C are considered to be the most damaging under hypoxic conditions (*12, 13*). Increasing concentrations of lysophosphatidylcholine (lysoPC) and free fatty acids soon after ischemia are indicative of catalysis by phospholipases of the A class (*14, 15*). For phospholipase A_2, this catalysis can be written as in Eq. 1.



Uncontrolled, this reaction leads (Fig. 1) to membrane phospholipid hydrolysis, to the consequent disruption of cell and mitochondrial membranes, to the release of free fatty acids (such as arachidonic acid), and to the further potentiation of ion redistributions (*12, 13*).

The overall impact of O_2 limitation to the brain, as summarized in Fig. 1, is similar in other tissues as well, although it may not be as rapid or extensive in more hypoxia-tolerant tissues. In myocardial ischemia, energy difficiencies and membrane failures are indicated by intra- and extracellular changes in $[Na^+]$ and $[K^+]$, as well as by a large influx of Ca^{2+}, a loss of sarcolemmal Ca^{2+}, and a disruption of mitochondrial Ca^{2+} homeostasis (*10, 16*). Analogous membrane failure with associated translocation of Ca^{2+} and other ions between intra- and extracellular pools is found in liver under O_2-limiting conditions and in acute renal failure in mammals and can be presumed to occur generally in hypoxia-sensitive mammalian organs and tissues during O_2 lack (*9, 18*).

Such ion-flux–initiated, self-reinforcing cascades also may be facilitated by the action of phospholipase C (*14, 15*), whose continued catalytic function under O_2-limiting conditions is indicated by increasing levels of stearoyl and arachidonoyl diacylglycerols coincident with decreasing levels of phosphatidylinositol (PI). The major metabolic pathways responsible for the formation and degradation of PI are illustrated in Fig. 2. Phosphatidylinositol is phosphorylated at the 4-position of its inositol head group by a specific kinase to form phosphatidylinositol 4-phosphate [PI(4)P]; this is in turn further phosphorylated at the 5-position to give $PI(4,5)P_2$, which is one of the inositol lipids located in the inner leaflet of the plasma membrane. The steady-state concentration of $PI(4,5)P_2$ is determined by the balance between the activities of these kinases and phosphomonesterases, which convert $PI(4,5)P_2$ back to PI (Fig. 2)—that is, by the operation of two linked metabolic cycles in which phosphates are constantly being added to and removed from the 4 and 5 positions of the inositol head group.

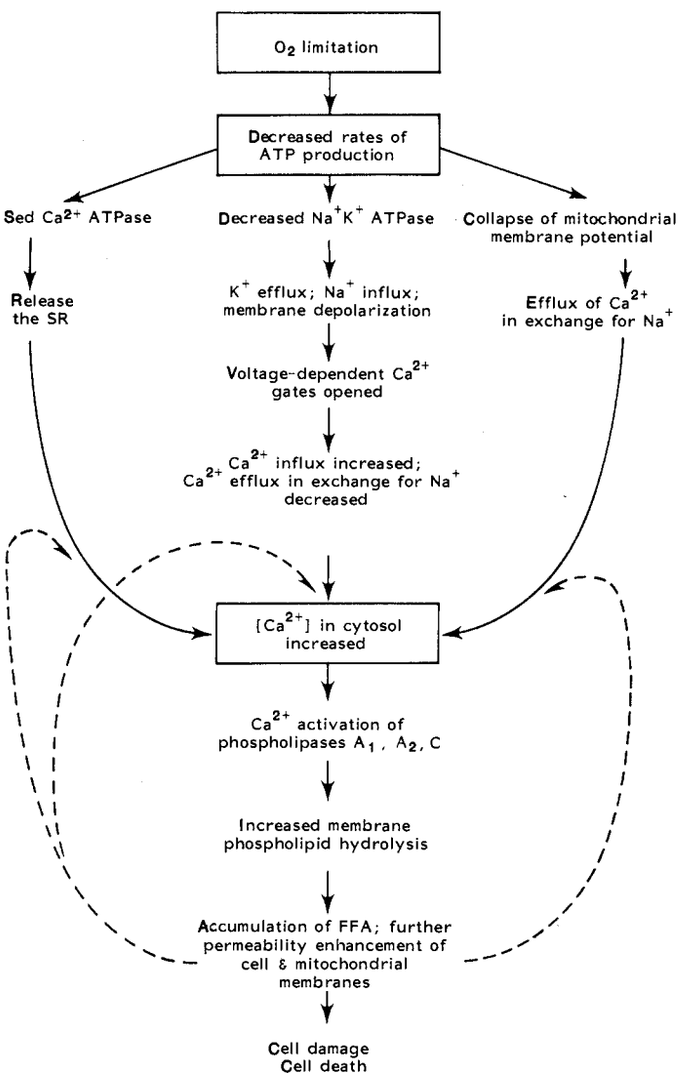
In response to various Ca^{2+}-mobilizing hormones, these two metabolic cycles are broken in a controlled way by preferential phospholipase C action on $PI(4,5)P_2$, releasing diacylglycerol plus water-soluble inositol triphosphate (IP_3) as a second messenger that signals release of Ca^{2+} from intracellular pools. In hypoxia, in contrast, these cycles are broken in an apparently uncontrolled way, presumably leading to the same end products (*14, 15*). Inositol triphosphate released in the process is thought to act as a secondary signal for opening Ca^{2+} channels and releasing sarcoplasmic Ca^{2+}, thus increasing cytosolic Ca^{2+} availability, a cascade that may again be self-potentiating (increasing Ca^{2+} availability favoring phospholipase C catalysis). Under normal circumstances, diacylglycerol is phosphorylated to phosphatidic acid which is then converted back to PI. However, as shown by Matthys *et al.* (*15*), diacylglycerol accumulates in hypoxia-sensitive tissues during O_2 lack and is thought to increase Na^+-H^+ exchange, thus effectively slowing down Na^+ exchange-based Ca^{2+} efflux (Fig. 2).

Such failure of membrane function does not occur at all, or develops comparatively slowly, in the brain and other organs of good ectothermic anaerobes during hypoxia (*19–21*) (Fig. 3, A to C). In hypoxia-sensitive tissues, as soon as continuous and high rates of ATP generation are reduced, intra- and extracellular ion concentration gradients are rapidly lost and tissue viability is at risk (Fig. 3A); in contrast, ionic concentration gradients do not fall to their thermodynamic equilibrium in homologous tissues of ectothermic anaerobes (Fig. 3, B and C) despite metabolically depressed states in anoxia and proportionately lower ATP turnover rates (*3*). Obviously, something about cell membranes in facultatively anaerobic ectotherms is different; under hypoxic conditions, either these membranes are more impermeable to ions, or ionic pumping capacity can pace thermodynamic drift of ions to electrochemical equilibrium.

Stabilized ion gradients almost certainly cannot be the result of accelerated ion pumping, because ATP turnover rates are lowered in the metabolically arrested states typical of animal anaerobes in anoxia. For this reason, we assume that membrane-based differences in the effects of hypoxia are due mainly to different permeability barriers and are an expression of a basic difference between cell membranes of hypoxia-tolerant and hypoxia-sensitive tissues. This would explain the above-mentioned comparative studies showing that even if ion gradients do decline during hypoxia in hypoxia-tolerant ectotherms, the process is much slower than in homologous hypoxia-sensitive cells (*20*), and that in the extreme, ion gradients remain stable after days of anoxia (*21*) even if ATP turnover rates are too low to explain the stability with ion pumping (*3*). Furthermore,

this interpretation is consistent with comparative studies of mammals and reptiles (*22*) indicating (i) that ATP turnover rates of homologous tissues in mammals are about five times higher than in ectothermic reptiles, (ii) that the ouabain sensitivities of metabolism are similarly greater in mammals, and (iii) that the "leakiness" of mammalian cell membranes (measured as Na^+ permeability of hepatocytes) is severalfold greater than that in reptiles. Else and Hulbert (*22*) argue that the latter explains the former; that is, one cost of endothermy is a higher rate of thermogenesis arising, in part at least, from leaky membranes and from the consequent necessity for higher ion pumping rates and higher ATP turnover rates. In this sense, leaky membranes are adaptive in endotherms as they are part of an O_2-fueled biological furnace (*22*).

The striking implication arising from these studies is that nonleaky membranes are a necessary but insufficient provision for the anoxia tolerance of good animal anaerobes and that for this reason ectotherms have a distinct advantage over endotherms in inherent resistance to O_2 lack. The counterargument, that leaky membranes although inherent to endothermy, cause an increased sensitivity to hypoxia in many tissues of endotherms, appears to supply the element—stabilized membrane functions—missing in earlier attempts to extend the hypoxia tolerance of mammalian tissues and cells (*10, 11*). In this view, a minimal requirement for establishing in mammalian tissues the hypoxia tolerance of ectothermic anaerobes is to couple metabolic arrest mechanisms with nonleaky membranes. In fact, intervention strategies designed to block uncontrolled Ca^{2+} fluxes [designed, in effect, to block one or more of the steps leading to a rise in cytosolic [Ca^{2+}] or to phospholipase activation (Fig. 1)] are used with increasing frequency in attempts to protect mammalian tissues against hypoxia (*9, 10, 16, 18*). Yet for prolonged protection, these are of limited success. The problem seems to be that such interventions sometimes do not include metabolic arrest, and when the latter strategy is used, depressed rates of ATP turnover are usually achieved by hypothermia (*10, 16*). Although hypothermia applied on its own to any mammalian tissue or organ so far investigated is "protective" against hypoxia for short periods, pro-



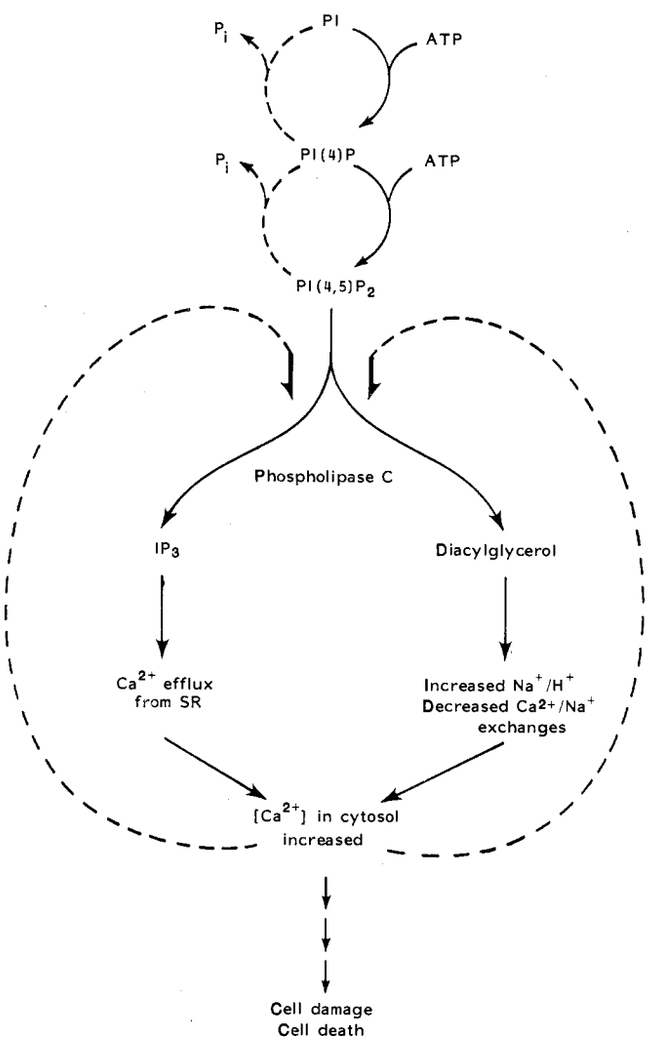


Fig. 1 (left). A summary of probable metabolic events progressing in hypoxia-sensitive cells from the initial energetic consequences of O_2 limitation to cell damage and cell death. The summary is based on analysis in the text and is constructed from various studies of hypoxia-sensitive mammalian tissues (*9–18*). Fig. 2 (right). Under O_2-limiting conditions, phosphoinositols decrease in concentration while diacylglycerol levels increase—for example, as shown by Matthys *et al.* (*15*) for the ischemic kidney. This is presumed to occur along with some accumulation of inositol triphosphate facilitating increased Ca^{2+} levels in the cytosol. This pathogenic cascade is thought to represent a normally closely tuned signal-transducing system running out of control during O_2 lack [see Berridge and Irvine (*14*) for further discussion of this control system].

longed hypothermia is not, presumably because in itself it strongly perturbs membrane and metabolic functions (see references below). Since hypothermia as such is damaging, and its disrupting effect in combination with hypoxia may well be exaggerated, it is by no means the metabolic arrest mechanism of choice. To understand why, it is necessary to examine the problem of hypothermia more closely.

Why Many Mammalian Cells Are Hypothermia-Sensitive

Most mammals are unable to survive at low body temperature (T_b) for extended time periods, but why should this be so when function at T_b near 0°C is so commonly observed in ectotherms? While there may well be many biochemical problems at low T_b that homeotherms cannot solve (*3*), the two most important ones thus far identified appear to be (i) maintaining regulated metabolism and (ii) maintaining ion gradients. Because the first of these has been analyzed (*23*), we emphasize only that two kinds of metabolic processes are disrupted in hypothermia: metabolic rate and metabolic regulation, partly as a result of direct thermodynamic effects and partly as a result of substrate limitation. As tissue-specific metabolic pathways may be expected to display unique thermal dependence (*3*), it is evident that if hypothermic metabolic depression is largely unregulated, the functional decline of one organ (for example, heart) need not parallel that of others (for example, respiratory system). That may explain why, in hypothermic hamsters, respiratory failure may occur more quickly and at higher temperatures than cardiac failure, whereas these functions are slowed down in synchrony during the regulated metabolic depression sustained in the hypothermia of hibernation (*23, 24*).

A disruption in metabolic regulation may also be illustrated by the breakdown of glucose homeostasis. For example, during imposed hypothermia, hamsters experience a hypoglycemia so severe that plasma glucose concentrations may fall to 1/20 of normal. Yet liver glycogen, the immediate precursor of plasma glucose, is retained at near-normal levels, a situation clearly indicating that normal regulatory mechanisms cannot be harnessed (*23*). Although much is yet to be learned about the details of such metabolic derangements, it is clear that imposed hypothermia may cause serious mismatching between substrate availability and substrate delivery, and ultimately between ATP requirements and ATP-generating capacities. In terms of cell survival, one of the critical functions influenced by this mismatch is ion regulation.

The regulation of K^+, Na^+, Ca^{2+}, or Mg^{2+} concentrations in intracellular fluid (ICF) and ECF is widely used as a criterion of cell survival because such ion regulation is required for the maintenance of cell volume and specific metabolic processes (for example, protein synthesis) and can be used as a convenient indicator of cell integrity and sustained function. In principle, although not all mammalian cells need be cold-sensitive, most are and lose K^+ at low temperature because the temperature coefficient for leakage of K^+ is less than the temperature dependence of ATP-dependent, active accumulation (or of energy metabolism). K^+ efflux at low cell temperatures, therefore, is necessarily blocked less than inward K^+ pumping; as a result, efflux exceeds K^+ influx so that ICF and ECF K^+ ions shift toward their equilibrium concentrations (*24, 25*). Na^+ moves in the opposite way, and if this process is not interrupted, we assume it ultimately leads to partial membrane depolarization, the opening of voltage-dependent Ca^{2+} channels (*12, 13, 17*), and the influx of Ca^{2+} (*26, 27*). Ca^{2+} influx may also be facilitated by Na^+-Ca^{2+} exchange or by damaged (that is, low-temperature modified) Ca^{2+} channels behaving as Na^+ channels, a process that would increase $[Na^+]$ in the ICF and set the stage for activated Na^+-Ca^{2+} exchange (*10, 16*). Low temperature may also lead to a loss of Ca^{2+} from the sarcoplasmic reticulum (SR), which is again at least in part caused by an imbalance between rates of SR Ca^{2+} uptake (by Ca^{2+} ATPase) and rates of Ca^{2+} efflux (*28*). In addition, a drop in cell temperature alters the fractional dissociation of imidazole groups on proteins; as a result, intracellular *p*H rises (*29*) and directly activates Ca^{2+} efflux from the SR (*28*). The net effect of all these processes would seem to facilitate gradually increasing $[Ca^{2+}]$ in the cytosol and thus lead to some (perhaps all) of the metabolic perturbations shown in Fig. 1.

An illustration of this kind of pathogenesis arises from studies of human platelets (*30*), where reactions 2 and 3 are known to proceed even at low temperature.

$$\text{PC} \xrightarrow{H_2O} \text{lysoPC} + \text{Arachidonic acid} \quad (2)$$

$$\text{PE} \xrightarrow{H_2O} \text{lysoPE} + \text{Arachidonic acid} \quad (3)$$

(PE is the abbreviation for phosphatidylethanolamine.) The end products of these reactions normally are formed after thrombin activation of platelets, but during hypothermia they proceed without thrombin, indicating a low temperature-mediated "activation" of phospholipase A_2 (*30*). From parallel studies it is known that the influx of Ca^{2+} occurs before the onset of lysoPC and lysoPE formation and the liberation of arachidonic acid (*30*). Thus thrombin activation and hypothermia can initiate similar chains of metabolic events (Eq. 4).

$$\begin{array}{l} Ca^{2+} \text{ influx} \longrightarrow \text{Rise in cytosolic } [Ca^{2+}] \longrightarrow \text{P-lipase } A_2 \text{ activation} \longrightarrow \\ \qquad\qquad \text{Membrane phospholipid hydrolysis} \begin{cases} \nearrow \text{Aggregation (thrombin-activation)} \\ \searrow \text{Cell damage (hypothermia)} \end{cases} \end{array} \quad (4)$$

The difference between the two pathways is that thrombin activation is a controlled process, whereas the hypothermia-mediated process is apparently largely uncontrollable. As in hypoxia, the pathogenic pathway favored by prolonged hypothermia may be autocatalytic in the sense that cell membrane damage may in turn accelerate further dissipation of ion gradients. Any cellular defense mechanisms requiring activated ion pumping would also require increased ATP turnover rates, so that an energy-depressed state (as may be expected in hypoxia), if prolonged, would probably aggravate the low temperature problem. Thus hypothermia coupled with hypoxia is probably an unsatisfactory combination. That it is often used clinically means that the metabolic-arresting effects of low temperature are of greater advantage in the short-term than are the disadvantages of membrane-destabilizing effects. In the longer term, however, one of the main reasons why many mammalian tissues and cells are cold-sensitive seems to be membrane failure at low temperatures; indeed, in the intact organism, respiratory blockade and cardiac fibrillation are possibly the most immediate causes of death during imposed hypothermia (*23, 24*), both being physiological manifestations of membrane failure at the cellular level.

How to Survive Hypothermia

From the above analysis, it is evident that the cells and tissues of hypothermia-tolerant mammals (such as hibernators) must maintain

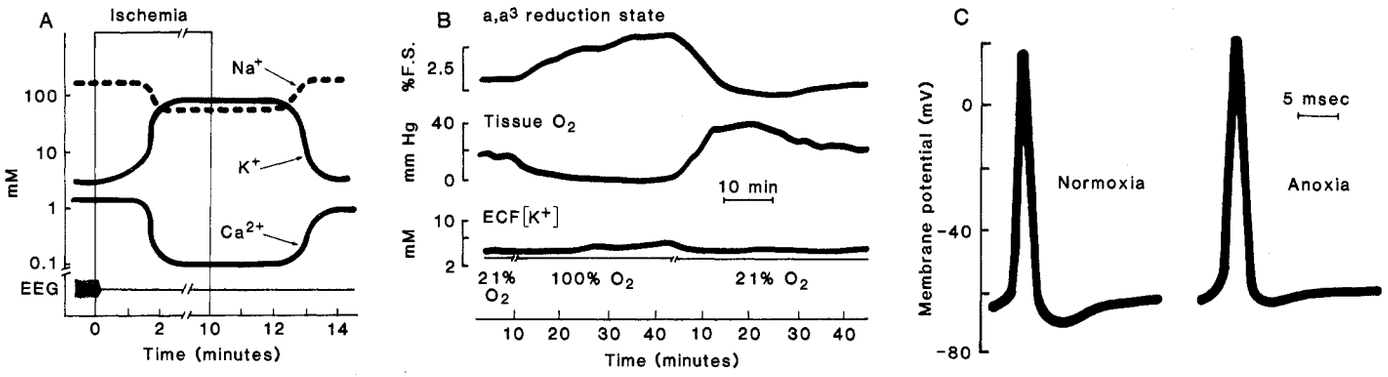


Fig. 3. (A) Extracellular ion concentration and EEG-activity in rat brain cortex at the onset and end of 10 minutes of cerebral ischemia [modified from Hansen (*12*)]. (B) Changes in the redox state of cytochrome aa_3, tissue organ tension, and extracellular concentration in turtle brain which resulted from switching inspired gas mixture from 21 percent O_2 to 100 percent N_2 [modified from Sick *et al.* (*20*)]. (C) Intracellular recorded action potentials in *Arenicola marina* giant axons. (Left) normoxia; (right) after incubation for 3 days in anoxia (in cyanide), indicating that normal ion gradients are retained by a direct functional criterion [modified from Surlykke (*21*)].

(i) regulated metabolism and (ii) regulated membrane functions despite low cell temperatures. Although the mechanisms are still not fully understood and are being actively researched (*31*), it has long been appreciated that metabolism during hypothermia of deep hibernation is not a simple resultant of thermodynamics; metabolism is slowed down but it is clearly and closely regulated (*3, 22, 24*). More recent studies (*24, 31*) furthermore indicate, that, as was assumed earlier, membrane functions are also maintained during hibernation despite a greatly depressed metabolism. Regulated metabolism and regulated membrane functions maintain myocardial function during deep hibernation, which thus sustains adequate blood pressure and contributes to thermogenesis during arousal.

Similarly, sustained contractions of skeletal muscles allow for maintained respiratory movements; in rodents, skeletal muscle metabolism also provides much of the heat for periodic arousal (*24*). The functions of other cells and tissues, particularly of the brain and peripheral nerves, are also patently protected on a long-term basis during the hypothermia of deep hibernation (*24*). Although some ionic redistribution occurs [for example, Mg^{2+} availability in the ECF increases (*32*)], the uncontrolled dissipation of ion gradients between different intracellular and extracellular compartments typical of cold-sensitive cells does not occur in hibernators. If it did, none of the above-mentioned membrane-based functions could be sustained. Evidently, something about cell membranes of hibernators differs from what occurs in membranes of cold-sensitive cells. Either, as in the ectotherm case mentioned above, cold-tolerant cells are fundamentally less permeable during hypothermia than cold-sensitive cells or they maintain higher ion pumping capacities. The latter mechanism is not very likely because metabolic rates during hibernation may be reduced by nearly two orders of magnitude (*3, 23*). Moreover, Na^+,K^+-ATPase studies (*31*) are illuminating; although the enzyme is cold-adapted in hibernators (displays higher catalytic capacities at low temperature, higher affinities for Na^+ and K^+, and probably higher affinity for ATP), as indeed would be expected for many enzymes in hibernators (*3, 29*), no single feature or combination of kinetic features is adequate to account for the low-temperature viability differences between cold-sensitive and cold-tolerant species. Thus we are led to the conclusion that cold-tolerant cells in hypothermia are able to maintain near-normal membrane functions (that is, maintain near-normal ion concentration differences between various internal and external compartments in spite of greatly reduced cell metabolic rates), in large part because they maintain cell membranes fundamentally less leaky than those in cold-sensitive cells. As argued above, this is the strategy utilized in anoxia by good animal anaerobes, and in both cases it can be viewed as a solution to a common problem; namely, that ATP synthesis capacities are potentially more depressed than membrane leaks. In the hypothermia of deep hibernation, the drop in ATP synthesis rates is due to a regulated metabolic depression at reduced cell temperatures, whereas in hypoxia it is due to reduced O_2 availability and the reversed Pasteur effect. To keep ion pump demands for ATP in balance with reduced ATP synthesis rates, animals seem to select for low-permeability membranes in response to both environmental parameters. The final problem, therefore, is how the two common adaptations are achieved. This in turn may supply insights into the application of analogous intervention strategies for protection against hypoxia (metabolic arrest mechanisms other than hypothermia).

Regulation of Membrane Permeability

Because regulated moment-to-moment changes in membrane permeabilities are requisite for normal function in most tissues and organs, any large permeability differences between homologous cold-tolerant and cold-sensitive tissues must be due to fundamental specializations in the way membranes are structured or in their regulated function. From what is currently known about membrane properties [see (*33*) for an excellent overview] such basic differences could arise from (i) change in the phospholipid composition and consequently in functional properties of membranes, (ii) change in the ratio of functional to nonfunctional ion-specific channels by cyclic nucleotide-controlled phosphorylation or by recruitment of channels from nonfunctional pools, or (iii) change in the abundance of "pores"; that is, in the density of ion-specific protein channels.

The first of these, requiring adjustments at the level of bilayer composition is probably of minimal importance, since a major function of bilayer adjustments is to lower the temperature for phase transition, thus allowing normal membrane fluidity and normal membrane functions despite lower cell temperatures. In fact, some adjustments in membrane-bound enzymes such as Na^+,K^+-ATPases may serve to facilitate function in the face of the new bilayer composition in the cold (*29*). In all animals such "homeoviscous adaptations" in effect contribute to functionally similar membrane functions at different cell temperatures and thus probably do not contribute heavily to any tissue permeability differences between cold-tolerant and cold-sensitive animals. This conclusion is consist-

Table 1. Several fundamental features of cells tolerant to hypoxia and hypothermia and of cells sensitive to hypoxia and hypothermia.

Hypoxia tolerant		Hypoxia sensitive
Metabolic arrest capacity (reversed Pasteur effect)		No metabolic arrest capacity (standard Pasteur effect)
Low-permeability membranes; low Na^+,K^+-ATPase	Intervention mechanisms ←	High-permeability membranes; high Na^+, K^+-ATPase
ATP synthesis rates equal ATP utilization rates	Transition from ectothermy to endothermy →	ATP synthesis rates cannot pace energy requirements for ion pumping
Stable membrane functions; stable ion gradients		Disrupted membrane function; dissipated ion gradients; Ca^{2+} influx
Hypothermia tolerant		**Hypothermia sensitive**
Regulated metabolic depression		Metabolic depression simply by Q_{10} effects
Low-permeability membranes	Intervention mechanisms ←	High-permeability membranes
ATP synthesis rates equal ATP utilization rates	Transition from hibernation to euthermy →	ATP synthesis rates cannot pace energy requirements for ion pumping
Stable membrane functions; stable ion gradients		Disrupted membrane function; dissipated ion gradients; Ca^{2+} influx

ent with results of tests showing no correlation between membrane fluidity as such and permeability to ions (*34*). This leaves the ion-channels option.

Regulation of Ion-Channel Densities

In assessing this matter, it is worth emphasizing, as Hille (*33*) has done, that even if bilayer adjustments influence permeability properties, they presumably do so mainly by changing the properties of proteins called ion channels. It is widely held that ions move across cell membranes through voltage-regulated or receptor-regulated aqueous pores or ion-specific channels, each having characteristic permeability, selectivity, and kinetics. In skeletal muscle and nerves, electrical excitation involves voltage- and time-dependent changes in Na^+, K^+, and Ca^{2+} permeabilities through Na^+-, K^+-, and Ca^{2+}-specific ion channels. At rest, Cl^- channels and other K^+ channels carry the dominant conductances [see (*35*) for example]. The Na^+ channel, perhaps the best understood of the identified ion channels, is now described down to subunit composition and is being analyzed at the subunit level. When solubilized, the Na^+ channel protein from both muscle and nerve (*36*) is about 316,000 daltons in size and consists of three nonidentical subunits. Transmembrane Na^+ flux is mediated by a hydrophilic pore containing a selective ion coordination site. By the selective use of neurotoxins, which bind with high affinity to specific sites on the Na^+ channel protein, it has become evident that Na^+ conductance through the channel is regulated or "gated" by controlling the rate and voltage dependence of opening and closing of the channel (*33, 36*).

It is generally held that such fundamental features as protein size, oligomeric structure, subunit composition, ion coordination site, pore size, and neurotoxin binding sites are common to the Na^+ channel protein in all cell membranes, from different tissues and from different species; that is, like channel proteins in general, Na^+ channels in particular appear to be highly conservative (*33, 36, 37*). Channel density per square micrometer of membrane surface, however, varies between and within tissues, and in different functional states within the same tissue. The number of channels per square micrometer in rat brain, for example, ranges from about 100 in unmyelinated axons to possibly over 10,000 at the nodes of Ranvier in myelinated nerves where the highest Na^+ fluxes are required (*36, 37*). In addition to the number of channels per unit area of membrane being regulated by long-term mechanisms, channel density may be modifiable on a moment-to-moment basis during transitions between different metabolic and physiological states. For example, recent studies of toad bladder indicate that antidiuretic hormone and aldosterone may influence the density of ion channels by controlled recruitment of preformed channels possibly stored in the cytosol (*38*). Similarly, the numbers of functional ion channels available for voltage-dependent activation are controllable by cyclic nucleotides and protein phosphorylation [see (*27, 33*) for further discussion of this subject]. Although additional studies on shorter term regulation of numbers of functional channels in membranes are needed, the evidence already available indicates that (at least in the long term) regulating channel density may be a universal way of meeting tissue-, cell-, and ion-specific permeability requirements in different microenvironments or different metabolic states. The common utilization of this strategy may explain the observed permeability differences in cell membranes of cold-tolerant and cold-sensitive mammalian cells (*24, 26, 31*) and in homologous tissues of good anaerobes versus hypoxia-sensitive endotherms (*20, 21*). Furthermore, exactly the same considerations hold in principle for membranes of mitochondria and of other intracellular organelles (*39*). That is why at least the tentative conclusion is that hypoxia- and hypothermia-tolerant tissues compensate for reduced ATP-dependent ion pumping capacities during O_2 lack or during cold exposure (i) by reducing the densities of functional channels per square unit of membrane surfaces in proportion to declining metabolic rate, or (ii) by maintaining the required low densities of functional ion channels all the time so as to be unstressed by declining ion pumping capacities. The net effect of either mechanism is to maintain the ratio of leakage rates to pumping rates at unity even during metabolic arrest of varying degrees.

Conclusions

From the above analysis, a simple and unifying picture of cellular mechanisms underlying tolerance to hypoxia and to hypothermia arises. In both conditions, an unavoidable (but patently desirable) depression in ATP synthesis rates is potentially greater than any

associated change in the passive leak of ions across membranes. In the absence of any additional adjustments, ATP synthesis rates could not match ATP requirements for sustained stable membrane functions. In cold-sensitive and anoxia-sensitive cells, this problem does not appear to be resolvable, and during prolonged anoxic or hypothermic exposure, ion gradients dissipate, intracellular Ca^{2+} concentrations rise and activate membrane phospholipid hydrolysis in a process that ultimately leads to cell damage or cell death. Anoxia-tolerant and hypothermia-tolerant cells void this Ca^{2+}-mediated pathogenic process by maintaining low-permeability membranes (possibly by means of lower ion-specific channel densities), so that the energy costs of ion pumping can be matched by the rates of ATP synthesis realizable under sustained hypoxic or hypothermic conditions (Table 1).

An insight arising from this interpretation is that critical provisions for tolerance of hypoxia overlap with provisions for tolerance to hypothermia. Cells and tissues protected against hypoxia should automatically be at least partially protected against hypothermia. It has been known for many years that the fetus and neonate in mammals display a greater hypoxia tolerance than the adult does (*40*); on the basis of the foregoing analysis, they should also be more tolerant of hypothermia, as is indeed observed (*41*). The reverse situation (mechanisms for protecting tissues against hypothermia being automatically protective against hypoxia) need not hold because other requirements (such as appropriate regulation of anaerobic metabolism, appropriate means for solving end-products problems, and so forth) may not be realizable. Still, it is perhaps worth noting that hibernating mammals have long been known to be unusually hypoxia-tolerant by mammalian standards (*8*). A part of this tolerance may arise from the metabolically depressed state of hibernation; another part may arise from the maintenance of less leaky cell membranes, at least in those tissues and organs whose sustained function is most crucial to successful hibernation.

Models such as the above are useful if they help to explain previously perplexing and poorly understood data and if they help in charting the way to further studies. The present model (Table 1) is helpful by the first criterion, but only future experiments can determine whether it is useful by the second. One experimental approach, for example, would interfere with processes utilized for sustained anoxic survival by relatively good anaerobes. It would aim to block or reverse metabolic arrest mechanisms typical of the anoxic state and preferentially open up ion-specific membrane channels so as to facilitate rapid dissipation of ion gradients. The present model would require that these manipulations convert anoxia-tolerant organisms, tissues, or cells (turtle as an example of an ectotherm system; fetus as an example of an endothermic one) simultaneously into anoxia-sensitive and hypothermia-sensitive ones. In metabolic terms, it would be tantamount to moving along the solid arrows in Table 1.

A second approach, the converse of the first, assumes that if ATP turnover rates in endothermic systems can be significantly curtailed and if "leaks" (that is, dominant ion-specific membrane channels) can be effectively blocked (so that ATP production rates can continue to pace rates of ATP utilization by ion pumping), then both anoxia tolerance and hypothermia tolerance should be greatly expanded. In metabolic terms, these manipulations would be analogous to moving along the dashed arrows in Table 1. If successful, studies along both experimental lines would have far-reaching theoretical and practical implications.

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