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CLASSICS

The origin of the 'channel arrest' hypothesis

Meldrum Robertson discusses Peter Hochachka's classic paper, 'Defense strategies against hypoxia and hypothermia', published in Science in 1986.

In mammals, the persistent demand for oxygen renders their tissues highly vulnerable to its absence, though this can be mitigated in hibernators, for example, by allowing body temperature to drop thus reducing metabolic rate. In contrast, invertebrates and some ectothermic vertebrates are remarkably resistant to hypoxia and their tissues can survive with low or no oxygen for prolonged periods. Understanding the cellular mechanisms responsible for this striking difference is important both to explain organismal diversity and to inform medical research aimed at discovering how to protect vulnerable tissues during oxygen insufficiency such as occurs during cardiac failure or stroke. By 1986, Peter Hochachka had already made many seminal contributions to our understanding of hypoxia tolerance in different organisms, notably promoting the concept that metabolic arrest is a particularly effective strategy for protecting tissues from a lack of oxygen. His 1986 article published in Science was an insightful analysis of the literature that led inescapably to the conclusion that much of the tolerance to hypoxia or hypothermia was a consequence of low ion permeability of cell membranes (Hochachka, 1986), providing a satisfying mechanism for the efficacy of metabolic arrest.

In re-reading this Hochachka classic, I was struck by the logic of his arguments, which epitomises the 'strong inference' method championed by John Platt (1964; see also Fudge, 2014) as being a way of thinking that enables rapid scientific progress. This was not just a review of the field at the time but a summary of diverse research, leading to clearly defined alternative hypotheses, which could be winnowed by argument to arrive at a conclusion that crystallized much of the debate at that time into a testable model. In the summary below, I follow the same progression but ignore much of the detailed discussion of the literature that Hochachka used to support his arguments.

The general approach in the article is to consider mechanisms of tolerance to hypoxia or hypothermia to determine the critical tissue vulnerabilities with an underlying goal of identifying targets for interventions that could protect tissues. The starting point is a summary of the arguments leading to the conclusion that metabolic arrest is the most effective organismal strategy to cope with hypoxia. As oxygen levels drop, tissues can switch from aerobic to anaerobic metabolism but a negative consequence is that the low efficiency of anaerobic metabolism results in increased fuel use if ATP production is to be maintained (the Pasteur effect). However, hypoxia-resistant organisms reverse the Pasteur effect by dramatically reducing ATP turnover rates as oxygen levels drop. This has the added benefit of reducing the production of potentially harmful end products. That artificially induced metabolic depression could be an important intervention strategy was suggested by clinical procedures used to reduce myocardial damage after cardiac arrest (Farber et al., 1981) and experimental manipulations of isolated organs like the kidney. Hochachka noted, however, that the protection afforded by such strategies is very much less than that possessed by anoxia-tolerant organisms. The next section in the article searches for the cause of the discrepancy.

With reference to neural function, which is particularly vulnerable to hypoxia, Hochachka marshals the evidence from the literature to conclude that the critical step leading to cell death under hypoxia is the failure of cell membrane function, resulting in a lethal influx of Ca^{2+} ions that disrupts several important metabolic cascades. Thus, for organisms to be anoxia tolerant, either the cell membranes are more impermeable to ions or ion pumping keeps pace with the tendency of ion gradients to be degraded. Of these alternatives, several arguments lead to the conclusion that non-leaky membranes are necessary for anoxia tolerance. Indeed, leaky membranes can be considered adaptive for endotherms because of their role in generating metabolic heat. Interventions that target Ca^{2+} influx generally had little success because they were not coupled with metabolic arrest, and it is the combination of non-leaky membranes and metabolic arrest that is protective in anoxia-tolerant organisms. Attempts to induce metabolic arrest usually use hypothermia, which will slow all biochemical reactions, but this approach has its own limitations because hypothermia also damages cell membranes, leading to a catastrophic loss of ion gradients.

At this point in the article, the reader has been led by argument and close analysis of the literature to the conclusion that cold tolerance (e.g. in hibernators) and anoxia tolerance (e.g. in anaerobes) share a solution to the common problem that, during exposure to their respective stresses, ATP synthesis is insufficient to cope with a leaky membrane. This solution is to select for low-permeability membranes (Fig. 1).

The final arguments in the article consider potential mechanisms that could account for impermeable membranes. Membrane fluidity is quickly discounted, which leaves the ion channels themselves. Given that mechanisms had been described for regulating the number and density of ion channels in the cell membranes of diverse tissues, and the observation that channel structure is relatively conservative across tissues and species, Hochachka concluded that 'regulating channel density may be a universal way of meeting

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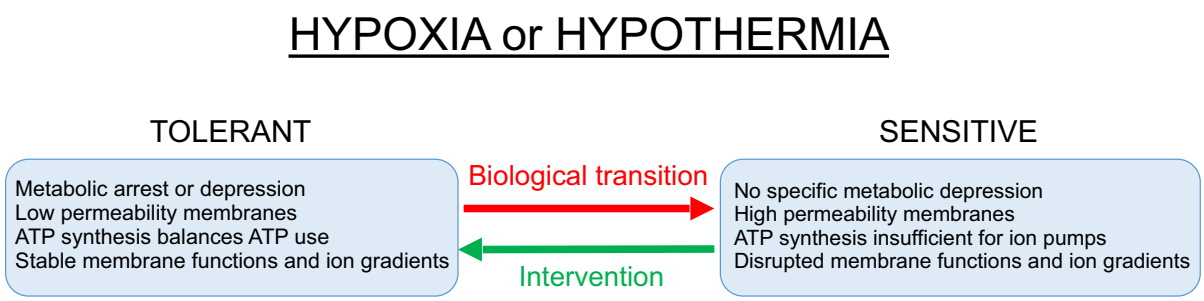

Fig. 1. Simplification of table 1 from Hochachka (1986), listing several fundamental features of cells that are tolerant or sensitive to hypoxia or hypothermia. The biological transitions (red arrow) imparting sensitivity are from ectothermy to endothermy (resulting in hypoxia sensitivity) and from hibernation to euthermy (resulting in hypothermia sensitivity). Hochachka suggested that sensitive tissues could be rendered more tolerant by interventions (green arrow) to block leaks through ion-specific membrane channels and thus reduce ATP turnover rates.

tissue-, cell-, and ion-specific permeability requirements in different microenvironments or different metabolic states'. This conclusion is eminently testable and he suggests two experiments: (1) block metabolic arrest mechanisms and dissipate ion gradients in anaerobes with the prediction that they would become anoxia and hypothermia sensitive; and (2) block ion leakage, which should increase tissue tolerance to anoxia and hypothermia.

This influential article is the origin of the 'channel arrest hypothesis' for anoxia and hypothermia tolerance. Interestingly, the phrase 'channel arrest' appears only once in the article: in the Abstract and in the context of an intervention strategy (i.e. experiment 2, above). The conclusion in the main body of the article focuses on variation in ion channel density in membranes as providing tolerance in organisms. Nevertheless, the phrase, and hypothesis, refers to the suite of mechanisms that would reduce ion flow across cell membranes and the article has a healthy citation history, receiving around 30 citations each year since it was published. The validity of the hypothesis was confirmed within a few years in the brain of anoxia-tolerant turtles (Edwards et al., 1989; Doll et al., 1991; Pérez-Pinzón et al., 1992) and in their hepatocytes (Buck and Hochachka, 1993). Not surprisingly, much subsequent research focused on CNS neurons, which are critically dependent on ion channels for function and are particularly sensitive to reductions in ATP supply because of the high energetic demands of the sodium pump. This led to an extension of the hypothesis to the notion of 'spike arrest', whereby neuronal activity is suppressed as a protective measure (see Jonz et al., 2015, for a review referencing both channel arrest and spike arrest).

My own interest in this classic article stems from my research into the effects of abiotic stressors on neural function in insects (locusts and fruit flies). Insects are renowned for their resistance to anoxia and hypothermia. During exposure to either stressor they enter a coma, which results in considerable metabolic arrest, and they can remain in such a coma for several hours without any obvious cellular damage or behavioural consequences (the damage-free duration is stress and species dependent). These comas are triggered by a phenomenon known as spreading depolarization (SD), during which there is an abrupt loss of ion gradients within neural tissue and a cessation of neural activity. Mechanisms of SD in insects are similar to those in vertebrate brains, and SD underlies several human brain pathologies (Spong et al., 2016). It is notable that the strategy is markedly different from channel and spike arrest by virtue of the partial, or complete, dissipation of ion gradients, rather than the preservation of them. Arguably this 'CNS arrest' can join spike arrest, channel arrest and metabolic arrest as a member of the group of arrest mechanisms that can protect organisms from environmental stress.

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