MEDICAL HYPOTHESIS

The physiology of artificial hibernation

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ABSTRACT

Incomplete understanding of the mechanisms responsible for induction of hibernation prevent translation of natural hibernation to its artificial counterpart. To facilitate this translation, a model was developed that identifies the necessary physiological changes for induction of artificial hibernation. This model encompasses six essential components: metabolism (anabolism and catabolism), body temperature, thermoneutral zone, substrate, ambient temperature, and hibernation-inducing agents. The individual components are interrelated and collectively govern the induction and sustenance of a hypometabolic state. To illustrate the potential validity of this model, various pharmacological agents (hibernation induction trigger, delta-opioid, hydrogen sulfide, 5'-adenosine monophosphate, thyroneamine, 2-deoxyglucose, magnesium) are described in terms of their influence on specific components of the model and corollary effects on metabolism.

Relevance for patients: The ultimate purpose of this model is to help expand the paradigm regarding the mechanisms of hibernation from a physiological perspective and to assist in translating this natural phenomenon to the clinical setting.

ARTICLE INFO

Article history:
Received: 5 May, 2015
Revised: 7 September, 2015
Accepted: 14 September, 2015
Published online: 30 September, 2015

Keywords:
anapyrexia
hypometabolic agents
hypometabolism
natural hibernation
topos
thermoneutral zone
hypoxia
body temperature
thermal convection
Arrhenius law

ABBREVIATIONS: 2-DG, 2-deoxyglucose; 5'-AMP, 5'-adenosine monophosphate; A;AR, A, adenosine receptor; A, anabolism; BAT, brown adipose tissue; C, catabolism; DOP, delta-opioid; H,S, hydrogen sulfide; POAH, preoptic anterior hypohalamus; Q, metabolism; Rx, pharmacological agent; S, substrate; TAM, thyroneamine; Tb, ambient temperature; Tb, core body temperature; Zn, thermoneutral zone.

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DOI: http://dx.doi.org/10.18053/jctres.201502.005
1. Introduction

Metabolic homeostasis is key to physical function, justifying its meticulous regulation and powerful governing mechanisms in every living cell. The ability to artificially and reversibly reduce metabolism could provide many advantages for medicine, sports, and aviation. However, despite a growing understanding of our ability to regulate the mechanisms that govern metabolism at the cellular level, translation of metabolic control in cells to a whole organism has remained beyond our reach.

In nature, reversible states of hypometabolism are a common trait. Members of eight species, including a variety of rodents, carnivores (bears), and primates (lemurs) are known to exhibit a type of hypometabolism [1]. Although biological vernacular varies between many different types of hypometabolism (Figure 1), two types can generally be distinguished within animals that have an endogenous thermoregulatory system: a deep and a shallow type of hypometabolism. The difference lies in the depth of the drop in body temperature ($T_b$) and the duration. Shallow hypometabolism represents a temporary type, as exhibited during torpor by e.g., the house mouse, whereas deep hypometabolism is a more sustainable type, as is found in e.g., the hibernating ground squirrel [1, 2].

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Classification of the different types of hyper- and hypometabolism and the official biological vernacular. Endogenous thermoregulation occurs through the modulation of the thermoneutral zone ($Z_{tn}$) and thermal effectors, whereas exogenous thermoregulation is dependent on the ambient temperature ($T_a$) and exogenous triggers but not the $Z_{tn}$.

The mechanism(s) responsible for the induction of hypometabolism remain(s) controversial [1]. Unfavorable environmental circumstances appear to be a common denominator in hibernating animals, including seasonal cooling, light deprivation, and prolonged starvation. However, the use of such external triggers to induce artificial hibernation in humans has proven to be of no avail. Irrespective of the external cues, an internal physiological signal, or perhaps a concerted cascade of signals, must initiate, propagate, govern, and sustain hypometabolic signaling *in vivo*. In an attempt to find such a signal, much research has focused on (bio)chemical signaling during hibernation and its induction, leading to the identification of several hypometabolic agents [3-7]. However, despite their discovery and extensive research in various animal models, none of the identified agents appear to induce a hypometabolic state ubiquitously across species, as a result of which not a single hypometabolic agent has made it yet to (pre)clinical application. Currently, the only metabolic control that is clinically employed is forced hypothermia-induced hypometabolism, although this type of hypometabolism fails to achieve the depth found in natural hibernators and comes with challenging limitations.

In an attempt to expand on current insights into hypometabolism, this review addresses the conditions necessary for the induction of hypometabolism and the possible initial (bio)chemical triggers. Accordingly, a theorem on the induction of hypometabolism is presented, whereby the relationship between key physiological and environmental factors is provided as a framework to explain the physiological cascade that leads to a sustainable and reversible state of hypometabolism in mammals. Readers should note that the focus of this paper is on mainly the physiological and biochemical conditions required for the induction of hypometabolism. Although neurological signal relay is key to convey, propagate, and sustain a state of hypothermia and hypometabolism, the responsible neurological pathways have only been superficially addressed. These pathways will be elaborated in more detail in a subsequent, separate review.

DOI: [http://dx.doi.org/10.18053/jctres.201502.005](http://dx.doi.org/10.18053/jctres.201502.005)
2. A model for hypometabolic induction

2.1. Control of metabolism through temperature and substrate availability

A pivotal step in the induction of artificial hypometabolism is gaining control over the most important factors that regulate metabolism (Q), namely the availability of substrate (S, i.e., oxygen and glucose) and the rate of adenosine triphosphate (ATP) production (anabolism, A) and consumption (catabolism, C), which are both influenced by the core body temperature (Tb). Hence, a direct relationship exists between Tb and Q (Figure 2) as well as between S and A (Figure 2). The relationship between Tb and Q essentially abides by Arrhenius’ law, which states that the chemical (i.e., enzymatic) reaction rate, Q, is reduced as a result of lowering of temperature (Equation 1).

![Figure 2](image)

**Figure 2.** Substrate and temperature effects on metabolism. The S—Q relationship relies on substrate (S) availability to support anabolism (A). The Tb—Q relationship is dictated by the Arrhenius equation (Equation 1, where k is equal to Q in this model), which governs the relation between body temperature (Tb) and chemical reaction speed (Q). Catabolism (C) is directly affected by Tb, but not by S.

**Equation 1**

Arrhenius equation:

\[
R = \text{universal gas constant (J·K}^{-1}·\text{mol}^{-1})
\]

\[
A = \text{prefactor (s}^{-1})
\]

\[
E_a = \text{activation energy (J·mol}^{-1})
\]

\[
T = \text{temperature (K), determined by } T_b \text{ in the proposed model (Figure 11)}
\]

\[
k = \text{rate constant (s}^{-1}) \text{, identical to } Q \text{ in the proposed model (Figure 11)}
\]

Although the magnitude of Q varies among enzymes, all have in common that Q is temperature-dependent and therefore relies on Tb. The directly proportional effect of Tb on Q is in turn affected by the ambient temperature (Tamb), which impacts Tb and hence Q through heat exchange (Figure 2). The (Tamb—Tamb)—Q relationship is widely exploited in the clinical setting, as exemplified by the contrived reduction in patients’ Tb through direct or indirect cooling (e.g., reduction in Tb by means of breathing cold air, cutaneous cooling, organ perfusion with a cold solution, or intravascular cooling) as a protective strategy in surgery [8, 9], neurology [10], cardiology [11], trauma [12], and intensive care [13]. The protective effects of mild to moderate hypothermia (Tb reduction to ~35-32°C) have been ascribed to lower radical production rates, ameliorated mitochondrial injury/dysfunction, reduced ion pump dysfunction, and cell membrane leakage, amongst others [14]. The majority of these factors is directly related to the rate at which chemical reactions proceed, whereby cytoprotection is conferred by a Tb-mediated reduction in Q in accordance with the Arrhenius equation (Equation 1).

The generally protective effects of hypothermia notwithstanding, the advantage of clinically forced hypothermia-induced hypometabolism is questionable in some instances. At mild hypothermia (~35°C), serum concentrations of norepinephrine start to rise in response to hypothemic stress, coagulopathy starts to develop, susceptibility to infections increases, and mortality rates are negatively affected [15-17]. When the Tb is lowered further to ~30°C, severe hypothermia-related complications may occur, including ventilatory and cardiac arrest [14, 18]. An even more profound reduction in Tb would require mechanical ventilation with extensive monitoring and would considerably increase procedural risks. Accordingly, in the last five decades, the limit of ~30°C has not been adjusted downward in the clinical setting as much as it has been refined, despite of successful animal experiments with much deeper hypothermia [19-22].

The detrimental effects associated with forced deep hypothermia reflect the limits of the practical implementation of the (Tamb—Tb)—Q relationship. It is evident that the (Tamb—Tb)—Q relationship must be differentially regulated in natural hibernators compared to humans. In natural hibernators the Tb—Q effects may be integratively mediated by endogenous signaling, such as by the release of biochemical agents or by hypoxia (both addressed in detail below). Humans essentially lack such endogenous pathways and do not exhibit hypothermia-related benefits from exogenously administered pharmaceuticals or hypoxia, as a result of which Q cannot be actively adjusted downward by other pathways than through the Tb—Q relationship. The distinctive responsiveness to hypothermia between humans and natural hibernators may be due to differential neurological and biochemical regulation of Tb, both of which act via mechanisms related to thermogenesis and heat loss. Thermoregulation and the role of thermogenic and heat loss effectors is therefore addressed in the following section.

2.2. Thermoregulation following a shift in the thermoneutral zone

The chief role of thermoregulation is maintenance of Tb to support an optimal thermodynamic environment for all chemical reactions in the organism, which is around 37°C in humans. Thermogenic control is believed to rely on several...
neurological pathways, which includes involvement of the preoptic anterior hypothalamus (POAH). Together, these pathways manage a thermoneutral zone (Z\textsubscript{tn}) which provides a range in which the T\textsubscript{b} is to maintain itself, and outside of which the T\textsubscript{b} is to be adjusted towards the Z\textsubscript{tn} through the use of thermogenic effectors and heat loss effectors (Figure 3).

Although it is difficult to ascertain that a single location among the thermoregulatory pathways can have an effect on the Z\textsubscript{tn}, it is generally accepted that the POAH exerts such an effect in hibernating and non-hibernating animals based on indirect experimental and clinical evidence [23,24]. In humans, incidental but selective destruction of the hypothalamic region is associated with dysfunctional thermoregulation, as evidenced by passive T\textsubscript{b} declines to as low as 29 °C [25-29]. Moreover, exposure of hypothalamiically impaired patients to low T\textsubscript{s}s causes a drop in T\textsubscript{b}, whereas the same conditions induce a rectifying rise in T\textsubscript{b} in ‘control’ subjects [28,30], attesting to impaired thermoregulatory capacity in hypothalamiically afflicted patients. Altogether, these reports provide compelling evidence for a temperature integration site in the hypothalamus through which management of Z\textsubscript{tn} and T\textsubscript{b} ensures maintenance of euthermia.

On the basis of these findings it can be concluded that POAH-affected subjects exhibit a sensory defect between Z\textsubscript{tn}—T\textsubscript{b} (Figure 3A), as a result of which T\textsubscript{b} will approximate T\textsubscript{a} in the absence of thermoregulation. Contrastingly, healthy subjects exhibit a reactive effect between Z\textsubscript{tn}—T\textsubscript{b}, whereby T\textsubscript{b} is sustained in conformity with the Z\textsubscript{tn} irrespective of the T\textsubscript{s} via activation of thermogenic effectors (Figure 3B). Accordingly, these data imply that, under normophysiological circumstances, Q is mainly regulated by the Z\textsubscript{tn} via Z\textsubscript{tn}—T\textsubscript{b}—Q such that the optimal thermodynamic conditions (37 °C) are at all times maintained. POAH-mediated thermoregulation also takes place in rodents that are capable of entering a state of torpor. Corroboratively, selective infarction of the anterior hypothalamus in rats coincides with the inability to regulate T\textsubscript{b}, indicating destruction of pathways that govern the Z\textsubscript{tn} and abrogation of thermoregulatory function [31].

As opposed to humans, Z\textsubscript{tn} management in smaller animals (e.g., rodents) may veer from a euthermic regime in some species due to specific changes in environmental conditions. One exemplary condition is hypoxia, which is addressed in section 2.3 to illustrate the relationship between S (oxygen) and Q.

Before moving to the S—Q relationship in the context of hypoxia, however, it is imperative to address hypothermia as a function of an organism’s surface:volume ratio, or the ease with which heat exchange between T\textsubscript{b}—T\textsubscript{a} can proceed. Small animals have a high surface:volume ratio compared to large animals, which allows for faster heat dissipation and results in subsequent lowering of T\textsubscript{b} when exposed to cold environments. A high convective efficiency is essential for the induction of hypothermia, and is dependent on the heat loss properties such as the animal’s skin phenotype, breathing pattern, the extent of skin exposure, and the animal’s posture and physical activity [32]. In addition to such effects, small animals require a higher metabolic rate than larger animals to sustain their T\textsubscript{b} (Kleiber’s law [33]), which causes small animals to become more easily affected by low T\textsubscript{S}s. As a result, it takes considerably less time to lower the T\textsubscript{b} and coincidentally the Q of a mouse compared to those of a human. This effect is reflected in the strong correlation between the ability and depth of hibernation and surface:volume ratio, which indicates that virtually all hibernating animals are small (i.e., high surface:volume ratio) and that increased body size is associated with a decreased depth of the T\textsubscript{b} drop during hibernation/torpor [34]. Hence it appears that environmental/biochemical modulation of metabolism is more prevalent in small animals and subject to the effectiveness of T\textsubscript{b}—T\textsubscript{a} heat exchange.

2.3. Hypoxia-induced hypometabolism: aligning anabolism with catabolism

The relationship between S and Q is to an extent regulated by the intracellular oxygen tension insofar as oxygen constitutes a vital S for Q (Figure 4). During hypoxia, Q is impaired because of insufficient oxygen availability for oxidative phosphorylation, resulting in reduced cytochrome c oxidase function [35] and cessation of ATP production (A). Under hypoxic but normothermic conditions, the ATP consumption rate (C) can be suppressed to match the ATP production rate, but only to a limited extent and for a short time [36]. In order to survive during a long period of normothermic hypoxia, an organism’s metabolism must remain active to fuel metabolically vital processes, which include protein synthesis (25-30% of total ATP consumption), ion homeostasis (23-36%), gluconeogenesis (7-10%), and ureagenesis (3%) [37]. The limited production yet active consumption of ATP during normothermic hypoxia therefore forces the organism to initially
switch to anaerobic respiration (Pasteur effect) – a switch that imposes limits on the maximally tolerable duration of hypoxia/anoxia due to inefficient ATP yields from glycolysis and the production of toxic metabolites such as lactic acid.

In several non-hibernating species, exposure to a prolonged period of hypoxia concurs with regulated hypothermia and hypometabolism, suggesting that hypoxia-mediated thermogenic and metabolic suppression constitutes a protective/coping mechanism for such life-threatening conditions [38-41].

This hypoxic stress response, illustrated in Figure 5, is in fact an effective survival mechanism in that the catabolic rate is realigned with the limited anabolic rate caused by hypoxia, which is in part achieved by the lowering of $T_b$ through the inhibition of thermogenesis and activation of heat loss mechanisms (explained in section 2.4). In that respect, the hypoxic stress response essentially embodies a pre-programmed manifestation of Arrhenius’ law. During this process, the $Z_m$ must either shift downward or be biochemically inhibited in order to resolve the incongruity between the hypothermic $T_b$ and the euthermically ranged $Z_m$. A reduction in $T_b$ (and consequently $Q$) resulting from a downward adjustment of the $Z_m$ is referred to as anapyrexia (Figure 6A), as opposed to pyrexia, which comprises an elevated $T_b$ as result of an elevated $Z_m$ (i.e., fever). How anapyrexia is mediated under hypoxic conditions in animals is elusive. The effect of anapyrexia on thermoregulatory effectors, on the other hand, is not and provides useful information on the hypoxia-anapyrexia signaling axis.
The onset of hypoxia, i.e., low substrate (S = oxygen) levels, is proposed to modulate the thermoneutral zone (Z\text{tn}) downward via a so-called hypoxic link. The lowering of the Z\text{tn} inhibits thermogenesis and activates heat loss mechanisms through the Z\text{tn}–T\text{b} relationship, allowing heat exchange between body temperature (T\text{b}) and ambient temperature (T\text{a}) to occur. The consequent reduction in T\text{b} slows down both anabolic (A) and catabolic (C) metabolism (Q) as described in Figure 2. (B) Pathways leading to anaperyxia via the R\text{ox}–Z\text{m} and S–Z\text{m} relationships. Although this relationship is exemplified for hypoxic conditions, where S comprises oxygen, it may also apply to conditions where another S is reduced, such as glucose during periods of starvation. Prolonged hypoglycemia is known to also induce hypometabolism, as addressed in section 2.5.4. A direct anaperyxia pathway is suggested for R\text{ox}–Z\text{m}, where a neuroactive agent such as delta-opioids directly lowers the Z\text{m} (section 2.5.5). Alternatively, an R\text{ox} such as H\text{2}S can also affect the Z\text{m} without affecting S availability by inducing hypoxic signaling through oxygen sensors such as carotid bodies (section 2.5.1).

2.4. The effect of hypoxia-induced anaperyxia on thermoregulatory effectors

Hypoxia-induced anaperyxia is found in a large number of species, including mice [39,42], hamsters [43], rats [38,44], pigeons [45,46], dogs [42,47], primates [41], and man [42], and manifests itself when the organism is concurrently exposed to low T\text{a}. A low T\text{a} appears to be a prerequisite for an anaperyxic response to hypoxia, as an anaperyxic response during hypoxic euthermic conditions is absent. The main question, however, is how hypoxic signaling decreases the Z\text{m} to facilitate hypothermia.

The answer may entail an effect of hypoxia on thermogenic effectors (Figure 6A), such as BAT and shivering (Figure 3B). The inhibitory effects of hypoxia on the intensity of cold-induced BAT activity include a lower afferent blood flow to BAT [48], reduced sympathetic nerve activity [49], desensitized response to norepinephrine (a potent BAT activating agent [50,51]) [52], and can eventually lead to a reduction in BAT mass during prolonged exposure to hypoxia [53,54]. In addition, hypoxia results in the inhibition of shivering upon exposure to low T\text{a} compared to normoxic controls in mice, dogs, and man [42]. Moreover, some species further reduce their T\text{b} through changes in behavioral patterns, such as disengagement from cold-induced huddling [55] or exhibiting an explicit preference for cooler environmental temperatures [56].

Considering these effects, it can be hypothesized that hypoxia either acts directly on BAT and muscle tissue (shivering) or indirectly inhibits these thermogenic effectors via central regulation, the Z\text{m}. The latter is a more likely mechanism of action since anaperyxic signaling controls both BAT and shivering in order to facilitate hypothermia. Poor blood oxygenation, a result of exposure to hypoxia, is relayed to the brain via the carotid bodies, which is described by a R\text{ox}–Z\text{m} relationship (Figure 6B). CBs are oxygen sensing bodies located alongside the carotid artery that contain oxygen-sensitive chemoreceptors through which they provide essential neuronal feedback on the arterial partial oxygen pressure [57]. Excitation of the CBs by reduced oxygen levels during hypoxia possibly induces lowering of the Z\text{m} to activate heat loss effectors (Figure 3B) so as to facilitate the induction of hypothermia with the sole purpose of aligning ATP consumption rates with ATP production rates as part of the survival response to stress conditions (section 2.3). Naturally, this response prevails in species that have a sufficiently high T\text{a}–T\text{b} convective efficiency to allow rapid manifestation of hypothermia and corollary reduction in Q (section 2.2), given that sustenance of life by anaerobic metabolism is time-limited. CBs are therefore an important instrumental component of the ‘hypoxic link’ in smaller species.

The existence of a ‘hypoxic link’ to the Z\text{m} (Figure 6) has been suggested [58] but lacks direct evidence other than the previously mentioned changes in thermal effectors. This link implies that, under cold but normoxic conditions, the Z\text{m} enforces an array of physiological tools that coordinate a thermogenic response, such as shivering, activation of BAT, vasoconstriction, and piloerection (Figure 3B). Under hypoxic conditions, however, these thermogenic responses are dampened or even absent and coincide with activation of heat loss effectors such as vasodilation, sweating, and panting (Figure 3B) [58]. Direct measurement of changes in the Z\text{m} range would be useful in substantiating the ‘hypoxic link.’ Unfortunately, due to incomplete knowledge of Z\text{m} functionality and technical difficulties related to reaching the neural pathways involved, it is currently very difficult to directly measure the range of the Z\text{m} or changes therein.

In summary, hypoxia (low S levels) leads to hypometabolism potentially by signaling anaperyxia through CBs, thereby allowing the body to cool via the S–Z\text{m}–(T\text{a}–T\text{b})–Q relationship. The hypoxia-induced anaperyxic component provides an advantage over the T\text{b}–Q relationship in that the anaperyxia allows the T\text{b} to drop below normothermia, preventing the stress response that would otherwise be needed to keep the body normothermic. Nevertheless, it is unlikely that these pathways constitute all necessary conditions for the induction of natural hibernation. A closer look at (bio)chemical agents (R\text{ox}) that have the ability to induce or mimic ‘anaperyxia-driven hibernation’ present additional pathways, namely through their effect on S availability, the relay of S availability to the Z\text{m}, and through direct effect on Z\text{m}.
2.5. Pharmacological agents and induction of artificial hypometabolism

Induction of hypometabolism in natural hibernators normally occurs in response to environmental triggers such as low T_b and light and/or food deprivation. The internal (bio)chemical trigger responsible for the subsequent propagation of this signal is key to understanding the hibernation process. It has been suggested that a yet to be characterized endogenous molecular compound, referred to as hibernation induction trigger (HIT), is responsible for inducing hibernation in vivo [59,60] via the R_T=Q(−T_b) relationship, whereby the drop in T_b is arguably a consequence of the reduced Q by the HIT (Figure 7). It should be noted that this mechanism would differ fundamentally from anapyrexic signaling, which requires Z_m downmodulation and subsequent T_s−T_b equalization as a precursor event for hypometabolic induction (Figure 6). In an effort to identify the HIT, different pharmacological agents (R_s) have been shown to trigger hypometabolism through CB signaling. Exogenously applied H_2S, via a soluble NaSH precursor, can mimic the production of this hypoxic signal in vitro [75], suggesting the utilization of the ‘hypoxic link’ (R_c−Z_m) by H_2S (Figure 6B). This is supported by the observed dichotomy between H_2S-induced effects in small versus large species, where H_2S produces hypometabolic effects in small species but not in larger species (Figure 8). The R_s−Z_m−T_b−Q relationship is dependent on a high T_s−T_b convective efficiency; a property that prevails strictly in small species due to their high surface:volume ratio (section 2.2).

2.5.1. Hydrogen sulfide

Exposure to H_2S consistently produces a hypometabolic state in small animals such as mice and rats (Figure 8, outer ring) [3,62-66]. However, the use of H_2S in larger animals such as pigs [67-70], sheep [65], and heavy rats [71] has failed to induce a hypometabolic response (Figure 8, inner ring). The current mechanistic paradigm of the hypometabolic effect of H_2S is based on its high membrane permeability and direct inhibitory effect on cytochrome c oxidase in the electron transport chain (i.e., through the R_c−A relationship) [72,73]. However, increasing evidence indicates the hypometabolic effects are the result of hypoxic signaling. For example, endogenously produced H_2S is necessary for CBs to signal hypoxia [74]. Exogenously applied H_2S, via a soluble NaSH precursor, can mimic the production of this hypoxic signal in vivo, suggesting the utilization of the ‘hypoxic link’ (R_c−Z_m) by H_2S (Figure 6B). This is supported by the observed dichotomy between H_2S-induced effects in small versus large species, where H_2S produces hypometabolic effects in small species but not in larger species (Figure 8).

2.5.2. Adenosine monophosphate

Intraperitoneal injections of 5′-AMP have been shown to induce an artificial hypometabolic state in mice and rats as evidenced by a profound drop in T_b (Figure 8) [61,76-78]. The putative contention is that intraperitoneal administration of high 5′-AMP concentrations (e.g., 500 mg/kg) lead to extensive 5′-AMP uptake by erythrocytes [79], after which the high intracellular levels of 5′-AMP drive the adenylate equilibrium (ATP + AMP ⇌ 2 ADP) towards production of ADP, thereby depleting erythrocyte ATP levels [76,77]. As a result, erythrocyte 2,3-disphosphoglycerate is upregulated, limiting the binding of oxygen to hemoglobin’s oxygen binding sites (referred to as oxygen affinity hypoxia) [76]. In addition to the already impaired oxygen transport, the severe cardiovascular depression following 5′-AMP administration has the potential to further exacerbate this hypoxic state (referred to as circulatory hypoxia) [78]. Although there is no conclusive evidence that these types of hypoxia have the ability to induce an anapyrexic state, the generally pervasive hypoxic state likely uses the S−Z_m−T_s(−T_a)−Q relationship (Figure 6A) to induce hypometabolism through CB signaling.

More recent studies have implicated a direct 5′-AMP signal transduction route to the central nervous system in seasonal hibernators, culminating in the induction of torpor [80-83]. 5′-AMP signaling occurs via the A_1 adenosine receptor (A_1AR), which is ubiquitously distributed throughout all tissues, but not the A_2AAR or A_3AR receptors. In the brain, A_1AR signaling leads to deceleration of metabolic rate and induction of a torpor state in arctic ground squirrels [84]. In this species, intracerebroventricular administration of the A_1AR antagonist...
Figure 8. Physiological effects of hibernation inducing agents. Overview of physiological effects in response to 2-deoxyglucose (2-DG), 5'-adenosine monophosphate (5'-AMP), hydrogen sulfide (H2S), hibernation induction trigger (HIT), delta-opioid (DOP), and thyronamine (TAM). Each effect is represented by a black dot (lowering of value), transparent square (equal value), or black triangle (rise of value). The effects are stratified according to the size of the animal, from outer ring to inner ring these are: < 0.1 kg (e.g., mouse), 0.1-1 kg (e.g., rat), 1-10 kg (e.g., macaque) and > 10 kg (e.g., pig). The inner white ring indicates the respective reference (number) and species (letter). Animals: A, house mouse (*Mus musculus*, Linnaeus); B, deer mouse (*Peromyscus maniculatus*, Wagner); C, djiangarian hamster (*Phodopus sungorus*, Pallas); D, common rat (*Rattus norvegicus*, Berkenhout); E, thirteen-lines ground squirrel (*Spermophilus tridecemlineatus*, Mitchill); F, domestic dog (*Canis lupus familiaris*, Linnaeus); G, rhesus macaque (*Macaca mulatta*, Zimmermann) or southern pig-tailed macaque (*Macaca nemestrina*, Linnaeus); H, domestic pig (*Sus scrofa domesticus*, Erxleben); I, sheep (*Ovis aries*, Linnaeus). The physiological parameters include: T, core body temperature; VO2, oxygen consumption; M, motion; RR, respiratory rate; HR, heart rate; U, urine production; BP, blood pressure; RQ, respiratory quotient; CO, cardiac output; CO2, carbon dioxide production; PP, pulmonary pressure. All parameters are expected to be reduced during hypometabolism.
cyclopentyltheophylline reversed spontaneous entrance into torpor during hibernation season, whereas administration of the A₁AR agonist N(6)-cyclohexyladenosine induced torpor [82]. Later studies confirmed the manifestation of 5'-AMP-driven torpor in non-hibernators, including the mouse [80] and rat [83], suggesting that the hypothermia- and hypometabolism-inducing potential of 5'-AMP is pleiotropically applicable across species. Although the signaling is of more direct neurochemical nature compared to the hypoxic signaling, the S—Zᵐ—inhibiting Tᵃ—Tₜ(—Tᵢ)—Q relationship (Figure 6A) also holds for the 5'-AMP-A₁AR signaling axis.

As indicated previously, the depth of the hypometabolic response is dependent on the Tᵇ—Tₜ convective efficiency (section 2.2). This is further supported by the extent of the drop in Tₜ that is observed in 5'-AMP-treated mice, which is proportional to the difference between Tₜ and Tₜ( i.e., lower Tₜs induce a greater drop in Tₜ), demonstrating the Tₚ—Tₜ dependency [76].

2.5.3. Thyronamines

TAMs are a thyroid hormone-derived group of compounds of which currently nine structural analogues have been identified [6]. Contrary to the structurally similar metabolism-enhancing thyronines (T₃ and T₄), exposure to TAM analogues triggers a transient Tₚ depression in small animals, epitomizing the induction of a hypometabolic phase (Figure 8) [6,85-88]. Although earlier studies in a canine model presented contradictory evidence with respect to metabolic effects compared to later studies (Figure 8, cardiac output), it is likely that this was a result of differences in synthesis methods and compound purity [87,89]. Nevertheless, the metabolic effects of TAMs remain obscure, regardless of the synthesis method.

Both in vivo and ex vivo studies have found the physiological effects of TAMs to be mainly cardiogenic in nature, producing severe hemodynamic depression, bradycardia, hypotension, and reduced cardiac output [6,85,90]. These effects result in reduced oxygen levels (affecting the Rₓ—S—Zᵐ relationship, Figure 6) by lowering the extent of blood oxygenation in accordance with Fick's principle, which describes an inverse relationship between cardiac output and oxygenation (circulatory hypoxia) [91, 92]. Although the effect of circulatory hypoxia on the Zᵐ has not been demonstrated, the presence of hypoxia in the broader sense may support the implication of the S—Zᵐ—Tₚ(—Tₜ)—Q relationship (Figure 6A) as a basis of the observed hypometabolism in smaller animals.

Given the magnitude of the hemodynamic collapse in small species, TAMs may additionally render the animal motionless (Figure 8, motion), which would facilitate a greater rate of thermal convection (Tₚ—Tₜ) and therefore accelerate the consequent reduction in Tₚ and Q (Figure 2).

2.5.4. Deoxyglucose

In addition to oxygen, S can also comprise glucose, as evidenced by the induction of a hypometabolic state upon glucose deprivation in hamsters and various types of mice [4, 93-95]. Shortage of food, and with that the ability to use glucose as energy substrate, has the potential to induce hypometabolism or alter its depth [94-96]. This principle has been demonstrated in hamsters using 2-DG, a glucose analogue that cannot undergo glycolysis as a result of the 2-hydroxyl group, which causes the animals to enter a hypometabolic state [4,93]. The hypometabolic response to 2-DG, measured by the drop in Tₚ, is reflective of the S—A relationship (Figure 4). Currently there is no evidence that supports the S—Zᵐ pathway for 2-DG.

2.5.5. Delta-opioids

Isolation and characterization of the HIT found in serum of hibernating woodchucks and squirrels revealed a resemblance to D-Ala-D-Leu-5-enkephalin, a non-specific DOP receptor agonist [5,97]. Consistent with this characterization, DOPs induce a hypometabolic state of similar depth as the HIT, whereas mu- or kappa-opioid receptor agonists show no effect [97,98]. The involvement of opioid receptors is further substantiated by the inability of DOP to induce hypometabolism upon exposure to naloxon, a non-specific opioid receptor antagonist [99-102]. These findings raise the question whether DOPs make use of a direct Rₓ—Q relationship or act via the Rₓ—Zᵐ (Figure 6). Growing evidence suggests the latter, as delta-opioids appear to be directly involved in hypoxic signaling. In mice, exposure to hypoxia has been suggested to decrease Zᵐ via delta-1-opioid receptor agonism [103-106]. Other studies have suggested that the delta-2 opioid receptor rather than a delta-1 opioid receptor is responsible for the effects on the Zᵐ [107-109], supported by the limited presence of delta-1-opioid receptors in the hypothalamic region [110,111]. However, in both cases the preferred pathway to Zᵐ modulation appears to involve a direct Rₓ—Zᵐ relationship.

2.6. Pharmacological agent properties and the feedback loop

As demonstrated by the different mechanisms discussed in the previous section, Rₓ can affect Q via three potential pathways, namely via Rₓ—Q(—Tₜ) (Figure 7, suggested for HIT), via Rₓ—S—Zᵐ—Tₚ—Q (Figure 6, suggested for H₂S, S'-AMP, TAM, and 2-DG), and via Rₓ—Zᵐ—Tₚ—Q (suggested for DOP). However, irrespective of the hypometabolic pathway, it is unlikely that the systemic release of a single Rₓ accounts for the hypothermic/hypometabolic induction process. Instead, as is the case in many biochemical pathways, it is more probable that the induction of hypometabolism is governed by a signal amplifying feedback loop (Figure 9).

With respect to the model, the ideal properties of an Rₓ regarding its regulatory function of the feedback loop encompass 1) endogenous production and/or release during induction of hibernation, 2) inhibitory effects on both A and C, 3) downregulatory effect on Zᵐ, 4) equal distribution throughout the body, and 5) availability of agonists and antagonists to accelerate and abrogate Rₓ-mediated signaling, respectively. Although currently there is no sound evidence for the existence of an Rₓ feedback loop, such a mechanism is theoretically
necessary to propagate a hypometabolic signal \textit{in vivo}.
Consequently, a mechanistic framework for such a feedback
loop will be elaborated for magnesium (Mg\textsuperscript{2+}), which appears
to play an important role in hibernation across different species.

\textbf{Figure 9.} Signal amplifying feedback loop during hypometabolic induction.
Theoretically, lowering of the body temperature ($T_s$) could be part of a feedback
system that triggers the release of a metabolism inhibiting agent ($R_x$) capable of
further lowering metabolism (Q) via direct inhibition of anabolism (A) and/or
catabolism (C). This process is embodied by the $T_s$—$R_x$—Q relationship.

Induction of hibernation coincides with a change in serum Mg\textsuperscript{2+} concentration (Figure 10). Serum Mg\textsuperscript{2+} levels increase by
an average of 1.6-fold upon induction of hibernation in different species compared to their summer active state, which is a considerably higher increase than observed for other electrolytes. The release of Mg\textsuperscript{2+} into the circulation occurs from storage pools that have formed prior to induction of
hibernation in cells that comprise muscle (Figure 10) and skin tissue [112, 113]. The translocation of Mg\textsuperscript{2+} from tissue to blood and subsequent systemic distribution is in conformity with the first $R_x$ property, i.e., the release of an endogenous agent during the induction of hibernation.

In regard to the second property, Mg\textsuperscript{2+} exerts an inhibitory effect on Q, affecting both A and C. Mg\textsuperscript{2+} acts as a necessary co-factor in over 300 enzymatic reactions [138]. When the Mg\textsuperscript{2+} concentration exceeds the saturating concentration required for occupying all substrate binding sites, Mg\textsuperscript{2+} becomes an inhibitor of enzymatic activity [138]. The inhibitory properties of Mg\textsuperscript{2+} are not limited to the inhibition of A, such as reduction of state III respiration (ADP-stimulated respiration) upon exposure to Mg\textsuperscript{2+} [139], but also include inhibition of C, such as reduction of Na/K-ATPase activity [140]. In addition, Mg\textsuperscript{2+} inhibits ion channels, such as the NMDA receptor ion channel and voltage gated ion channels [141-143]. Although inhibition of A and C are essential in sustaining a prolonged state of hypometabolism, as occurs during hibernation, this $R_x$ property has been largely ignored in reports on conventional hibernation-inducing $R_x$
agents (i.e., H\textsubscript{2}S, 5'-AMP, TAM, 2-DG, and DOP).

\textbf{Figure 10.} Electrolyte changes during induction of natural hibernation.
Analysis of blood levels of magnesium (Mg\textsuperscript{2+}; serum $n = 23$, muscle $n = 9$),
calcium (Ca\textsuperscript{2+}; serum $n = 19$, muscle $n = 5$), sodium (Na\textsuperscript{+}; serum $n = 16$, muscle $n = 6$), potassium (K\textsuperscript{+}; serum $n = 25$, muscle $n = 9$), and chloride (Cl\textsuperscript{-}; serum $n = 9$, muscle $n = 3$) from summer active state to hibernation
($< 1$, reduction upon induction into hibernation; $1$, no change; $> 1$, increase in electrolyte concentration). Black bars correspond to serum electrolyte levels, grey bars indicate electrolyte levels in muscle tissue. Animals included in this figure are: European hedgehog (Erinaceus europaeus, Linnaeus) [114-116], long-eared hedgehog (Hemiechinus auritus, Gilminel) [117], golden hamster (Mesocricetus auratus, Waterhouse) [118-122],
common box turtle (Terrapene carolina, Linnaeus) [123], pond slider (Trachemys scripta, Thunberg) [123], painted turtle (Chrysemys picta, Schneider) [124-127], European ground squirrel (Spermophilus citellus, Linnaeus) [128], thirteen-lined ground squirrel (Spermophilus tridecemlineatus, Mitchill) [121, 122, 129], groundhog (Marmota monax, Linnaeus) [130, 131], yellow-bellied marmot (Marmota flaviventris, Audubon & Backman) [132], Asian common toad (Buettaphrynus melanostictus, Schneider) [133], little brown bat (Myotis lucifugus, LeConte) [122, 134, 135], big brown bat (Eptesicus fuscus, Palisot de Beauvais) [122,134], American black bear (Ursus americanus, Pallas) [122], common musk turtle (Sternotherus odoratus, Latreille) [136], desert monitor (Varanus griseus, Daudin) [137]. Statistical analysis was performed in MatLab R2011a. Intragroup analysis of serum versus muscle electrolyte levels (Mann-Whitney U test: p-value): Mg\textsuperscript{2+}, $p < 0.001$; Ca\textsuperscript{2+}, $p = 0.395$; Na\textsuperscript{+}, $p = 0.299$; K\textsuperscript{+}, $p = 0.067$; Cl\textsuperscript{-}, $p = 0.315$. Intergroup analysis of serum electrolyte levels, indicating statistical differences (Kruskal-Wallis test): Mg\textsuperscript{2+} versus Ca\textsuperscript{2+}, Na\textsuperscript{+}, K\textsuperscript{+} and Cl\textsuperscript{-}, ($p < 0.05$).

The third property of an $R_x$ is its downward adjusting effect on the $Z_{in}$. In case of Mg\textsuperscript{2+} there appears to be conflicting evidence; intracerebroventricular perfusion with a solution containing a supraphysiological concentration of Mg\textsuperscript{2+} does not result in a hypothemic response in hamsters [144], rats [145], cats [146-148], and primates [149, 150] but does result in a hypothemic response in pigeons [151], dogs [152], and sheep [153]. However, with this perfusion approach it cannot be
guaranteed that intracerebroventricular perfusion solely affected the neural pathways involved in thermoregulation. A more accurate assessment can be made on the basis of the thermal effectors, in which case Mg\textsuperscript{2+} exerted an inhibitory effect on shivering in cold-exposed dogs [154], reduced post-anesthetic shivering in patients [155-157], lowered the cold-
induced shivering threshold in healthy human subjects [158], and promoted heat loss effectors in rats [159]. Essentially, these reports constitute indirect evidence for the Mg$^{2+}$ lowering property of Mg$^{2+}$, which is manifested through the activation of heat loss mechanisms and inhibition of thermogenic mechanisms (R$x$—Z$m$—T$b$, Figure 6).

Fourth, an R$x$ must distribute throughout the entire body. Although self-evident, this property is often omitted in common theories on the induction of hibernation by pharmacological agents. Heterogeneously distributed receptors of an R$x$ deter widespread propagation of hypometabolic signaling, and instead support hypometabolic signaling through an interposed effect such as the lowering of the Z$m$ (e.g., DOP) or the availability of S (e.g., TAM). The systemic distribution of Mg$^{2+}$ is unclear, but is expected to be ubiquitous given the role of this cation in many enzymatic reactions, including in the hexokinase-mediated conversion of glucose to glucose 6-phosphate, which occurs in every somatic cell type as part of sugar metabolism.

Finally, it is important that an R$x$ is sensitive to stimulation and inhibition for the induction and abrogation of hypometabolic signaling, respectively. The natural factors that trigger hibernation include lowering of T$a$ and dietary change, both of which are able to increase plasma Mg$^{2+}$ via cold-stimulated muscular and dermal release of Mg$^{2+}$ that is stored during the pre-stavation diet period [160]. The subsequent rise in plasma Mg$^{2+}$ can promote inhibition of thermogenic activity and activation of heat loss mechanisms by lowering of the Z$m$, potentiating heat exchange (T$a$—T$b$). In addition, direct effects on shivering via Mg$^{2+}$-mediated inhibition of neuromuscular transmission facilitates lowering of T$b$ [161]. The consequent cooling releases more Mg$^{2+}$ stored in muscles and skin, further adding to the increase in serum Mg$^{2+}$ through a positive feedback loop. As discussed in section 2.2, a feedback loop of this type would be most efficient in small animals due to their high body surface: size ratio and result in a less profound T$b$ drop in larger animals.

2.7. Hypothermia and hypometabolism research and clinical implementation: important considerations

The complete model on the induction of hibernation, presented in Figure 11, is in part hypothetical and requires additional research to validate every relationship. Given the supportive experimental evidence discussed in the previous sections, the model provides a starting framework for interpreting observations made in future in vivo experiments concerning hypothermia and hypometabolism, particularly in the context of integrative physiology. There are several important considerations regarding this type of research that must be accounted for, especially when data are interpreted on the basis of the model.

As implied in sections 2.1 and 2.2, prevention of stress signaling upon exposure to a cold stimulus is crucial to safe lowering of metabolism, underscoring the need for validation of the R$x$—(S)—Z$m$ relationship. This would require knowledge on both the location and function of the Z$m$, which, as alluded to previously, is currently beyond our reach. However, by investigating the impact of a stimulus such as T$a$ or R$x$ on thermogenic effectors, heat loss effectors, and behavioral thermoregulation (Z$m$—T$b$, Figure 3B), the Z$m$ issues can be circumvented while still gaining insight into the Z$m$ lowering potency. Z$m$-related research is presently conducted in this fashion, whereby ancillary parameters (effectors and behavior) are used as a gold standard to gauge Z$m$ [58].

Due to their high T$b$—T$s$ convective efficiency, small animals constitute ideal subjects for screening the anapyrexic potential of an R$x$ or investigating the effects of hypoxia. In larger animals, the lower T$s$—T$b$ convective efficiency necessitates the use of active cooling to accommodate induction of hypothermia and corollary hypometabolism. If active cooling in larger animal models is omitted, an anapyrexic agent or hypoxia may yield hypometabolic results in small animals but induce limited or no effect in larger animals. A totally T$s$-independent R$x$ (i.e., R$x$—Q; T$b = 37 \, ^{\circ}C$) would be in contradiction to this model and in fact disprove the necessity of T$s$—T$b$ convection. It is our opinion, however, that hypometabolism cannot occur under normothermic conditions – an opinion that is supported by extension of the Arrhenius law.

When translating these principles to a clinical setting, the use of the R$x$—(S)—Z$m$ relationship suggests that better outcomes would be achieved if hypothermic patients were pretreated with an anapyrexic agent (R$x$—Z$m$—T$s$—T$b$) or subjected to hypoxia (S—Z$m$—T$s$—T$b$)). The fact that clinical practice deviates from these approaches may contribute to the increased

Figure 11. Model for induction of (artificial) hypometabolism. Depicted parameters: Q, overall metabolism defined as chemical reaction speed (i.e., similar to k in Equation 1); C, catabolism; A, anabolism; T$s$, core body temperature; T$a$, ambient temperature; Z$m$, thermoneutral zone; S, substrate; R$x$, (bio)chemical agent able to induce hypometabolism. The relationships: T$s$—Q, Arrhenius law; T$s$—T$b$, heat exchange; Z$m$—T$s$, thermogenesis and heat loss mechanisms; T$a$—Z$m$, sensory input; S—Z$m$, hypoxic link; R$x$, S, hypoxia/hypoglycemia induction; R$x$—C, catabolic modulation; R$x$—A, anabolic modulation; R$x$—Z$m$, anapyrexic signal; S—A, metabolic substrate supply; T$s$—R$x$, positive/negative feedback loop.
comorbidity in patients as a result of hypothermia-inflicted stress responses during trauma-induced and perioperative hypothermia [15,162]. Presently, none of the strategies aimed to resolve these responses in patients encompass guidelines for $Z_m$ modulation. As a result, many patients are placed on 100% $O_2$ and symptomatic treatment of shivering without a clear rationale. According to our model, a more cautious approach in oxygenating hypothermic patients could be beneficial, as reflected by the $S-Z_m-T_b$ signaling axis. By subjecting a hypothermic patient to a hypoxic signal ($S-Z_m$) or anaplerotic agent ($R_4$), the cold-induced stress response may be mitigated by reduction of $Z_m$ through CB signaling and alignment of $T_b$ with $T_s$. As exposure to a cold stimulus readily activates thermal effectors such as shivering and BAT [163,164], such an effect would be promptly visible. However, to date no anaplerotic agents or hypoxic signaling mechanisms have been reported in a clinical setting.

3. Conclusions

In conclusion, the lack of understanding of the induction mechanisms underlying natural hibernation stands in the way of successful application of artificial hibernation in biotechnology and medicine. Accordingly, a model was developed to assist in finding the means to translate the physiological changes observed during natural hibernation to its artificial counterpart. Summarized in Figure 11, six essential elements form the basis of our model, which were extrapolated from literature. The relationships between these elements dictate their values and collectively govern the induction and sustenance of a hypometabolic state. To illustrate the potential validity of this model, various $R_4$ ($HIT, DOP, H_2S, 5^\prime-AMP, TAM, 2-DG, Mg^{2+}$) were described in terms of their influence on the intervariable relationships and effects on $Q$.

Although the ultimate purpose of this hypothetical model was to help expand the paradigm regarding the mechanisms of hibernation from a physiological perspective and to assist in translating this natural phenomenon to the clinical setting, our model only comprises a part of the vastly complex biological systems that underlie anapleraxis and hypometabolism. Moreover, readers should note that concepts as ‘set point’ are model-based phenomena, rather than neurobiological constructs. The key to the mechanistic underpinning of anapleraxis signaling currently rests on the shoulders of neurobiology, which is slowly unveiling the neurological signaling pathways. In that respect, thermal reflexes (cold defense, fever, anapleraxis, hibernation, etc.) are mediated by changes in the discharge of neurons in neural circuits controlling thermoregulatory effectors, and understanding how and through which neurochemical mediators these reflexes are effected will only be accomplished through detailed neurobiological experimentation. Accordingly, basic elucidation of the neurochemistry of anapleraxis is needed for the identification of useful Rxs [81, 82].

Acknowledgements

We thank Inge Kos-Oosterling for support with the graphics.

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