Is Human Hibernation Possible?

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Abstract

The induction of hypometabolism in cells and organs to reduce ischemia damage holds enormous clinical promise in diverse fields, including treatment of stroke and heart attack. However, the thought that humans can undergo a severe hypometabolic state analogous to hibernation borders on science fiction. Some mammals can enter a severe hypothermic state during hibernation in which metabolic activity is extremely low, and yet full viability is restored when the animal arouses from such a state. To date, the underlying mechanism for hibernation or similar behaviors remains an enigma. The beneficial effect of hypothermia, which reduces cellular metabolic demands, has many well-established clinical applications. However, severe hypothermia induced by clinical drugs is extremely difficult and is associated with dramatically increased rates of cardiac arrest for nonhibernators. The recent discovery of a biomolecule, 5′-AMP, which allows nonhibernating mammals to rapidly and safely enter severe hypothermia could remove this impediment and enable the wide adoption of hypothermia as a routine clinical tool.

INTRODUCTION

The word hibernation is often associated with bears taking a long winter rest. However, some investigators would argue that bears do not really hibernate but rather enter a state of torpor. In mammals, the term torpor is often used when body temperature drops below 31°C. Hibernation is often described as deep torpor. Although both words are widely used in the scientific literature to reflect different degrees of a hypometabolic state, their physiological definitions are not clear. In addition, some reptiles and amphibians can undergo a hypometabolic process known as estivation to survive challenging environmental conditions. As articulated by Heldmaier et al. (1), "The physiological properties of daily torpor, hibernation and estivation are very similar. The classification of hibernation, daily torpor or estivation simply represents gradual difference in the timing, the duration and the amplitude of physiological inhibition." In essence, these are hypometabolic behaviors used by animals for energy conservation. On the basis of oxygen consumption measurements, it was demonstrated that the biochemical and physiological events that inhibit endogenous thermoregulation occur rapidly prior to the onset of hypothermia (1). In contrast, hypothermia is driven by heat loss from the body to the environment. This process depends on several parameters including surface/volume ratio, the amount of fur and fat insulation, and the temperature gradient between the body and the environment. The molecular and biochemical mechanisms underlying the natural shutdown of metabolic activities remain largely unknown.

The goal of this article is not to recapitulate the physiology of hypometabolism that occurs naturally, as there are excellent reviews in the literature (1). Instead, I focus on recent attempts to mimic the induction of a hypometabolic state in mammals, which may have potential clinical applications. I also offer some hypotheses on how hypometabolism could occur in nonhibernating mammals. Whether chemically induced hypometabolism has any connection with the natural process is not critical; observations from these studies can be drawn upon to reveal the underlying biochemical and molecular processes of hypometabolic behaviors.

Clinically, only mild hypothermia of 32–34°C can be induced safely by a cocktail of paralytic drugs and anesthesia (2, 3). Severe hypothermia (28°C or lower) induced by such drug cocktails results in a high cardiac arrest rate in nonhibernating mammals (4). To date, three classes of molecules will result in reversible severe hypothermia when administered to small mammals such as mice and hamsters. Two of these are metabolic inhibitors: 2-deoxyglucose and hydrogen sulfide (H2S). The third, recently identified in my laboratory, is the end metabolite 5′-adenosine monophosphate (5′-AMP). It is the first natural biomolecule to have this effect (5).

2-DEOXYGLUCOSE AND HYDROGEN SULFIDE

Hamsters are known to undergo daily torpor to conserve energy upon prolonged exposure to low environmental temperature and short photoperiod, which mimic seasonal changes (6). This torpor behavior of hamsters is circadian-driven. This was demonstrated by experiments in which ablation of the suprachiamastic nucleus (SCN), the central circadian structure, disrupts the animal's temporal rhythm (7). After receiving the glycolytic inhibitor 2-deoxyglucose, hamsters readily undergo torpor even when kept in long photoperiod (8). In contrast, inhibition of fatty acid metabolism by mercaptoacetate did not induce torpor in hamsters kept in long photoperiod (6). These observations implicate impediment of glucose utilization as a key event for hypometabolism. However, the biochemical mechanism underlying 2-deoxyglucose induction of torpor remains unclear.

Another metabolic inhibitor, H2S, was recently found to enable mice to enter severe

hypothermia or suspended animation at a low dosage of 80 ppm (9). Mice given H2S could be cooled down to 15°C into a state of suspended animation for up to six hours. Arousal was spontaneous, and no apparent detrimental outcome was observed after recovery. It has been thought that a core body temperature below 20°C in nonhibernators will lead to cardiac fibrillation (10). Our lowest recording of core body temperature in mice during torpor induced by fasting was about 26°C even when the ambient environmental temperature was maintained at 8°C (J. Zhang & C.C. Lee, unpublished observations). Thus, the ability to drop a nonhibernating mammal's core body temperature to 15°C is a major step forward. It suggests that nonhibernators are fully capable of withstanding extreme hypometabolism.

As with 2-deoxyglucose, the mechanism underlying H2S induction of severe hypothermia remains unclear. H2S is a specific and reversible inhibitor of cytochrome c oxidase, a key component of the mitochondria respiratory complex IV (9). Inhibitors of the mitochondria respiratory chain are toxic to mammals because they disrupt ATP production by oxidative phosphorylation. Similarly, 2-deoxyglucose inhibits glycolysis, which is another biochemical process involved in the generation of ATP and NADH from glucose outside of the mitochondria. Thus, the actions of both H2S and 2-deoxyglucose affect the cellular production of ATP. In turn, the lower ATP level would downregulate biochemical reactions necessary for thermal regulation defenses. It has been observed that during hibernation, erythrocytes and organs have significantly lower ATP levels (11, 12). In particular, the ATP level of erythrocytes from a hibernating animal is ~50% of the level observed in a euthermic state (11). Such a large decrease in ATP level in the erythrocyte would significantly compromise its function in regulating oxygen/carbon dioxide molecules necessary for maintaining high metabolic activity of the major organs. Under such conditions, it is highly possible that the metabolic rate of organs will slow, and heat loss from the body to the environment will not be adequately controlled. This reasoning is consistent with the hypothesis that decreased ATP production or utilization is involved in hibernating behaviors (1, 12). How these biochemical observations can be reconciled with the widely held dogma that the preoptic area of the hypothalamus controls thermal regulatory response in mammals is not clear (13).

SEARCH FOR AN ENDOGENOUS MOLECULE FOR HYPOMETABOLISM

For hibernators to achieve a severe hypothermic state, the basic principles of metabolic biochemistry must be preserved to ensure energy homeostasis (12). This raises the possibility that such biochemical processes may be retained in all mammals. Mammalian organs can be maintained in a hypothermic but highly hypoxic state for many hours (14), as we see when donor organs are transported in coolers for organ transplantation. Upon transplantation, the restoration of blood flow and its rewarming revive the basic functions of the donor organ. In addition, examples from accidental hypothermia have suggested that under certain conditions, humans can recover fully from prolonged periods in severe hypothermia. Critically, these observations suggest that nonhibernators' organs are inherently capable of withstanding extreme hypoxic stress if their metabolic demands are reduced. Thus, a project was initiated to probe the possibility of identifying genes from nonhibernating mammals that are activated in an environment encountered during hibernation.

It is widely recognized that mammals enter hibernation in an environment of constant darkness (1). Using liver mRNA, gene expression in mice exposed to regular 12:12 h cycles of light:dark (LD) was compared to gene expression in mice kept in constant darkness or dark:dark (DD). From microarray analysis, a gene encoding procolipase was identified to be highly expressed in the liver of the DD mouse. Previous studies have demonstrated

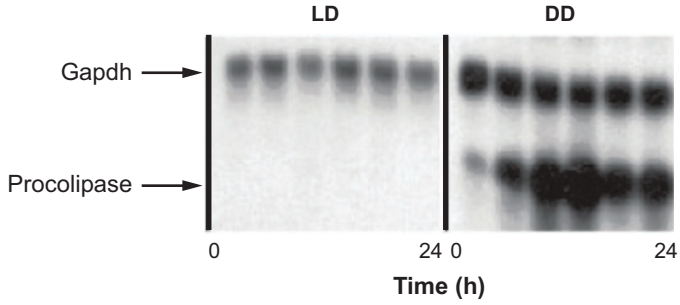

Figure 1

Activation of procolipase expression by a constant-darkness environment. The Northern blot analysis shows a 4-h time course of the liver mRNA species of Gapdh and procolipase obtained from mice kept in 12:12 h light-dark (LD) cycles or in dark-dark (DD) cycles, i.e., constant darkness. Gapdh is used as an internal control. The genes were identified by radiolabeled cDNA's probe for Gapdh and procolipase, respectively.

that procolipase gene expression is highly specific to the pancreas and gastrointestinal tract, consistent with its primary role of breaking down dietary fat into fatty acids (15). The exceptions are hibernating ground squirrels, in which procolipase was activated in other peripheral organs (16). Confirming the microarray findings, an independent method of gene detection based on Northern blot analysis demonstrated that the procolipase gene was indeed activated in DD mice but not in LD mice (**Figure 1**). Tissue analysis revealed procolipase was activated in a circadian manner in the majority of the peripheral organs in DD mice. Exposure of these mice to white light resulted in the shutdown of procolipase expression in the various organs (5). Together, these findings suggested that the endogenous signaling mechanism is mediated by a circulatory factor.

IDENTIFICATION OF 5′-AMP AS AN ACTIVATOR OF PROCOLIPASE EXPRESSION

The above observations indicate that this endogenous regulator must be a circulatory molecule that displays a circadian profile in its activity. It could behave either as an activator or a repressor. If it is an activator, then injection into LD mice will induce procolipase expression in the major organs. If it acts as a repressor, then injection into DD mice will abolish procolipase expression in the peripheral organs.

Through a series of careful experiments, our search for this circulatory molecule was narrowed to the soluble aqueous fractions of blood extract. These studies led to the identification of a circulatory nucleotide displaying clear circadian oscillation that matched the hypothesized profile described above (5). Characterization of this molecule based on its spectral absorption, its migration distance on high-performance liquid chromatography (HPLC) relative to known chemical nucleotide standards, and enzymatic analysis indicated the molecule was adenosine 5′-monophosphate (5′-AMP). To confirm this, synthetic 5′-AMP was injected into LD mice; procolipase expression was detected by reverse transcriptase polymerase chain reaction (RT-PCR) in all tissues sampled with the exception of the brain (**Figure 2*a***). These findings demonstrate that the level of circulatory 5′-AMP was involved in regulating procolipase expression. However, the induction of procolipase by 5′-AMP was not immediate. Its prolonged time course suggests that it was an indirect effect. Unexpectedly, mice that received 5′-AMP were severely hypothermic, with core body temperatures as low as 25°C when kept in ambient room temperatures of about 23–24°C (**Figure 2*b***). The severe hypothermic period was transient; animals reentered a thermogenic period and core body temperature was restored several hours later. Further monitoring of these animals over several months revealed no apparent deficit. The impact of 5′-AMP as reflected internally by the heart rate indicated a very rapid decline in metabolic activity. The heart rate fell from ~600 beat/min (normal for a mouse) to ~200 beat/min within 5 min after 5′-AMP was given (Z. Tao & C.C. Lee, unpublished observations). As core body temperature dropped from 37°C to 25°C, there was a direct correlation between this hypometabolic state and the reduced heart and respiration rates. Similarly,

as the animal aroused from the hypometabolic state, the core body temperature gradually rose toward euthermic state (~37°C), along with increasing heart and respiration rates.

To determine whether 5′-AMP plays a similar role in natural torpor, DD mice were fasted to induce natural torpor. Mice in fasting-induced torpor had approximately a two- to three-fold higher level of 5′-AMP in the blood than mice that did not enter torpor (5). The kinetics of natural torpor is slower than that induced by synthetic 5′-AMP; this can be accounted for by the slower buildup of natural 5′-AMP generated by fasting and the physical barriers that regulate heat loss from the body to the environment. It appears that 5′-AMP must inhibit or retard thermoregulation, as hypothermia is a result of undefended heat loss from the body to the environment. How low the core body temperature of a nonhibernator can be reduced and yet maintain viability is unclear. Hibernators such as arctic ground squirrels can withstand drops in core body temperature to several degrees below freezing (17). However, larger mammals that are known to hibernate prefer core body temperatures that are much higher. In the winter, the core body temperature of bears reduces to ~32°C, and the tropical Malagasy lemur, a primate that hibernates in the dry season of the tropics, drops its core body temperature to around 25°C (1). These observations suggest that each mammalian species has an optimum core body temperature range for such hypometabolic activities. A temperature compensation mechanism is used to maintain the core body temperature necessary for biochemical reactions. Thus, when an animal's core body temperature drops below the ideal range for its species, its metabolic rate increases rather than slows down further (1).

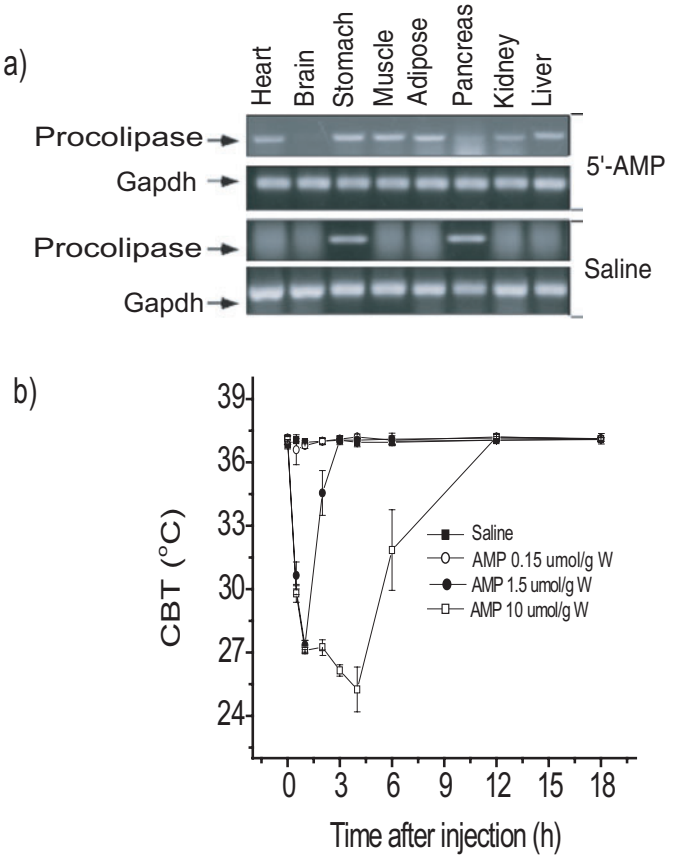

Figure 2

Induction of procolipase expression and hypothermia by synthetic 5′-AMP. (*a*) RT-PCR detection of procolipase and Gapdh expression in mice kept in 12:12 h light:dark cycles given saline or 5′-AMP. (*b*) Hypothermic response of mice injected with various dosages of 5′-AMP.

POSSIBLE MECHANISMS OF 5′-AMP–INDUCED HYPOMETABOLISM

Although ATP is the cellular energy currency, 5′-AMP occupies the unique position that determines salvage and catabolism of the adenine nucleotides (**Figure 3**). In all cells, the adenylates' biochemical equilibrium, ATP + 5′-AMP ↔ 2ADP, which is regulated by the enzyme adenylate kinase, controls cellular energy charge (18). By and large, the ratio of ATP to 5′-AMP determines the energy state of the cell. Therefore, excess 5′-AMP can either be salvaged via ADP or is catabolized. In the catabolic pathway, 5′-AMP can be degraded via two pathways. The major pathway is via inosine 5′-monophosphate (IMP) control by the enzyme AMP deaminase. IMP is rapidly catabolized to inosine and hypoxanthine and then to uric acid, which in humans

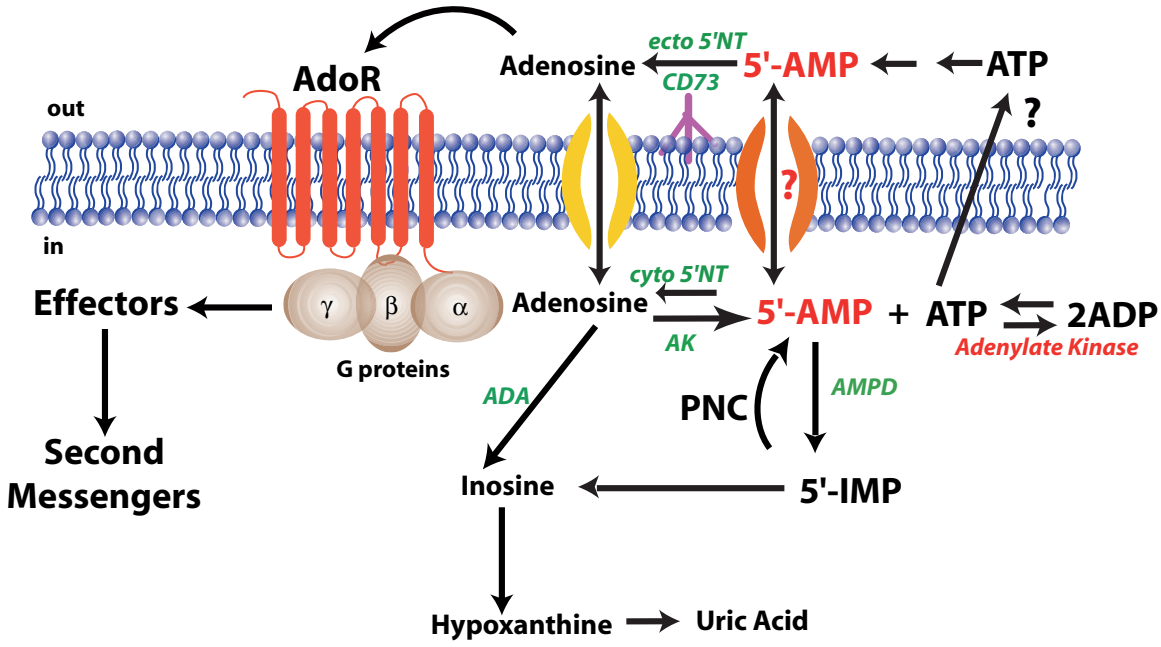

Figure 3

5′-AMP is pivotal in adenine nucleotide salvage and catabolism. The diagram illustrates the extracellular and intracellular roles of 5′-AMP. AdoR, adenosine receptors; CD73/ecto 5′NT, ecto 5′-nucleotidase; ADA, adenosine deaminase; AK, adenosine kinase; cyto 5′NT, cytosolic nucleotidase; AMPD, AMP deaminase; PNC, purine nucleotide cycle; 5′-IMP, inosine 5′-monophosphate; AMP, adenosine 5′-monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate. It is unclear how ATP gets out of the cell. Whether 5′-AMP is transported in and out of the cell is also unclear.

and other primates is excreted. In most other mammals, the uric acid is further catabolized into allantoin before being excreted. However, IMP can be salvaged to regenerate 5′-AMP through the purine nucleotide cycle via an intermediate, adenylosuccinate. In the minor pathway, 5′-AMP can be dephosphorylated directly into adenosine extracellularly by ecto-5′-nucleotidase (CD73) and intracellularly by cytosolic 5′-nucleotidase. Adenosine is then degraded to inosine by the enzyme adenosine deaminase (ADA) and eventually to uric acid. Intracellularly, the rephosphorylation of adenosine to 5′-AMP is undertaken by the enzyme adenosine kinase.

Extracellularly, adenosine, resulting from dephosphorylation of 5′-AMP into adenosine by CD73, can either enter the cell via transporters or bind its receptors. The latter involves binding to four known adenosine-activated G protein–coupled receptors: A1, A2a, A2b, and A3. These receptors have different affinities for adenosine and can play a key role in either activating or inhibiting cascades regulated by adenylyl cyclase (19). Alternatively, adenosine is taken up by the cell via multiple types of transporters such as equilibrative nucleoside transporters (ENTs) and concentrative nucleoside transporters (CNTs) (20, 21). The ENTs are gradient-driven, whereas the CNTs are active transporters that involve exchange of a cation such as sodium. These transporters are very important to certain cells, such as erythrocytes, that are deficient in organelles and cannot perform de novo synthesis of nucleoside. To maintain its cellular nucleotide pool, an erythrocyte has extremely high levels of nucleoside transporter to overcome this lack of de novo synthesis of nucleoside (20). Although the

identification of an AMP receptor has been reported recently (22), it remains controversial (23). The direct uptake of adenine nucleotides such as cAMP by erythrocytes (24, 25) and of 5′-AMP by intestinal cells (26) has been reported. Several genes for adenine nucleotide transporter/translocase have been identified, but to date their functions have been demonstrated only for organelles such as mitochondria and peroxisomes (27, 28).

The injection of 5′-AMP and adenosine both induce procolipase expression in organs of mice (5). However, the solubility of adenosine in water is very low, and the amount required to induce procolipase expression in a mouse has to be dissolved in an organic solvent, DMSO. The solubility consideration alone would argue strongly against adenosine as the primary activator. In addition, circulating adenosine largely remains very low throughout the diurnal cycle (5). The extracellular level of 5′-AMP is regulated by the enzyme ecto-5′nucleotidase (CD73), expression of which is under circadian control (29, 30). In addition, DD mice have a significantly dampened expression level of CD73 compared with LD animals (5). Thus, the photoperiod effect and circadian profile of circulating 5′-AMP levels is linked to the endogenous clock control of CD73 expression.

The induction of procolipase by 5′-AMP or adenosine was blocked in animals that had received a prior injection of dipyridamole, a broad-based transporter inhibitor (5). This observation indicates that the underlying mechanism involves an uptake of adenosine or 5′-AMP rather than being mediated through the adenosine receptors. Our studies do not exclude adenosine receptors' role in the regulation of the adenosine transporter function. Recent studies have shown that nucleoside transporter activity can be controlled by the adenosine receptors, as for example CNT2 is controlled by the adenosine A1 receptor (31). However, once inside the cell, adenosine is rapidly phosphorylated into 5′-AMP because the K_m of adenosine kinase (AK) for adenosine is one to two orders of magnitude smaller than that of ADA for adenosine (32). Alternatively, 5′-AMP could be transported into cells known to take up adenine nucleotides directly, such as erythrocytes (24, 25). With either uptake mechanism, a rapid buildup of 5′-AMP inside the cell results, which in turn affects the adenylates' biochemical equilibrium (ATP + 5′-AMP ↔ 2ADP). To counter the rise of 5′-AMP, the enzyme adenylate kinase drives the formation of ADP, which leads to a depletion of ATP. The decrease in ATP slows cellular function, which would mimic the drop in ATP levels observed in erythrocytes of hibernating mammals (11). However, ATP cannot decrease indefinitely if 5′-AMP levels continue to rise, as this would result in the complete shutdown of cellular function. The AMP deaminase K_m for 5′-AMP is higher than that of adenylate kinase for 5′-AMP (12). Thus, excess 5′-AMP above and beyond the K_m of adenylate kinase is rapidly catabolized by AMP deaminase to IMP, thus limiting overdepletion of ATP in the cell.

ALLOSTERIC REGULATION OF METABOLIC ENZYMES BY 5′-AMP

In addition to its pivotal position in the purine biochemical pathways, 5′-AMP also acts as an allosteric regulator of many rate-limiting metabolic enzymes (18) (**Figure 4**). One such allosteric enzyme is AMP-dependent protein kinase (AMPK). AMPK, which is activated by 5′-AMP, has been implicated as a positive regulator of glucose transport in muscle, glycolysis, and fatty acid oxidation (33). Its vast cellular role is beyond the scope of this review. Our studies showed that the 5′-AMP activation of procolipase expression in LD mice is reciprocally linked to blood glucose (5). 5′-AMP is known as an allosteric regulator of two key enzymes that control glucose homeostasis: It is a positive regulator of fructose 1,6 phosphatase (FDP) and a negative reglator of

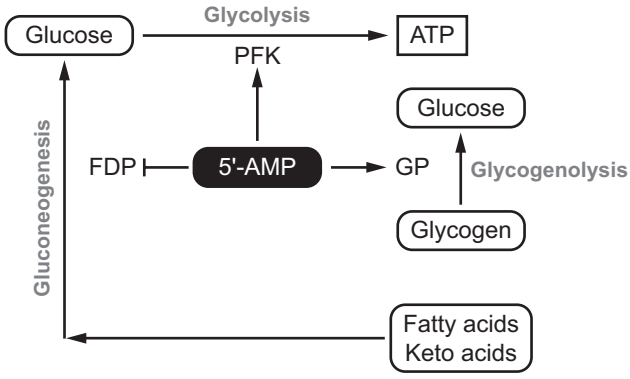

Figure 4

Allosteric regulation key metabolic enzymes by 5′-AMP. The rate-limiting enzymes for gluconeogenesis (FDP), glycolysis (PFK) and glycogenolysis (GP, glycogen phosphorylase) are allosterically regulated by 5′-AMP.

phosphofructose kinase (PFK). FDP is a rate-limiting enzyme for gluconeogenesis, and it converts fructose 1,6 phosphate to fructose 6 phosphate (18). FDP binding of 5′-AMP inhibits its enzymatic activity, thereby limiting gluconeogenesis. In the reverse direction, PFK is a rate-limiting enzyme for glycolysis. It converts fructose 6 phosphate into fructose 1,6 phosphate by utilizing an ATP molecule (18). Unlike FDP, the activity of PFK is enhanced by 5′-AMP, thereby increasing the rate of glycolysis. For both enzymes, the allosteric effects of ATP are opposite to those of 5′-AMP because these adenylate nucleotides bind competitively to the same regulatory motif (18). As proposed above, the elevated level of cellular 5′-AMP after its uptake will alter adenylate biochemical equilibrium to favor ADP production, thus reducing the cellular ATP level. The drop in ATP will slow cellular biochemical processes and is postulated to reduce thermoregulatory activity and allow hypometabolism to set in. An animal must eventually exit from the hypometabolic state, and the catabolism of 5′-AMP to IMP by AMP deaminase or by the cytosolic nucleotidase into adenosine can provide only part of the means for exit. The regeneration of ATP from ADP via glycolysis in various cells, including the erythrocytes, must occur to reestablish the euthermic adenylate biochemical equilibrium. The decline in the ratio of ATP to 5′-AMP that leads to the hypometabolism eventually activates PFK, thereby increasing glycolysis, which utilizes the accumulated ADP in the cell to regenerate ATP. This restores the adenylate biochemical equilibrium to its euthermic state, completing the metabolic events initiated by 5′-AMP.

CONCLUSION

The experimental investigation of hypometabolism induced by 5′-AMP is ongoing. I have taken this opportunity to articulate some of the current hypotheses that are testable using existing biochemical and pharmaceutical tools. In addition, mouse genetics could be used to dissect the 5′-AMP–driven biochemical pathways of hypometabolism in animal models. Finally, our identification of a natural biomolecule that allows rapid initiation of hypometabolism in mammals may eventually result in clinical applications where hypothermia has been shown to have tremendous lifesaving potential, such as trauma, heart attacks, strokes, and many major surgeries (3).

DISCLOSURE STATEMENT

A patent application has been filed by the University of Texas Health Science Center, Houston on behalf of the author on the potential utilities of 5′-AMP.

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