AMP Deaminase 3 Deficiency Enhanced 5′-AMP Induction of Hypometabolism

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Abstract

A hypometabolic state can be induced in mice by 5′-AMP administration. Previously we proposed that an underlying mechanism for this hypometabolism is linked to reduced erythrocyte oxygen transport function due to 5′-AMP uptake altering the cellular adenylate equilibrium. To test this hypothesis, we generated mice deficient in adenosine monophosphate deaminase 3 (AMPD3), the key catabolic enzyme for 5′-AMP in erythrocytes. Mice deficient in AMPD3 maintained AMPD activities in all tissues except erythrocytes. Developmentally and morphologically, the Ampd3−/− mice were indistinguishable from their wild type siblings. The levels of ATP, ADP but not 5′-AMP in erythrocytes of Ampd3−/− mice were significantly elevated. Fasting blood glucose levels of the Ampd3−/− mice were comparable to wild type siblings. In comparison to wild type mice, the Ampd3−/− mice displayed a deeper hypometabolism with a significantly delayed average arousal time in response to 5′-AMP administration. Together, these findings demonstrate a central role of AMPD3 in the regulation of 5′-AMP mediated hypometabolism and further implicate erythrocytes in this behavioral response.

Introduction

It is widely known that in response to severe metabolic stress, some mammals enter a period of torpor or hibernation, physiological states of severely reduced metabolic rates, to survive [1]. The biochemical and physiological processes by which torpor is induced and maintained are poorly understood. We have previously shown that the natural metabolite adenosine monophosphate (5′-AMP), when given in sufficient dosages to mice or other mammals, can induce a state of transient hypometabolism [2][3]. During this hypometabolic state, the animal’s core body temperature (Tb) can be safely and readily reduced from C, the Tb of mice could be reduced to 1–2 C above Ta for 6–9 h and the process is reversible [3]. Questions have been raised as to whether this hypometabolic state associated with the Tb decline is the alteration in erythrocyte adenylate ratios by 5′-AMP uptake that is the underlying biochemical mechanism initiating the physiological responses that result in the hypometabolic state.

Cellular catabolic pathways for 5′-AMP could either occur through its deamination to inosine monophosphate (IMP) by AMP deaminase (AMPD), or its dephosphorylation to adenosine [7]. Cellular adenosine can either be phosphorylated to 5′-AMP by adenosine kinase or deaminated to inosine by adenosine deaminase (ADA). However, the Km for adenosine of ADA is more than an order of magnitude larger than that of adenosine kinase, favoring adenosine phosphorylation to 5′-AMP over deamination to inosine by ADA [7]. Thus, the majority of cellular 5′-AMP is catabolized by AMP deaminases. In mice, there are three known isozymes of AMP deaminase that are encoded by three different genes: the muscle isozyme (AMPD1), the liver isoform (AMPD2) and the erythrocyte isoform (AMPD3) [8]. Many tissues express various levels of all three AMPD isozymes. However, AMPD3 is the only known isozyme present in erythrocytes [9]. Therefore, we reasoned that disrupting AMPD3 function should alter the catabolism of 5′-AMP specifically in erythrocytes. An Ampd3−/− mouse would allow for the examination of the erythrocyte’s role in mediation of 5′-AMP induced hypometabolic behavior. Hence, we undertook a study to disrupt the gene for AMPD3 in mice. Our findings demonstrate that the loss of AMPD3 significantly enhanced the efficacy of injected 5′-AMP to induce a hypometabolic response in mice.

Materials and Methods

Creation of AMPD3 deficient mice

ES cells (C57Bl/6 background) containing the vector to generate the AMPD3 deficient mice were purchased from the
NIH Knockout Mouse Project (KOMP) [10], via the KOMP repository at the University of California, Davis. The ES cells from KOMP were amplified and verified using a PCR genotyping protocol provided by the supplier. Chimeric mice were generated using the ES cells at the Transgenic and Stem Cell Service Unit of the Brown Institute of Molecular Medicine, Houston, TX. Briefly, the ES cells were injected into blastocysts of pseudopregnant C57Bl/6 females to generate chimeric offspring that carry the Ampd3 gene targeted allele. Eight male chimeras (C57Bl/6 wild type, Balb/c Ampd3 knockout) were generated, and all were mated with C57Bl/6 wild type females purchased from Harlan Laboratories, Houston, TX. Of the eight chimeras, two were able to produce germline transmission to create Ampd3+/− mice, which were then mated to produce the Ampd3+/−, Ampd3−/− and Ampd3−/− mice used in experiments.

All animal studies were carried out following the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal research protocols (HSC-AWC-09-138 and HSC-AWC-13-012) were approved by the Animal Welfare Committee (AWC), the Institutional Animal Care and Use Committee (IACUC) for the University of Texas Health Science Center at Houston.

In vivo Metabolic Rate Measurement

Metabolic rates of live mice assessed by their oxygen consumption (VO2) and/or carbon dioxide production (VCO2) were measured in a Comprehensive Lab Animal Monitoring System (CLAMS) from Columbus Instruments, Columbus, OH. Each animal was housed individually in an airflow cage with gases going in and out of the cage measured by high-speed gas sensors on an Open Circuit Oxymax Calorimeter, (Columbus Instruments, Columbus, OH). The airflow into and out of the cage was calibrated against a known gas mixture before each experiment. Samples were taken every minute, cage by cage, with one extra minute for re-calibration at every four samplings. The animals had access to food and water ad libitum during the entire study.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Heart tissues were harvested from WT and Ampd3−/− mice. Total RNA was isolated using Trizol (Invitrogen), following the vendor’s instructions. First-strand cDNA synthesis was carried out using 1 μg of total RNA and oligo (dT) primer with SuperScript II reverse transcriptase (Invitrogen). Then the first strand cDNA was used for PCR using a kit (Roche USA), with forward primer, 5’-GATGTCAGGCTTGCCTACGAC; and reverse primer, 5’-AATAAGGGGTTCCTGCGGAGT. The cycling condition was: 95°C for 2 min, followed by 30 cycles of 95°C for 30 sec, 59°C for 30 sec, and 72°C for 1 min, followed by incubation at 72°C for 5 min. The resultant PCR products were visualized on a 1.5% agarose gel in 1X TAE buffer [11].

AMP Deaminase Assay

AMP deaminase activity was assayed as previously described [12]. Briefly, AMPD activity was quantified by measuring formation of IMP when protein samples were added to reaction mixtures with saturating amounts of 5’-AMP. Each 100 μl reaction mixture contained 20 μg soluble cell lysate, 25 mM imidazole, pH 7.0, 0.2 mg/ml bovine serum albumin (BSA) and 150 mM potassium chloride. The addition of 20 mM 5’-AMP at 37°C initiated the enzymatic assay. A 50 μl aliquot was removed at 60 min and frozen at −80°C. Samples were then analyzed by high performance liquid chromatography (HPLC) (Alliance 2995 Separations Module with 2998 Photodiode Array Detector, Waters, Millipore Corp.) using a 5 μm C18 reversed phase column and elution by a mobile phase of 20 mM ammonium phosphate, pH 5.1 with a methanol gradient of 0–4 min 0% methanol, 4–6 min 0–8% methanol, 6–8 min 8–20% methanol and 8–18 min 20% methanol.

Analysis of adenine nucleotides in erythrocyte lysates

Erythrocytes were isolated from whole blood by removing serum and washing 3 times in ice-cold phosphate-buffered saline. Cells were lysed by freeze thaw and then extracted with 3 volumes of ice-cold 70% methanol overnight. The supernatant was removed after centrifugation at 12,000 xg at 4°C for 10 min. The supernatant was evaporated to dryness and then resuspended in 200 μl mobile phase solvent. The protein pellet was used to determine protein concentration. Nucleotides in the extracts were separated by HPLC (Alliance 2695 Separations Module with 2998 Photodiode Array Detector, Waters, Millipore Corp.) using a 5 μm C18 reversed phase column (Sunfire, Waters, Millipore Corp.) with a mobile phase of 150 mM KH2PO4,150 mM KCl, pH 6.0, and a superimposed gradient using 15% acetonitrile (ACN) programmed as 0–20 sec 0% ACN, 20 s – 21 min 0–1.35% ACN, 24 min – 28 min 1.35–15% ACN, 29 min – 35 min 0% ACN. Nucleotides were quantified against standard curves of purchased standards (Sigma-Aldrich, St. Louis, MO).

Blood Glucose Measurements

Mice were fasted overnight. Blood glucose was measured from whole blood with a One Touch Basic glucose meter (Life Scan, Inc).

Data analysis

Standard statistical analyses are applied to assays of multiple samples yielding a value representing the mean ± standard error of the mean (SEM) [SEM = SD/(square root of sample size)]. The sample sizes (n) for each assay are shown in the corresponding figure legend. Asterisk (*) indicates statistical significance (p<0.05) in the two-tailed Students “t” test.

Results

Generation of Ampd3−/− Mice

To take advantage of the existing genetically engineered mouse resources in the scientific community, we searched for Ampd3 gene targeting projects from the KOMP catalog of mice and embryonic stem (ES) cells (C57Bl/6 background). An ES clone with Ampd3 gene loci disrupted by a targeting vector, generated by The Sanger Center, was available. The vector applied the “knockout-first” conditional gene knockout technology to insert an en2-LacZ-neo cassette into an intron downstream from exon 5 of the AMPD3 gene locus (Figure 1A). With the “knockout-first” design, the initial targeted allele is predicted to result in a null allele by splicing the upstream exon of the targeted gene into a lacZ trapping element contained in the targeting cassette [13][14]. By including a mouse En2 splice acceptor and the SV40 polyadenylation sequence in the trapping cassettes, this strategy has proven to be reliable in creating null alleles in mice. The outcome of the AMPD3 targeting is that the 3’ end of exon 5, containing the first ATG of the 14 coding exons, is spliced into the splice acceptor (SA) site of the en2-LacZ-neo cassette, disrupting and destabilizing the Ampd3 transcript. As a result, there should be no functional Ampd3 mRNA produced. These Ampd3−/− ES cells were used to generate the AMPD3 deficient mice.
Once the putative Ampd3<sup>-/-</sup> mice were generated, the animals were apparently developmentally normal based on gross morphological examination. In order to verify if the Ampd3<sup>-/-</sup> mice expressed Ampd3 mRNA, Ampd3 specific RT-PCR analysis was carried out with heart mRNA from wild type and Ampd3<sup>-/-</sup> mice. Ampd3 is the dominant isoform in heart tissue and is highly expressed. As shown in Figure 1B, RT-PCR analysis showed the expected transcript band was present in wild type but not in Ampd3<sup>-/-</sup> mice. As a control, Gapdh transcripts were detected in all Ampd3<sup>-/-</sup> samples when cDNA was made (+RT), and were absent in all samples when cDNA was not made (-RT, the negative controls). The RT-PCR analysis shows that the Ampd3<sup>-/-</sup> but not the wild type animal heart samples were deficient in the Ampd3 transcript.

Absence of AMP Deaminase Activity in Ampd3<sup>-/-</sup> Erythrocytes

To further verify the RT-PCR findings, we then assayed AMP deaminase activity in various tissues obtained from siblings of wild type and Ampd3<sup>-/-</sup> genotypes. To assay for AMP deaminase activity, cell lysates prepared from various tissues obtained from wild type and Ampd3<sup>-/-</sup> mice were incubated with ATP and the amount of inosine monophosphate (IMP) formed was determined by HPLC. The enzymatic formation of IMP was then normalized to the amount of total lysate protein used in the reaction.

In most tissues sampled, the levels of AMP deaminase activity did not differ significantly between wild type and Ampd3<sup>-/-</sup> mice (Figure 2A). This is expected since the other isoforms of AMP deaminases are present in varying levels in various tissues. However, there was no detectable AMP deaminase activity in erythrocyte lysates from Ampd3<sup>-/-</sup> mice in contrast to the wild type erythrocyte lysate. Consistently, there was also no detectable AMP deaminase activity in the serum fraction of blood obtained from Ampd3<sup>-/-</sup> mice. In the heart, where AMPD3 is a major isozyme, the AMP deaminase activity was significantly lower in Ampd3<sup>+/+</sup> mice when compared to wild type. The erythrocyte lysates of heterozygous Ampd3<sup>+<//-</sup> mice had significantly less AMP deaminase activity than the wild type (Figure 2B). Together, these results demonstrate that there is no measurable AMP deaminase activity in the erythrocytes of Ampd3<sup>-/-</sup> mice.

ATP and ADP are elevated in Ampd3<sup>-/-</sup> erythrocytes

In humans, the loss of AMP deaminase 3 is associated with an increased steady-state level of ATP in erythrocytes [15]. Therefore, we measured whether the erythrocytes of Ampd3<sup>-/-</sup> mice displayed a similar increase in ATP. HPLC analysis was performed to quantify the levels of ATP, ADP and AMP in methanol extracts of erythrocytes obtained from wild type and Ampd3<sup>-/-</sup> mice (Figure 3A, 3B).

Adenine nucleotides levels were quantified against calibration standards for ATP, ADP and AMP, and normalized to the protein concentration in each erythrocyte lysate sample. HPLC analysis revealed that 5'-AMP levels in Ampd3<sup>-/-</sup> erythrocytes were not significantly different from wild type, while ATP and ADP levels of Ampd3<sup>-/-</sup> erythrocytes were increased more than 2-fold. In addition, HPLC analysis revealed that the relative levels of adenosine in both wild type and Ampd3<sup>-/-</sup> samples were comparable (Figure 3B). The observed rise in ATP and ADP, but not AMP, could suggest that the adenylate equilibrium was favoring the formation of ATP and ADP when the major pathway for 5'-AMP catabolism via AMPD3 was blocked. Alternatively, the rise in ATP levels in Ampd3<sup>-/-</sup> erythrocytes could reflect increased glycolysis due to allosteric activation of phosphofructokinase by AMP. Such a possibility would correlate with a decreased level of blood glucose in Ampd3<sup>-/-</sup> compared to wild type mice. To address this possibility, fasting blood glucose was measured in the wild type and Ampd3<sup>-/-</sup> mice (Figure 3C).
Animals were fasted for 12 hr prior to blood glucose measurement. The average fasting blood glucose levels of wild type, Ampd3+/− and Ampd3−/− mice were 69 mg/dl, 70 mg/dl, and 73 mg/dl, respectively. The blood glucose levels were not significantly different between the three genotypes. This observation suggests that a decrease in glycolysis is an unlikely explanation. Rather, the elevated ATP and ADP levels likely reflect a reduced degradation of adenine nucleotides in the Ampd3−/− erythrocytes.

Figure 2. AMPD activity is not detectable in erythrocytes (rbc) and is reduced in the heart of Ampd3−/− mice. A. AMP deminase assays were carried out with cell lysates from the indicated organs. For all samples, except serum (WT n = 10; Ampd3−/− n = 5), heart (n = 8) and erythrocytes (WT n = 15; Ampd3−/− n = 7), the data represent the average of tissue samples (n = 3). B. The level of AMPD3 activities in erythrocytes from wild type, Ampd3+/− and Ampd3−/− mice. Error bars, mean ± SEM. T-test: *p<0.05.

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Figure 3. Erythrocytes of Ampd3−/− mice have elevated levels of ATP and ADP but normal levels of AMP. A. Nucleotides from methanol extracts of erythrocyte lysates from wild type (n = 4) and Ampd3−/− (n = 4) were separated by HPLC and quantified by calibration with standards. B. Representative HPLC chromatograms from wild type and Ampd3−/− erythrocyte nucleotide extracts. C. Fasting glucose levels in Ampd3−/− (n = 16) and Ampd3−/− mice (n = 9) and WT (n = 14) mice. Error bars, mean ± SEM. T-test: *p<0.05.

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Ampd3−/− mice have increased sensitivity to 5′-AMP induced hypometabolism

A major objective for generating the Ampd3−/− mice was to test our hypothesis that erythrocytes play an important role in mediating 5′-AMP induced hypometabolism. Previously, we reported that, following an injection of 5′-AMP, there are two distinct phases of metabolic reduction [3]. The initial phase I of metabolic rate drop was very rapid and independent of the Tb of the animal. During this phase I response, VO2 consumption dropped from the euthermic range of 5000–7000 ml/kg/h to about 1500 ml/kg/h within minutes of 5′-AMP administration. This was followed by a more gradual phase II response of metabolic decline that was dependent on Tb and was influenced by Tb. In mice, we observed that phase II corresponds to a VO2 below 1500 ml/kg/h. We previously reported that a dosage of 5′-AMP that is less than 0.2 mg/gw produced a phase I but minimal phase II response in the majority of wild type mice at a Tb of 15°C [3]. Here, we observed that, with a dose of 0.15 mg/gw 5′-AMP, the majority of wild type mice displayed a phase I metabolic decline but a minimal phase II response (Figure 4A). By contrast, the same dosage given to Ampd3−/− mice produced both phase I and a significant length of phase II decline. Both male and female Ampd3−/− mice displayed a similar length of phase II, which was significantly longer than that of wild type siblings of the same sex (Figure 4B, C). These studies show that Ampd3−/− mice displayed a longer state of hypometabolism when given a dosage of 5′-AMP that was less effective for their wild type siblings.

Next we examined the effect of a higher dosage of 5′-AMP on both wild type and Ampd3−/− mice. The two genotypes were given 0.5 mg/gw of 5′-AMP and maintained at 15°C Tb. At 2 h or 4 h post-5′-AMP administration both genotypes were in similar deep hypometabolism with comparable average VO2 profiles (Figure 4D, E). At 6 h and 8 h post-administration of 5′-AMP, arousal from deep hypometabolism was evident especially in wild type and their average VO2 profile had increased to about 2× that of Ampd3−/− mice. At 10 h post-5′-AMP administration, only 2 out of 16 wild type compared to 11 out of 15 Ampd3−/− mice were still in deep hypometabolism. Thus, at a higher 5′-AMP dosage, the average arousal time from deep hypometabolism was significantly delayed in Ampd3−/− compared with wild type siblings. Together, these mouse genetic studies demonstrate that AMP deaminase 3 plays a major role in modulating the effects of 5′-AMP mediated hypometabolism.

Discussion

Mammals are warm-blooded and must maintain a relatively constant Tb of about 37°C. Under metabolic stress, some mammals are able to decouple euthermic control to undergo torpor or hibernation [1]. During torpor, the drop in Tb causes significant slowing of the biochemical and physiological processes allowing the animal to conserve energy and negate the effects of metabolic stress. The biochemical mechanism of natural torpor remains poorly understood. However, it has been noted that the biochemical events that initiate torpor behavior occur rapidly prior to the reduction in Tb [1]. Our studies demonstrated that 5′-AMP, when given to mammals, rapidly induced a transient state of hypometabolism that allows for the Tb to be safely reduced towards Tb [3]. The biochemical mechanism of 5′-AMP induced torpor remains to be conclusively proven. We have previously reported that mice deficient in the extracellular ecto-nucleotidase CD73 have an enhanced response to 5′-AMP for induction of torpor indicating that the extracellular dephosphorylation of 5′-AMP into adenosine is not necessary for the mechanism [3]. We have observed that following 5′-AMP administration, the production of 2,3-BPG is enhanced in the circulation. The critical mutase enzyme for 2,3-BPG synthesis has been identified only in erythrocytes and placenta [16]. Since both male and female mice respond similarly to 5′-AMP induced torpor, the rise in 2,3-BPG implicates the erythrocyte as a cellular target of 5′-AMP. Through isotopic tracking, we and others have observed that erythrocytes readily accumulate radiolabeled 5′-AMP which is converted to ADP using cellular ATP to maintain the adenylate equilibrium [3][17]. A decrease in erythrocyte ATP has been shown to increase 2,3-BPG production [18]. We proposed that the phase I response, the rapid reduction of VO2 following 5′-AMP administration, reflects increased 2,3-BPG allosteric inhibition of hemoglobin oxygen binding. The length of the VO2 phase II reflects the uncoupling of thermogenic control allowing Tb to cool towards Tb thereby reducing the amount of cellular oxygen needed to match its reduced availability. Our proposed mechanism for the erythrocyte’s role is supported by observations that hypoxia can induce torpor in pocket mice [19]. In addition, exanguination is known to be associated with a reduction in Tb [20]. Together, these observations support the role of erythrocytes in mediating hypoxia regulation of Tb.

To test our hypothesis for the erythrocyte’s role in torpor, we used mouse genetics to exploit our current understanding of 5′-AMP catabolism. The major catabolism pathway for 5′-AMP is via its deamination to IMP by AMP deaminase. Of the three isozymes of AMP deaminase, AMPD3 is a major isozyme in heart and is the only isozyme in erythrocytes [8][9]. Our hypothesis predicts that AMPD3 deficiency should confer increased sensitivity to 5′-AMP induced hypometabolism if the erythrocyte is a major mediator of this behavior. We have generated an Ampd3−/− mouse line with a targeted disruption of the Ampd3 gene. Our RT-PCR analysis showed that there is no detectable Ampd3 mRNA in the heart tissue of Ampd3−/− mice unlike their wild type siblings. Enzymatically, the Ampd3−/− and wild type sibling mice displayed comparable AMPD activities in all organs except heart and erythrocytes. In the heart, the level of AMP deaminase activity was reduced by 70%. In erythrocytes, the AMPD3 deficient mice displayed no detectable AMP deaminase activity. Further, no detectable activity was observed in serum from Ampd3−/− blood. Thus, based on enzymatic activity and transcript expression, we have generated mice deficient in AMP deaminase 3. In humans, individuals with a complete lack of AMPD3 are clinically asymptomatic [15]. The erythrocytes of AMPD3 deficient individuals display significantly enhanced steady-state ATP levels. Our analysis of Ampd3−/− mouse erythrocytes also found elevated levels of ATP and ADP but not 5′-AMP. Recently, an independently derived line of Ampd3−/− mice was also reported to have greater than two-fold elevated erythrocyte ATP and ADP levels [21]. The study further reported a 3.5-fold elevated level of 5′-AMP that we did not observe in our HPLC analysis. We cannot explain the 5′-AMP level discrepancy between our analysis and those previously reported. We have provided our HPLC analysis trace as evidence to support our conclusion (Figure 3B). We tested the possibility that increased glycolysis could have converted excess 5′-AMP to ADP and ATP. Our analysis revealed that fasting blood glucose levels were not significantly altered and were within the normal physiological range in the AMPD3 deficient mice. Our findings are in line with the report that fasting blood glucose levels of AMPD3 deficient mice were similar to wild type [21]. A possible explanation for our observation is that the adenylate equilibrium preferentially maintains the cellular pool of adenine nucleotides as ATP and ADP rather than 5′-AMP. Typically, adenylate kinase maintains the adenylate equilibrium at a set ratio with ATP levels.
An Induced Hypometabolic State in Mammals

A.

\[ \text{VO}_2 \text{ (mL/kg/hr)} \]

\[ \text{Time (hr)} \]

\[ \text{PI} \]

\[ \text{PII} \]

\[ T_a = 15^\circ \text{C} \]

B.

\[ \text{Time VO}_2 \text{ below 1500 mL/kg/hr/min} \]

\[ \text{WT} \]

\[ \text{Ampd3}^{-/-} \]

C.

\[ \text{Time VO}_2 \text{ below 1500 mL/kg/hr/min} \]

\[ \text{WT} \]

\[ \text{Ampd3}^{-/-} \]

D.

\[ \text{5'-AMP injection} \]

\[ \text{Wild type (n=16)} \]

\[ T_a = 15^\circ \text{C} \]

\[ \text{VO}_2 \text{ (mL/kg/hr)} \]

\[ \text{Time (hr)} \]

\[ \text{5'-AMP injection} \]

\[ \text{Ampd3}^{-/-} (n=15) \]

\[ T_a = 15^\circ \text{C} \]
several fold higher than ADP and at least 10-fold higher than 5'-AMP [22]. In most mammalian erythrocytes, the conversion of 5'-AMP to IMP is irreversible due to the absence of the enzyme adenylosuccinyl synthetase that converts IMP to 5'-AMP [23][24]. Therefore, the rise in the adenine nucleotide pool in the Ampd3−/− erythrocytes reflects the loss of a major catabolic pathway for 5'-AMP even when catabolism via dephosphorylation to adenosine remains intact.

The generation of the Ampd3−/− mice allowed the testing of our hypothesis that erythrocytes are the major target of 5'-AMP in this model of induced hypometabolism. Consistent with our hypothesis, we observed that AMPD3 deficient mice displayed significantly enhanced sensitivity to 5'-AMP for induction of hypometabolism. Using a lower dose that produced only a phase I response in wild type mice, the AMPD3 deficient animals displayed both phase I and phase II responses regardless of gender. Further, when a higher dosage of 5'-AMP was given, the arousal time from deep hypometabolism for the majority of Ampd3−/− mice was significantly delayed compared with wild type mice. With an intact cellular adenylate equilibrium, we reasoned that the AMPD3 deficient mice undergo 5'-AMP induced hypometabolism with the same basic mechanism as wild type mice. We have previously measured ATP, ADP and 5'-AMP in these animals are more responsive to 5'-AMP than their WT cools at similar rates [3][25]. Consistent with previous findings, we and others have observed that A1AR deficient, unlike wild type mice, did not develop bradycardia following administration of 5'-AMP [26][25]. These observations argue against an induction of bradycardia by adenosine as the underlying mechanism for 5'-AMP induced hypometabolism.

In summary, we have generated AMPD3 deficient mice and these animals are more responsive to 5'-AMP for induction of hypometabolism. These findings support our proposal that the erythrocyte is a target of 5'-AMP's effect on hypometabolism.

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Author Contributions

Conceived and designed the experiments: CCL ZZ. Performed the experiments: ISD WGO VN ZZ. Analyzed the data: CCL ISD ZZ WGO. Wrote the paper: CCL ISD ZZ.

Previous it had been proposed that dephosphorylation of 5'-AMP to adenosine and activation of the A1 adenosine receptor (A1AR) causing bradycardia is an underlying mechanism of 5'-AMP induction of torpor [4]. Our current studies cannot rule out the possibility that decreased AMP deaminase activity in the heart could have contributed to the increased sensitivity of Ampd3−/− mice to 5'-AMP. Previously, we reported that A1AR deficient and wild type mice have similar responses to 5'-AMP as their Tb cools at similar rates [3][25]. Consistent with previous findings, we and others have observed that A1AR deficient, unlike wild type mice, did not develop bradycardia following administration of 5'-AMP [26][25]. These observations argue against an induction of bradycardia by adenosine as the underlying mechanism for 5'-AMP induced hypometabolism.

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