The role of endogenous H2S production during hibernation and forced hypothermia

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Endogenous H$_2$S Production is Crucial in Maintaining Torpor-arousal Cycles and Preserving Renal Integrity in the Hibernating Syrian Hamster

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ABSTRACT

Background: Mammalian hibernation is characterized by repetitive cycle of profound reduction in metabolism and core body temperature known as torpor followed by restoration to euthemic level (arousal). Despite cycling through the stress of torpor and arousal, no sign of organ injury has been reported upon arousal from torpor. Hydrogen sulfide (H$_2$S) has recently been implicated in inducing a hibernation-like state in small mammals without organ injury or behavioral changes upon full recovery. The present study seeks to investigate the involvement of endogenous H$_2$S and its role in natural hibernation.

Methods: Syrian golden hamsters (Mesocricetus auratus) were housed in a climate controlled chamber at 5°C under dim red light to induce torpor. Movement of all animals was continuously monitored with passive infrared detectors. Some groups of hamsters were euthanized during torpor and arousal phases. Other groups of torpid hamsters were implanted with osmotic mini-pumps i.p. filled with saline or a non-specific H$_2$S inhibitor, amino-oxyacetic acid (AOAA; 100mg/kg/day) after receiving an i.p. bolus injection of AOAA 70mg/kg or saline, causing arousal in all animals. Summer euthermic- and winter euthermic animals underwent a similar protocol. Hamsters were euthanized in the normothermic state ~ 4 days following the implantation by an overdose of pentobarbital. Blood and kidney samples were collected.

Results: Core body temperature dropped from 36.5 ± 0.5°C in summer euthermic animals to 10.0 ± 2.0°C during torpor and returned to euthermic temperature during arousal. Despite this significant reduction in temperature, renal function as determined by serum creatinine levels, renal morphology and ROS production, is not different from summer euthermic, torpor and arousal. Also, endogenous H$_2$S production is increased, potentially through upregulation of renal CBS, CSE and 3-MST during torpor. H$_2$S production was restored to near euthermic level upon arousal from torpor. Further, Pharmacological inhibition of H$_2$S production using AOAA precluded torpid hamsters to enter the subsequent torpor bouts, whereas torpid hamsters infused with saline re-entered torpor following pump implantation. Moreover, torpid animals infused with AOAA showed substantial renal damage, as indicated by profound changes in renal morphology and increased expressions of KIM-1, α-SMA, ED-1, HIS-48, high levels of serum creatinine and renal ROS production, decreased expression levels of H$_2$S-producing enzymes as well as a hundred-fold decrease in endogenous H$_2$S production. Also, renal damage was observed in both winter euthermic groups. However, renal morphology remained well preserved during hibernation in the saline and non-hibernating summer control groups (± AOAA) and correlated positively with upregulation of H$_2$S-producing enzymes and subsequent endogenous H$_2$S production.
**Conclusion.** Our results demonstrate that renal H\textsubscript{2}S system seems crucial in maintaining torpor-arousal cycle in Syrian hamsters and preserving their kidney morphology and function through regulation of H\textsubscript{2}S-producing enzymes under these physiological extremes. These findings might be relevant for a number of clinical conditions such as therapeutic hypothermia (e.g. during cardiac and brain surgery and following cardiac arrest) or organ preservation for transplantation medicine.

**INTRODUCTION**

During hibernation, mammals undergo repetitive cycles of metabolic depression and reduced core body temperature (Tb) known as ‘torpor’ and restoration of metabolism and Tb known as ‘arousal’. Typically, in small rodents such as European and golden-mantled ground squirrels, oxygen consumption decreases by 97-98\% of euthermic value (Barnes, 1989; Boyer and Barnes, 1999) and Tb drops to 2-10°C (Kenagy et al., 1989; Hut et al., 2002). In addition, heart rate drops substantially, e.g. in the marmot (*Marmota flaviventris*) from 200–300 bpm in the summer euthemeric state to 3–10 bpm during torpor (Zatzman, 1984). Despite cycling through extreme physiological situations during hibernation, organ injury is absent in the kidney, (Zancanaro et al., 1999; Jani et al., 2011), brain (Drew et al., 2001; Zhou et al., 2001), the gut and liver (Kurtz et al., 2006) and the heart (Camici et al., 2008) after the hibernation season. However, organs of torpid animals undergo major changes in architecture and/or the expression of specific molecular markers resembling those observed in chronic diseases as found in brain (Popov and Bocharova 1992, Arends et al., 2011) and lung (Talaei et al., 2011). Nevertheless, such changes are quickly reversed during arousal bouts, thus preventing permanent organ injury and ensuring proper organ function after hibernation. In fact, it has been speculated that arousals principally serve to reverse organ changes contracted during torpor (Fisher, 1964; French, 1982a, Daan et al., 1991). In addition to its reversal, hibernators may be intrinsically protected from substantial organ damage because of metabolic adaptations (Pan et al., 2013; Stenvinkel et al., 2013; Bogren et al., 2014), and/or suppression of the immune response (Bouma et al., 2010; Bouma et al., 2011).

The molecular mechanisms that convey the rapid repair and/or intrinsic protection of torpid tissues in hibernators are essentially unknown, although hibernators show changes in gene and protein expression. Recently, we identified the production of endogenous hydrogen sulfide (H\textsubscript{2}S) to be a crucial player in the restoration of lung architecture during arousal (Talaei et al., 2012), thus extending the previously disclosed protection of cells and organs from cooling and rewarming injury by the CBS/H\textsubscript{2}S system (Talaei et al., 2011). Moreover,
Revsbech et al. (2014) also reported high levels of unbound free sulfide in the blood of hibernating Scandinavian brown bears. These observations suggest that \( \text{H}_2\text{S} \) mechanism may form an integral part of hibernation. The potential involvement of \( \text{H}_2\text{S} \) in hibernation is further supported by its ability to induce a hibernation-like state in non-hibernating rodents such as mice for several hours without negative behavioral changes upon reversal (Blackstone et al., 2005; Blackstone and Roth 2007; Aslami et al., 2009; Bos et al., 2009). Moreover, \( \text{H}_2\text{S} \) has emerged as an endogenous molecule with potent cyto- and organoprotective properties (Kimura and Kimura 2004; Whiteman et al., 2004; Elrod et al., 2007; Bos et al., 2009; Szabo et al., 2011; Lobb et al., 2014).

Therefore, we hypothesized that endogenous \( \text{H}_2\text{S} \) is involved in entrance into natural hibernation and in preserving organ integrity during the repetitive cycle and stress of torpor-arousal. We first examined the endogenous \( \text{H}_2\text{S} \) system and organ damage in kidney - an organ highly vulnerable to hypothermia and hypoxia – in natural hibernation and found \( \text{H}_2\text{S} \)-producing enzymes to be substantially upregulated, particularly in torpor. In a second experiment, endogenous \( \text{H}_2\text{S} \) production was blocked during torpor and the subsequent arousal using continuous infusion of amino-oxyacetic acid (AOAA), a non-specific inhibitor of \( \text{H}_2\text{S} \) producing enzymes (Asimakopoulou et al., 2013). Effects of AOAA on the torpor-arousal cycle and kidney damage were assessed.

**MATERIALS AND METHODS**

*Induction of hibernation*

Hibernation was induced and monitored in male Syrian hamsters (Mesocricetus auratus) as described previously (Bouma et al., 2011). Periods with ≥ 24h of inactivity were considered to be torpid phases. Hamsters were euthanized on day 1 upon entering torpor (TE: torpor early; \( n = 4 \)), on day 3 during deep torpor (TL: torpor late; \( n = 4 \)), 1.5 h after onset of arousal (AE: arousal early; \( n = 5 \)) and 8 h after reaching full euthermia at ~37°C (AL: arousal late; \( n = 5 \)). In a second experiment, TL animals were implanted with osmotic mini-pumps filled with saline (0.9%, \( n = 5 \)) or amino-oxyacetic acid (AOAA; 100 mg/kg/day, \( n = 6 \)) under 2.5% isoflurane anesthesia. As AOAA precluded animals to return to torpor, all animals were euthanized during at euthermia ~4 days following implantation, which required induction of arousal 4 h prior to euthanization in animals which re-entered torpor following pump implantation. Summer euthermic hamsters (SE, 14h:10h light:dark cycle, ambient temperature of 21°C) and winter euthermic animals (WE, complete darkness, ambient temperature of 5°C) implanted with or without mini-pumps filled with AOAA served as controls. Summer euthermic and winter euthermic animals did not show any torpor behavior. Animals were euthanized by 1.5 ml 6% sodium pentobarbital i.p.. Blood samples were obtained and kidneys tissue was
either snap-frozen in liquid nitrogen and stored at –80°C or fixated in 4% paraformaldehyde for histopathological analysis. The experiments were approved by the Animal Experiments Committee of the University of Groningen (DEC5456).

**Histopathology**

Paraffin sections (4 µm) were dewaxed and stained with periodic acid-Schiff (PAS) reagent and counterstained briefly with Meyer’s hematoxylin. Renal sections were examined blindly by 2 independent observers (Gross et al., 2006). Glomerular damage was scored semiquantitatively in 100 glomeruli from 0 to 4 (El Nahas et al., 1991) and tubulointerstitial damage was quantified on the basis of tubular dilatation, atrophy of epithelial cells and widening of tubular lumen (Gross et al., 2006).

**Immunohistochemistry**

Sections were stained for kidney injury molecule (KIM-1, diluted 1:50), a marker for renal tubular damage (goat polyclonal, Santa Cruz, Te Huissen, Netherlands), ED-1 (diluted 1:500), a marker for macrophages (mouse monoclonal, Serotec Ltd, Oxford, UK), HIS-48, a marker for neutrophils (mouse monoclonal, generously provided Prof. F.G.M. Kroese, ProHisto foundation, Groningen, The Netherlands), α-SMA (diluted 1:100), a marker for fibrosis (mouse monoclonal, Dako, Glostrup, Denmark) as previously described (Sandovici et al., 2006).

**Western Blot**

The expression of renal CBS, CSE and 3-MST in all groups of hamsters was analyzed by western blot (Talaei et al., 2011). In brief, proteins on the nitrocellulose membranes were detected with CBS, CSE and 3-MST antibodies (1:1000) overnight at 4°C, washed three times with Tris buffered saline with 0.004% Tween and treated with the respective secondary antibody (1:1000, 2 h at room temperature). Protein bands were visualized using a Gene Genome and band intensities were quantified using Gene Tools software (Westburg B.V., Leusden, The Netherlands).

**ROS Measurement**

Reactive oxygen species (ROS) were determined by spectrophotometrical measurement of MDA levels in renal tissue (Kim and Vaziri, 2010) following homogenization with PBS containing butylatedhydroxytoluene (BHT, Cell Biolabs, Inc, Te Huissen, Netherlands) and expressed as micromoles of MDA per milligram of tissue.

**H2S Measurement in Blood**

Sulfide antioxidant buffer was prepared from 25 g of sodium salicylate, 6.5 g of ascorbic acid and 8.5 g of sodium hydroxide in 100 mL of distilled water at pH 13. Next, 100µL
of the sulfide antioxidant buffer was added to 100 µL whole blood. A sulfide ion selective electrode was immersed into the mixture and the electrode potential was measured. The sulfide ion concentration of the serum was calculated using a standard curve of Na₂S·9H₂O in the sulfide antioxidant buffer, according to the manufacturer’s instructions (Lazar Research Laboratories, Inc. Los Angeles, CA, 90036 USA).

**Statistical Analysis and Data Presentation**

Variables are expressed as mean ± SEM. Differences between groups were tested for significance using a One-Way ANOVA with Bonferroni Post-Hoc testing for normally distributed values or a Kruskal-Wallis ANOVA on ranks. P-values < 0.05 were considered statistically significant (SPSS version 22).

**RESULTS**

*Renal function is preserved during hibernation*

Hibernating Syrian hamsters (*Mesocricetus auratus*) underwent repetitive cycles of torpor and arousal, while summer euthermic hamsters remained fully active (supplemental figure S1). Renal function was preserved during torpor-arousal cycle, as shown by unchanged serum creatinine levels throughout the torpor-arousal cycle in comparison with summer euthermic controls (figure 1A; *p > 0.05*). In accord, (immune)histochemistry of renal tissue showed no changes in morphological features, and the absence of markers for tubular damage (KIM-1), influx of immune cells (ED-1, HIS-48) and fibrosis (α-SMA), and preservation of mitochondrial structure in glomerular and proximal tubular cells throughout the hibernation phases (supplementary figure S1).

*Blood H₂S and renal H₂S-producing enzymes are increased during torpor*

To investigate the role of H₂S during hibernation, blood H₂S level and the renal expression of 3 H₂S-producing enzymes (CBS, CSE and 3-MST) were measured. The blood H₂S level increased about 2-fold during early torpor and increased even further towards the end of torpor, followed by a substantial decrease during arousal close to summer euthermic levels (figure 1B). In kidney, CBS expression increased about 2-fold during early and late torpor compared to summer euthermic hamsters, reduced during early arousal and increased again during late arousal (figure 1C,D). Unlike CBS, CSE expression was significantly upregulated only during late torpor (figure 1C,E). Finally, 3-MST expression followed a pattern similar to CBS and increased 3-fold during early and late torpor, normalized during early arousal and increased two-fold during late arousal (figure 1C,F). Thus, the blood H₂S level and expression of H₂S-producing enzymes are increased during torpor and decreased upon arousal.
Inhibition of H₂S production precludes subsequent torpor bouts

To further substantiate the involvement of H₂S during natural hibernation, summer euthermic (SE), winter euthermic (WE) and hibernating animals (HIB) were i.p. implanted with mini-pumps delivering AOAA, a non-selective inhibitor of H₂S production. In HIB, AOAA was infused from the start of arousal. Importantly, AOAA precluded hibernating hamsters to re-enter torpor in contrast to saline-infused HIB animals (figure 2A, S2E,F; p < 0.001). Hence we decided to euthanize animals at similar body temperatures, i.e. 4 hours after induction of arousal in saline-infused hamsters. Blood H₂S levels were similar in saline-infused HIB and SE, but were reduced below detection in saline-infused WE (figure 2E; p < 0.01). Blocking H₂S production by AOAA dramatically reduced blood H₂S, ranging from being non-detectable in HIB to a 6-fold reduction in SE. Thus, AOAA strongly reduced blood H₂S levels during euthermia and precluded HIB animals from entering a subsequent torpor bout.

Inhibition of H₂S production lowers renal expression of all H₂S-producing enzymes and produces kidney damage
Figure 2. Inhibition of endogenous H$_2$S production precludes torpor bouts and induces renal injury. (A) AOAA infusion in hibernating animals reduced torpor duration as animals failed to enter subsequent torpor bouts. (B) Representative sections of the kidney (magnification x400) from all groups showing PAS, ED-1, α-SMA KIM-1, and HIS-48 stainings. Arrows point to positively stained areas (in brown). (C,D) Significant increase in serum creatinine level and renal MDA in WE and AOAA-infused HIB animals compared to SE animals. (E-H) H$_2$S inhibition by AOAA resulted in a substantial decrease in blood H$_2$S and its synthesizing enzymes in SE, WE and AOAA-infused HIB animals compared to saline-infused HIB animals. (I-M) quantification of immuno(histochemical) stainings of PAS, ED-1, α-SMA, KIM-1 and HIS-48. PAS= Periodic Acid Schiff; ED-1 = Macrophage marker; α-SMA = fibrosis marker; KIM-1 = Kidney Injury Molecule; HIS-48 = Neutrophil marker. SE = Summer Euthermic; WE = Winter Euthermic; HIB = Hibernating.
Next, we substantiated whether \( \text{H}_2\text{S} \) protects the kidney from damage during natural hibernation. In saline-infused groups, the renal expression of CBS, CSE and 3-MST was similar in HIB and SE, but strongly reduced in WE (figure 2F-H). AOAA infusion dramatically reduced the renal expression of all three \( \text{H}_2\text{S} \)-producing enzymes in SE and HIB to levels also found in WE, with much larger downregulation in CSE expression compared to CBS and 3-MST (figure 2F-H). Expression of \( \text{H}_2\text{S} \)-producing enzymes was mirrored by kidney damage, as determined by levels of serum creatinine and kidney damage markers. Creatinine and damage markers were considerably increased in saline-infused WE compared to HIB and SE (figure 2C,D,I-M). AOAA infusion strongly increased these parameters in HIB to levels similar to those found in AOAA-treated WE. In contrast, no increase in creatinine, ROS or damage markers was found in AOAA-infused SE. Thus, AOAA infusion strongly reduced renal expression of \( \text{H}_2\text{S} \)-producing enzymes and produced renal damage in hibernating hamsters.

**DISCUSSION**

In the present study we identified an important role for \( \text{H}_2\text{S} \) in the induction of torpor and preservation of renal integrity during hibernation in the Syrian hamster. Natural hibernation was characterized by an increase both in circulating \( \text{H}_2\text{S} \) and in the renal expression of all 3 \( \text{H}_2\text{S} \)-producing enzymes during torpor, and by the absence of kidney damage in all phases of hibernation. In addition, pharmacological inhibition of \( \text{H}_2\text{S} \) by AOAA lowered serum \( \text{H}_2\text{S} \), precluded hibernating animals from re-entering a torpor bout and induced renal injury, as shown by an increase in serum creatinine levels, renal ROS formation and increased levels of markers of kidney damage. The AOAA-induced increase in renal injury also attenuated the upregulation of \( \text{H}_2\text{S} \)-producing enzymes observed in saline-infused hibernating animals. Thus, endogenous \( \text{H}_2\text{S} \) and its synthesizing enzymes are crucial in the induction of torpor as well as in preserving renal integrity during the torpor-arousal cycle.

*Endogenous \( \text{H}_2\text{S} \) is pivotal in induction of torpor during hibernation*

Our data strongly implicate \( \text{H}_2\text{S} \) as a key player in natural hibernation. A first major finding of this study is that \( \text{H}_2\text{S} \) seems to be of pivotal importance in the induction of torpor. Blood levels of \( \text{H}_2\text{S} \) are substantially regulated during the hibernation cycle, with the highest values found in the torpor phase. Moreover, blockade of endogenous \( \text{H}_2\text{S} \) production by infusion of the non-specific inhibitor, AOAA, substantially lowered blood \( \text{H}_2\text{S} \) levels below detection limits, precluded hibernating animals from re-entering torpor. Induction of torpor is characterized by an active and rapid inhibition of metabolism with subsequent lowering of body temperature towards the ambient temperature (Heldmaier et al., 2004). \( \text{H}_2\text{S} \) is a good candidate to convey the initial suppression of metabolism in torpor, since the compound suppresses metabolism
by reversibly inhibiting oxidative phosphorylation through inhibition of mitochondrial complex IV (cytochrome c oxidase), the terminal enzyme in the mitochondrial electron transport chain (Beauchamp et al., 1984, reviewed in Szabo et al., 2014), particularly under normoxic conditions (such as at the entrance of torpor). However, H$_2$S actually supports mitochondrial function at the level of coenzyme Q (Goubern et al., 2007) and may help in maintaining ATP levels under hypoxic conditions (Fu et al., 2012, Modis et al., 2013; reviewed in Szabo et al., 2014), as encountered during the torpor bout. The decreasing rate of H$_2$S oxidation as tissue oxygen level drops during torpor may further assist in the maintenance of biologically active H$_2$S in the tissue (Olson, 2008). Thus, H$_2$S seems a prime candidate to manage both facets of torpor as increased levels during torpor may both assist in torpor induction and in the maintenance of tissue viability during torpor by safeguarding ATP production.

The importance of H$_2$S in natural torpor is in keeping with observations in non-hibernating mouse kept under low ambient temperatures, in which the exogenous administration of H$_2$S gas induces a hibernation-like state (Blackstone et al., 2005; Blackstone and Roth, 2007). Curiously, the level of blood H$_2$S of torpid hamster is of the same order of magnitude as the one reported in H$_2$S-treated mice, i.e. both in the low µM range. Importantly, reversal of the artificial hibernation-state produced by exogenous H$_2$S is dependent on increasing the ambient temperature to induce rewarming in the animal. In contrast, rewarming in natural hibernation is initiated from within by heat generated by uncoupled oxidative phosphorylation in brown fat (Zivadinovic et al., 2005), leaving open the option that the induction of arousals in natural hibernation may be primarily depend on the initiation of brown fat oxidation.

Endogenous H$_2$S is produced from L-cysteine in various mammalian tissues by a variety of biochemical pathways catalyzed by two cytosolic enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Wang, 2002; Kimura, 2011) and a mitochondrial enzyme, 3-mecaptopyruvate sulfurtransferase (3-MST) (Olson et al., 2010; Kimura, 2011). All 3 H$_2$S-generating enzymes are highly expressed in the kidneys, mainly in the renal proximal tubules (Ishii et al., 2004; Li et al., 2006; Kimura, 2011). These observations are in agreement with previous reports in which lung H$_2$S and CBS increase during torpor in hibernating Syrian hamsters (Talaei et al., 2012) and with the reported elevation of unbound free sulfide with high cysteine levels in the blood of hibernating Scandinavian brown bears (Revsbech et al. 2014).

**Blood H$_2$S levels**

The 2-3 fold increase in the expression levels of H$_2$S-producing enzymes in kidney during torpor closely matches the 2-3 fold increase in endogenous H$_2$S production, which level was also reduced following arousal. Consequently, H$_2$S levels in blood throughout hibernation closely match those of the expression of H$_2$S-producing enzymes in kidney. It is still not clear
what constitutes the source of blood H$_2$S. Apart from the kidney, H$_2$S-producing enzymes are also abundantly expressed in the brain, liver and the cardiovascular system (Wang, 2002; Szabo, 2007; Kimura, 2011). Further, H$_2$S is also produced by CSE in erythrocytes and released into circulation (Bearden et al., 2010). Therefore, one could surmise that the H$_2$S-producing enzymes in these sources may contribute to the high levels of circulating H$_2$S during torpor.

Renal H$_2$S formation and kidney damage

A second obvious finding of the current study implicates H$_2$S as a key player in precluding organ damage during the torpor-arousal cycle. The expression of all 3 H$_2$S-producing enzymes in the kidney is strongly increased during torpor. During arousal, the expression patterns differ substantially; whereas CSE is normalized to SE levels, expression of CBS – and to a lesser extent that of 3-MST – remain increased compared to SE. These observations in kidney are consistent with our previous findings in lung tissue, where both CBS expression and lung H$_2$S production is increased during torpor and normalized upon arousal (Talaei et al., 2012). Further, blocking the H$_2$S production with AOAA significantly reduced the expression levels of all 3 H$_2$S-producing enzymes in kidney, which was accompanied by a marked increase in serum creatinine, renal production of ROS, pathological changes in glomerular and tubular structures, increased expression of the damage markers such as α-SMA and KIM-1, and the influx of neutrophils and macrophages. The observed renal protection by H$_2$S is in agreement with the organoprotective property of exogenously applied H$_2$S in several animal studies (Esechie et al., 2008; Bos et al., 2009; Bos et al., 2013; Lobb et al., 2014). The mechanism by which H$_2$S induces organ protection is not completely understood. Blocking endogenous H$_2$S during torpor in the present study resulted in a substantial increase in the level of ROS production, suggesting that H$_2$S may act as an antioxidant to inhibit ROS production during torpor-arousal cycle and prevent oxidative stress in the kidney. Alternatively, increased H$_2$S may recruit additional antioxidant pathways e.g. through induction of the expression of various antioxidant genes upon activation of nuclear factor erythroid 2-related factor (nrf2) (Calvert et al., 2009). Therefore, both H$_2$S antioxidant properties or activation of endogenous antioxidant pathways may protect the kidney during hibernation.

A third and remarkable finding in the current study is that WE hamsters show very low levels of blood H$_2$S and reduced expression of all renal H$_2$S-producing enzymes. Moreover, in contrast to hibernating hamsters, WE hamsters displayed a substantial increase in serum creatinine, increased renal ROS production and, importantly, kidney damage. Curiously, these phenomena were not observed during (late) arousal in hibernating animals, which are at similar body temperature (and presumably metabolic rate) as WE. Possibly, this difference is related to the duration of the housing under 5°C ambient temperature, lasting only about 4
days for arousals, whereas amounting several weeks in WE, exhausting defense mechanisms and provoking damage in the latter. Further, AOAA infusion induces (excess) renal damage in WE and HIB and decreases the expression of H\textsubscript{2}S-producing enzymes in the kidney. In contrast, AOAA infusion in SE did not provoke kidney damage, although blood H\textsubscript{2}S level and expression of renal H\textsubscript{2}S-producing enzymes were significantly reduced. Important to note is that HIB and WE animals have a substantial higher metabolic rate compared to SE due to low ambient temperatures. Taken together, these data suggest that H\textsubscript{2}S provides renal protection at high metabolic demand, possibly by fueling ATP production (Modis et al., 2013; reviewed in Szabo et al., 2014), whereas it is of minor significance under baseline metabolic conditions.

**Potential applications of H\textsubscript{2}S-induced hibernation-like state in biomedicine**

The induction of torpor by H\textsubscript{2}S and the cell protection by upregulation of its synthesizing enzymes holds a substantial promise in human medicine. H\textsubscript{2}S has been found to protect cells and organs against inflammatory responses in animal models of inflammatory diseases (Whiteman and Winyard 2011). More recently is the observation of cyto- and organoprotective properties of H\textsubscript{2}S in the field of hypoxic- and ischemia/reperfusion injury (Lobb et al., 2012; Lobb et al., 2014). Therefore, elucidating the exact mechanisms of H\textsubscript{2}S-induced hibernation-like state will add to novel therapeutic approaches biomedicine.

In conclusion, we have demonstrated for the first time that the H\textsubscript{2}S system plays a pivotal role in the induction of torpor and the regulation of the torpor-arousal cycle in mammalian hibernators. Moreover, it is of major importance to protect kidney under extreme physiological conditions. Threats encountered during hibernating conditions resemble specific clinical situations, such as accidental hypothermia, hypothermic organ preservation for transplantation and ischemia/reperfusion injuries. Therefore, hibernation may represent a unique natural model providing strategies to cope with such conditions. Hence, unraveling the exact molecular mechanisms involved in the induction of torpor, the timing of arousal and the preservation of organ function may lead to novel pharmacological strategies relevant to human medicine.

**CONFLICT OF INTEREST STATEMENT**

There are no conflicts of interest.

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Supplementary figure (S1). Renal integrity is preserved during torpor-arousal cycle. Representative sections of the kidney (magnification x400) from hamsters in various stages of hibernation showing PAS, KIM-1, α-SMA, ED-1, and HIS-48 stainings showing preservation of the glomerular and interstitial compartments of the kidney during the torpor-arousal cycle. PAS = Periodic Acid Schiff; ED-1 = Macrophage marker; α-SMA = fibrosis marker; KIM-1 = Kidney Injury Molecule; HIS-48 = Neutrophil marker. SE = Summer Euthermic; TE = Torpor Early; TL = Torpor Late; AE = Arousal Early; AL = Arousal Late.

Supplementary figure (S2). Full immunoblots of the renal expression of the three H₂S-producing enzymes. Expression was measured for CBS, CSE and 3-MST in the kidney. (A) The expression levels of all three H₂S-producing enzymes increased during torpor and reduced following arousal. (B) AOAA infusion decreased the expression levels of all three H₂S-producing enzymes in SE, WE and HIB animals. SE = Summer Euthermic; TE = Torpor Early; TL = Torpor Late; AE = Arousal Early; AL = Arousal Late; WE = Winter Euthermic; HIB = Hibernating.
Body temperature (ºc)

Time (h)

Legend?