Explorations of the therapeutic potential of influencing metabolism during critical illness

Aslami, H.

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Potential Applications of Hydrogen Sulfide–induced Suspended Animation

Hamid Aslami[1], Marcus J. Schultz[1,2], Nicole P. Juffermans[1,2]

[1] Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.)

[2] Department of Intensive Care Medicine, Academic Medical Center, Amsterdam, The Netherlands

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Abstract

A suspended animation–like state has been induced in rodents with the use of hydrogen sulfide, resulting in hypothermia with a concomitant reduction in metabolic rate. Also oxygen demand was reduced, thereby protecting against hypoxia. Several therapeutic applications of induction of a hibernation–like state have been suggested, including ischemia–reperfusion injury. More recently, hydrogen sulfide has been found to be protective in states of exaggerated inflammatory responses, such as acute lung injury. Possible mechanisms of this protective effect may include reduction of metabolism, as well as reduction of inflammation. In this manuscript, the methods of inducing a suspended animation–like state in experimental models using hydrogen sulfide are described. We discuss the effects of hydrogen sulfide–induced hypo–metabolism on hemodynamic, metabolic and inflammatory changes in animal models of various hypoxic and inflammatory diseases. In addition, potential therapeutic possibilities of hydrogen sulfide–induced hibernation are outlined.

Introduction

In October 2006, the first known case of a human going into “hibernation” was described. After slipping and breaking his pelvis, a hiker survived 24 days in a mountain forest without food or water. He was found unconscious, with a body temperature close to 22°C, a weak pulse and suffering from blood loss (1). After referral to a hospital, he made a full recovery. His treating physicians believed that his survival was a result of going into a state akin to hibernation, as mountain temperature dropped as low as 10°C. This report does not stand alone. Other cases of survival of accidental deep hypothermia have been mentioned, including patients after a prolonged cardiac arrest and resuscitation (2–6).

Organ damage occurs after more than five minutes of circulatory arrest as a reaction to low oxygen levels (hypoxia) or no oxygen (anoxia). Hypoxia leads to the generation of free radicals, release of excitatory amino acids and calcium shifts lead to mitochondrial damage and apoptosis, causing cerebral injury (7–9). In nature, tolerance to hypoxia is seen in hibernating mammals (10). These animals can transiently arrest cellular processes as a results of unfavorable environmental changes by changing their metabolism, substrate selection, oxygen consumption, heart rate and body temperature (10–14). During hibernation, body temperature can drop to ambient temperature even below freezing points. Under these circumstances, hibernating animals are resistance to otherwise lethal hypoxia.

Several animals adapt to hypoxic or anoxic atmospheres (15–19). Some invertebrates exhibit an arrest of development and a reduction in protein synthesis in the absence of oxygen (17). A clinically relevant example of adaptation to anoxia was provided in a dog model of prolonged exsanguination–induced cardiac arrest, followed by flushing 4°C saline in the aortic arch, resulting in profound hypothermia. After resuscitation, a complete recovery without neurological damage was observed (20), suggestive of a mechanism to adapt to a lack of oxygen when energy supply and energy demand is limited.

From a philosophical point of view, these adaptive mechanisms may be a rudiment from early development of life on earth, when the atmosphere lacked oxygen, but was full of sulfur–containing molecules, such as hydrogen sulfide (21;22). Primordial organisms may have generated their energy supply by using hydrogen sulfide, resembling the way that modern life–forms use oxygen (23). In some bacteria, sulphur based respiration is still present (24;25). Indeed, hydrogen sulfide is produced endogenously in humans (26). By binding to cytochrome c oxidase, hydrogen sulfide can competitively inhibit the oxidative phosphorylation pathway in the inner mitochondrial membrane in the presence of oxygen (27). The ability of hydrogen sulfide to compete with oxygen may have become part of an intrinsic cellular program to naturally slow or stop oxidative phosphorylation under anoxic conditions (27;28).

Recently, it was shown that a hibernation–like state can be induced in animals that do not naturally hibernate (29). Mice exposed to hydrogen sulfide gas experienced a drop in core body temperature to 15°C, along with the ambient temperature, together with a drop in metabolic rate. This was termed a *suspended animation like state*. As described above, comparable physiological changes have been seen in naturally hibernating mammals (11).
The observations in animal models of anoxia and hypoxia suggest that inhibiting metabolism on demand is feasible. Although humans are considered to be homeothermic mammals, the anecdotal reports on otherwise healthy people surviving accidental deep hypothermia and circulatory arrest with no or minimal cerebral impairment, suggest that the ability to hibernate may have persisted throughout mammalian evolution (23).
If mammalian hibernation proves to be possible, induction of a hypo–metabolic state may be beneficial in a number of situations when supply of oxygen is jeopardized. An example of the protective effect of a treatment that reduces metabolic processes is induced hypothermia. Hypothermia increases the tolerance of the brain to ischemia. Induction of deep hypothermia has been applied in the clinical setting successfully in the last decades as a means of protecting the brain from ischemic injury when circulatory arrest is required, e.g. during cardiothoracic surgery (30–33). Moderate hypothermia is now an accepted clinical treatment in patients after cardiac arrest and is associated with better neurological outcomes in these patients (34). Although the mechanisms of the protective effect of induced hypothermia are complex, reduction of cerebral oxygen consumption is likely to contribute (35–38).
In this review, we will discuss the methods of induction of suspended animation with H_2S in non–hibernating animals, as well as the metabolic, hemodynamic and inflammatory effects of H_2S in different animal models. Furthermore, we speculate on potential applications of hydrogen sulfide–induced suspended animation.

Methods – systematic search of the literature

The following keywords were used to obtain papers published in the Medline database; *hypothermia, hydrogen sulfide, suspended animation, metabolism,* and *hypoxia*. The relevance of each paper was assessed using the online abstracts. In addition, the reference list of the retrieved papers was screened for potentially important papers.

Induction of a suspended animation–like state using H_2S

Hydrogen sulfide (H_2S) is a flammable gas, which has long been considered an environmental hazard because of its cytotoxic effects (39). However, H_2S is also endogenously produced in small amounts and plays a significant role as a second messenger in cellular signaling.

H_2S is synthesized in the cysteine biosynthesis pathway under the enzymatic regulation of cystatione b–synthase (CBS) and cystathione g–lyase (CSE) (26). The substrate of CBS and CSE is L–cysteine, which is derived from nutritional sources or synthesized from L–methionine. The activity of CBS and CSE depends upon pyridoxal 5'–phosphate as a cofactor. Production of H_2S generating enzymes is organ–specific. CBS is expressed in tissue of the nervous system, whereas CSE is expressed in vascular muscle tissue. The major metabolic detoxification of sulfide is hepatic oxidation to sulfate (SO_4^{2-}) and the subsequent elimination of sulfate in the urine (40;41). H_2S may also be methylated (CH_3SCH_3) by the liver.

Biological effects of H_2S.

Like nitric oxide (NO) and carbon monoxide (CO), H_2S is a gas transmitter, which is highly soluble in aqueous and lipid environments, readily passes between cells and is rapidly oxidized. H_2S induces a variety of posttranslational protein modifications, ranging from cytotoxic to cytoprotective actions. Biological activities of H_2S include dilation of blood vessels and smooth muscle cells (42) and modulation of neurotransmission (43;44). Furthermore, it displays both pro– and anti–inflammatory activities.

Similar to NO and CO, H_2S can inhibit mitochondrial respiration. Mitochondria utilize most of total body O_2 to produce adenosine triphosphate (ATP). Oxidative phosphorylation is the last step in the generation of ATP in the mitochondrial membrane, a process which involves the reduction of O_2 and which is catalyzed by cytochrome c oxidase. At high doses, H_2S competes with O_2 in binding to cytochrome c oxidase, thereby reducing cellular oxygen consumption and resulting in profound hypo–metabolism, with metabolic and cardiovascular responses consistent with the physiology of hibernating.

Induction of a suspended animation–like state using H_2S gas.

The first experiments showing that a suspended animation–like state can be induced by H_2S in non–hibernating mammals were done in conscious mice (29). In this study, mice were exposed to H_2S gas in concentrations ranging from 20 to 80 ppm and 17.5% O_2 in a customized sealed glass cage. The metabolic rate, measured by CO_2– production and O_2–consumption, were analyzed using portable gas analyzers. A dose–dependent reduction of metabolic rate by 50% was observed. Body temperature dropped as low as 15°C, which was 2°C higher than the ambient temperature. Animals ceased all movement and respiratory rate declined to 10 breaths per minute. After cessation of H_2S exposure, the mice awoke, without displaying any neurologic or behavioural deficits. Further experiments showed that pre–treatment with H_2S increased the survival rate of mice exposed to hypoxia, whereas hypoxia led to death in all the control mice (45). The authors speculated that hypoxic damage stems from the consequences of inefficient mitochondrial activity in the presence of low oxygen tension, including limited energy supplies and free radical formation. In

suspended animation, high levels of hydrogen sulfide may directly interfere with oxygen sensing through competitive inhibition of cytochrome c oxidase, thereby reducing damaging free radical formation. Studies performed in *Caenorhabditis elegans* embryos, which enter into suspended animation in response to anoxia (17), may support this notion. Exposure to CO protected *C. elegans* from hypoxic damage (46).
After these experiments, suspended animation in non–hibernating mammals was also achieved by others, using the same experimental set up with awake mice, exposed to 80 ppm of H_2S in a chamber (47).

Induction of a suspended animation–like state using H_2S–donors.
Parenteral solutions have a number of practical advantages: ease of administration, no need for an inhalation delivery system, no risk of exposure of gas to personnel and no issues related to the characteristic odor of H_2S gas. In aqueous solutions, H_2S readily dissociates to give HS^- which then exists in equilibrium with H_2S and S^{2-} according to the equations (48;49): $NaHS \rightarrow Na^+ + HS^-$ (1st reaction), $2HS^- \leftrightarrow H_2S + S^{2-}$ (2nd reaction), $HS^- + H^+ \leftrightarrow H_2S$ (3rd reaction).
Parental solutions have been used to inhibit metabolism in animal models. In anesthetized pigs, Na_2S was used, of which a bolus of 0,2 mg/kg was given, followed by an infusion of 2 mg/kg/hour. This dose resulted in reduction of energy expenditure, exemplified by reduced O_2–uptake and CO_2–production (50). In preliminary experiments performed in our laboratory, we used NaHS to induce hypo–metabolism in an anesthetized and mechanically ventilated rat model. Continuous infusion at a dose of 2 mg/kg/hour resulted in a reduction in body temperature, heart rate and exhaled CO_2 (unpublished data).
Whether parenteral solutions of H_2S donors in doses that induce hypo–metabolism are less toxic then gaseous H_2S, remains to be explored. It has been shown that bolus injections of very high doses of NaHS (10 mg/kg) cause acute lung injury (51).

Induction of suspended animation in larger animals.
It should be pointed out that small animal models of metabolism have important limitations. The metabolic rate of mammals increases with decreasing body mass, resulting in a faster exhaustion of energy reserves. Due to a large surface/mass ratio, small animals can reduce core body temperature rapidly when challenged with hypoxic conditions or other noxious insults (52). Thermal inertia of large mammals and humans may resist reductions in body temperature. However, in the experiments discussed above, H_2S depressed metabolic rate within minutes, whereas a decrease in body temperature to ambient temperature occurred gradually, over the course of hours. Therefore, a large body mass may not impede H_2S–induced suppression of metabolism. H_2S–induced suspended animation has been studied in several larger animal models, yielding contrasting results so far.

In anesthetized pigs, a parenteral formula of an H_2S–donor in a dose of 2 mg/kg/hour, resulted in a reduction of heart rate and body temperature, as well as a reduction in O_2–uptake and CO_2–production, indicating a reduction of metabolism (50). Others have not succeeded in inducing suspended animation–like state in pigs. In anesthetized and paralyzed animals, H_2S gas was delivered via mechanical ventilation (53). Ambient temperature was kept at 23°C. Ventilation with 20 to 80 ppm of H_2S resulted in a drop of O_2 consumption and CO_2 production, as well as a decrease in body temperature, in both H_2S exposed animals and controls exposed to air. However, as all metabolic effects were related to a decrease in body temperature, the authors concluded that H_2S did not influence metabolic processes. In sheep, H_2S gas was supplied through a silicone, plastic respiratory mask, which was adapted to each animal (54). The sheep were sedated with ketamine, were breathing spontaneously and lying calm on their side. Airflow was measured by a pneumotachograph. Gas from the mask was drawn for CO_2 measurements. Also in this study, H_2S 80 ppm did not reduce O_2–consumption or exhaled CO_2.

Effects of H_2S on circulation

H_2S is endogenously generated in blood vessels, under control of the enzyme CSE. H_2S induces relaxation of arterial blood vessels and cardiomyocytes, thereby regulating vascular tone and myocardial contractility (26;42;55). Similar to the gas transmitter NO, H_2S predominantly exerts vasodilatation, an effect that is mediated by opening of K_{ATP} channels (56;57). Indeed, an intravenous bolus injection of H_2S in rats transiently decreased blood pressure (41). The vasodilatory effect of H_2S depends on oxygen tension. At high concentrations of oxygen, H_2S has been found to contract arterial vessels in vitro (58).

The cardiovascular effects of H_2S depend on its concentration. The cardioprotective effect of sulfide (59–61), which may be mediated – at least in part – by ERK and phosphatidylinositol 3 – kinase (PI3K)/AKT pathways (49;62), was dose–dependent, ranging from 0,1µM to 1 µM of NaHS. Increasing the dose to 10 µM of NaHS did not further reduce infarct size, but rather aggravated myocardial injury. The cardioprotective effect appeared to be bell–shaped, with the optimal effect at hibernation inducing concentrations. A similar biphasic response in the infarct limiting effects of NO and NO donors has been reported (63). Cardioprotection probably involved the ability of H_2S to activate myocardial K_{ATP}channels, as evidenced by an abolishment of the beneficial effect by K_{ATP}channels blockers (49;59). Other pathways of cardioprotection have also been reported. High doses of sodium sulfide administered to mice after myocardial ischemia and reperfusion injury, resulted in a reduction in infarct size, an effect which was associated with a reduction in apoptosis and concomitant preservation of mitochondrial function (60). An anti–apoptotic effect was also observed in a pig model of myocardial infarction (61).

High doses of H_2S that inhibit cytochrome c oxidase, induce distinct cardiovascular responses, as seen during hibernation (47). Mice exposed to high doses of H_2S gas showed a decrease in heart rate by 50%, returning to baseline value after cessation of H_2S exposure. H_2S induced a sinus bradycardia with sinus arrest, which recovered to a slow sinus rhythm within 5 minutes after cessation of exposure. The reduction in heart rate did not result in a change of mean arterial pressure. Echocardiographic analysis showed a decrease in cardiac output by 60%, while systolic volume remained unaffected, suggestive of systemic vasoconstriction. However, this is not in line with the vasorelaxant properties of H_2S. Alternatively, a low body temperature could have resulted in systemic vasoconstriction. However, all hemodynamic effects occurred independently of body temperature, in hypothermic as well as normothermic mice. Therefore, it seems unlikely that hypothermia alone accounted for the observed cardiovascular effects. Alternatively, vasoconstriction may have been triggered by baroreceptors to compensate for a low cardiac output.

The sulfide–induced hemodynamic effects were confirmed in an anesthetized and mechanically ventilated pig model of ischemia–reperfusion injury (50). Infusion of an H_2S donor resulted in a parallel fall in both heart rate and cardiac output, whereas stroke volume did not change. The animals showed a drop in body temperature, concomitant with lower O_2 uptake and CO_2 production. High doses of a H_2S donor have also been reported to lower arterial blood pressure during ischemia–reperfusion injury (61). Also in this experiment, H_2S preserved left ventricular function.

Contrasting hemodynamic effects were found in anesthetized pigs exposed to high doses of H_2S gas (53). In this study, heart rate and cardiac output did not change, whereas arterial blood pressure increased after exposure to H_2S, suggestive of a stimulating effect. However, body temperature and oxygen consumption did not differ from controls, suggesting that hibernation was not induced in these animals. Overall, in doses that influence metabolism, a reduction in heart rate and cardiac output is most consistently noted, while myocardial function seems to be preserved. No conclusion can be made about oxygen demand from these experiments. Therefore, whether high doses of H_2S result in a better balance between oxygen supply and demand remains to be determined.

Effects of H_2S on inflammation

Hydrogen sulfide exerts a wide range of biological effects, which, like NO, may be due to the absence of a specific target receptor. Both pro– and anti–inflammatory activities of hydrogen sulfide have been reported. In several models of septic shock as well as in septic patients, levels of H_2S were elevated (64;65), together with an increase in the expression of the H_2S generating enzyme CSE (66), pointing to an increased endogenous production of H_2S during inflammation. In accordance, an inhibitor of CSE was found to abolish the increase in H_2S (48;67). H_2S has been implicated as a mediator of inflammation, as inhibiting endogenous

H_2S production with the use of a CSE inhibitor was found to reduce organ damage, as well as myeloperoxidase activity (67). Blocking CSE also reduced the production of pro–inflammatory cytokines and chemokines in a model of sepsis (51), an effect which was mediated by the activation of NF–kB (68). Inflammatory effects of H_2S are thought to occur at the leukocyte–endothelium interface, as inhibition of endogenous H_2S synthesis reduced leukocyte rolling and adherence to blood vessels (7;69). In addition, H_2S has been found to regulate the production of pro–inflammatory neuropeptides (substance P and calcitonin gene related peptide) during sepsis, thereby contributing to the inflammatory response (8;9). Therefore, during inflammation, endogenous H_2S seems to act as an important endogenous regulator of leukocyte activation and trafficking during an inflammatory response.

However, endogenous hydrogen sulfide has also been found to have anti–inflammatory effects. In a model of carrageen–induced inflammation in the rat, a CSE–inhibitor enhanced edema formation as well as the infiltration of inflammatory cells (7;69). Also, endogenous H_2S seems to play a role in healing of gastric ulcers, as inhibition aggravated gastric injury (70).

Administration of exogenous H_2S resulted in anti–inflammatory activities in multiple experimental designs. In vitro, neutrophils pretreated with 2 mM sulfide showed suppressed calcium dependent cytoskeleton activities such as chemotaxis and granule release (71). Furthermore, a cytoprotective effect has been demonstrated during myocardial ischemia reperfusion injury (60), which related to peroxynitrite ($ONOO^-$) scavenging capabilities of H_2S (72), thereby decreasing intracellular tyrosine nitration and oxidative stress. Similar to the protective effect of inhibiting endogenous H_2S, the addition of exogenous H_2S improved healing of gastric ulcers. Rats exposed to 160 ppm H_2S in a water–immersion and restraint stress model, were protected from the occurrence of gastric lesions (73). In addition, H_2S diminished local inflammation and neutrophil activation, as measured by a decrease in myeloperoxidase activity. In a mouse model of trauma–induced lung injury, treatment with NaHS (2 mg/kg) reduced the level of pro–inflammatory cytokine IL–1 in the lung compared to control animals, while increasing IL–10 concentration (74). Furthermore, burn and smoke inhalation injury increased the presence of protein carbonyl formation in the lung, while administration of hydrogen sulfide reversed this effect and reduced protein carbonyl formation, indicative of an overall anti–oxidant effect. In contrast, in mechanically ventilated pigs in suspended animation, a H_2S donor did not affect pro–inflammatory cytokine levels or markers of oxidative stress (50).

Therefore, H_2S can exert both anti–inflammatory and cytotoxic effects, which appear to be dose dependent. The considerable differences in design of the models hamper definite conclusions on the inflammatory effects of H_2S.

Potential applications of H_2S induced suspended animation

Organs for transplantation.

The success of organ transplantation is critically dependent on the quality of the donor organ. Donor organ quality, in turn, is determined by a variety of factors including donor age and pre–existing disease, the mechanism of brain death, donor management prior to organ procurement, the duration of hypothermic storage, and the circumstances of reperfusion (75). Measures to prolong viability of donor tissue comprise infusion of a preservative solution and placement on ice during transportation. Cold ischemic times which are accepted for transplantation in a recipient vary from 4 hours (heart) to 30 hours (kidney) (76). After this time window, ischemic injury causes early non–function or delayed graft function, which plays an important role in the development of chronic graft failure and late graft loss. A more detailed mechanism of ischemic donor organ injury includes the development of tissue edema, cellular and mitochondrial swelling and loss of cell membrane integrity. Cellular swelling is initiated by inhibition of Na^+ K^+ ATP–ase, followed by suppression of oxidative phosphorylation, ATP depletion, and an increase in the cytosolic calcium concentration. Mitochondrial calcium overload follows this increase in cytosolic calcium and is associated with the opening of the permeability transition pore, and mitochondrial swelling (77;78), a process which correlates with apoptosis (79;80).

In the last decades, different preservation solutions and techniques have been developed. Some experiments have used H_2S in their solution. Hu *et all* (81) reported an improvement in preservation of isolated rat hearts by adding 1 µmol/L NaHS. The solution showed comparable preservation effects with St. Thomas solution, but had a better ATP production and a reduction in apoptosis, as showed by TUNNEL staining. It can be speculated that organs placed in a suspended state by adding H_2S, may preserve their viability for days, which may reduce the number of unused organs due to mismatching or too long transportation time. Of interest, H_2S has been shown to reduce metabolic demand during excessive blood loss, thus in a state of low oxygen supply (82). Indeed, exposure of mice to H_2S resulted in survival during hypoxic conditions that otherwise would have been lethal (45).

Patients undergoing coronary artery bypass surgery.

Deep hypothermia (to 20°C) is already applied during cardiothoracic surgery to protect organs from ischemia–induced organ damage. Interestingly, patients with coronary arterial disease can develop transient cardiac failure, which recovers after perfusion is restored. This phenomenon is termed myocardial hibernation, which has been postulated as an adaptive response to an oxygen deficit (83;84). To identify molecular adaptations responsible for myocardial hibernation, swines were instrumented with a chronic left anterior descending coronary artery stenosis (85). After 5 months, myocardial analysis showed an intrinsic down regulation of many of the mitochondrial enzymes responsible for oxidative metabolism and

electron transport. Also, a chronic up–regulation of stress proteins coupled with a transient increase in cytoskeleton proteins was seen. These myocardial adaptations support the notion that the molecular mechanisms operative in hibernating myocardium are dynamic, protecting myocytes from irreversible injury, which does not always lead to fibrosis. Inducing a suspended animation–like state with H_2S in patients undergoing coronary artery bypass surgery may not only be beneficial due to hypothermic effects, but could also influence mitochondrial enzymes responsible for energy production, thereby protecting myocytes from injury. In addition, H_2S in non–hibernating doses has shown cardioprotective effects in various models of myocardial ischemia–reperfusion injury, which may involve a vasorelaxant effect of coronary arteries via opening of K_{ATP}–channels and a reduction of apoptotic cell death (41;49).

Patients suffering a cardiac arrest.

Mild hypothermia (to 32–34°C) improves neurological outcome when applied in patients after a cardiac arrest. Ischemic brain injury results from a loss of ATP production and dysfunction of membrane ATP–dependent pumps, resulting in cell swelling and global cerebral dysfunction, as well as overproduction of free oxygen radicals and induction of apoptosis (86;87). The ability of hypothermia to affect multiple points of the injury cascade may contribute significantly to its success as an intervention. It should be noted that induced hypothermia and reduction in body temperature due to H_2S exposure are different mechanisms. Hypothermia can be induced by external cooling of the body with the use of ice–cold infusions and with cooling devices, such as a cooling mattress. H_2S associated suspended animation in animal models inhibits metabolism, followed by a drop in body temperature. However, although a distinct condition, hypothermia is a significant characteristic of suspended animation and may be a consequence of competitively inhibiting oxidative phosphorylation in the presence of oxygen. Remarkably, a 17 month old patient with cytochrome c oxydase deficiency presented with several severe episodes of hypothermia (31°C – 33°C) during daytime when the child was normal active (88). Also, hypothermia has been observed in children with other mitochondrial respiratory chain dysfunctions.

Possibly, H_2S–induced suspended animation could also improve neurological outcome after a circulatory arrest, which might not only be due to a hypothermic effect, but also to protection against hypoxia as described earlier. Several observations may support this notion. The H_2S–regulating enzyme CBS is highly expressed in the brain. H_2S enhances sensitivity of aspartate receptor to glutamate and plays a central role in long–term potentiation of the neuronal circuitry. H_2S also regulates synaptic activity by modulating the activity of both neurons and glia cells. A neuroprotective effect of exogenous H_2S has been reported. H_2S decreased oxidative stress in neurons *in vitro*, by increasing levels of the antioxidant glutathione in

neurons (43;44;89). Research into mitigating brain damage after cardiac arrest has shown outcome benefits in clinically relevant dog models of a prolonged exsanguination–induced cardiac arrest as described earlier (20;90). In these dogs, a suspended state was achieved with the use of cold fluids within the first 5 minutes of no flow, resulting in profound hypothermia. Resuscitation after 90–120 minutes resulted in complete recovery of function and normal histology. We speculate that the addition of H_2S may increase the effectivity of induced suspended animation in these models, or prolong the time after arrest in which inducing suspended animation is possible.

Critically ill patients with multiple organ failure.
In critical illness, a hyper–metabolic response to insults is a common clinical entity. Sepsis is characterized by an exaggerated systemic inflammatory response to infection. In the absence of infection, a systemic inflammatory response syndrome (SIRS) can occur as a reaction to a variety of non–infectious insults, including trauma, cardiothoracic surgery and ischemia–reperfusion injury. The dysregulated host inflammatory response in sepsis and SIRS results in multiple organ failure (91;92). A deficiency in tissue oxygen delivery cannot solely account for the development of organ failure. In the presence of an adequate tissue oxygen tension, metabolic dysfunction persists, suggestive of a disturbance in cellular metabolic pathways (93;94). Although disparate results of mitochondrial function have been reported in short–term models of sepsis, a decrease in mitochondrial activity has been a consistent finding in longer term sepsis models (95;96) including a decrease in oxidative phosphorylation capability with subsequent decreased oxygen consumption and ATP synthesis. In agreement, a depletion of skeletal muscle ATP concentrations, a marker of mitochondrial oxidative phosphorylation, was found in septic patients, which was associated with worse outcome (96;97).
Preservation or optimization of residual mitochondrial function may prevent ATP levels from dropping below threshold levels that induce apoptosis and cell death. Treatment of multiple organ failure traditionally consists of supportive care, ensuring that high metabolic demands are met. Also, regulating mitochondrial substrate may provide a protective measure in the critically ill. Intensive insulin therapy aimed at maintaining strict normoglycemia has been found to preserve mitochondrial ultra structure and function (98) and improve outcome (99;100) in critically ill patients. It can be hypothesized that inducing a hypo–metabolic state during critical illness may limit organ injury by protection from prolonged energetic failure, enabling a faster recovery when the inflammatory insult has resolved.

Patients with acute lung injury.
Acute lung injury (ALI) is a common finding in the critically ill patients. ALI is characterized by hypoxia, for which intubation and mechanical ventilation is often indicated (101;102).

However, mechanical ventilation can induce or aggravate ALI. The use of lower tidal volumes has been shown to be protective in patients with ALI (103). In addition, lowering the number of breaths/minute reduced pulmonary inflammation in an animal model of ALI (104). Protective mechanical ventilation using low tidal volumes and respiratory rate is limited by low minute ventilation, resulting in elevated CO_2 and severe hypercapnia. H_2S–induced suspended animation may be a promising treatment strategy to reduce damage inflicted by mechanical ventilation, by decreasing CO_2 production and O_2 demand, with a concomitant lower minute ventilation needed for adequate gas exchange, thereby allowing for lower tidal volumes and less breaths per minute. Experiments with H_2S in ALI are limited. In a model of combined burn and smoke inhalation, high doses of H_2S attenuated lung injury, by inhibition of inflammatory mediators and oxidative stress (74). The effect on gas exchange was not studied.

Discussion and recommendations for future research

Reducing the metabolic rate on demand using H_2S seems to be feasible in naturally non–hibernating mammals. The anecdotal reports on people surviving deep accidental hypothermia and anoxic conditions suggest that the ability to hibernate might be latently present. Before clinical application of inducing hypo–metabolism can be considered, a number of issues need to be resolved in pre–clinical experiments.

The conflicting results in larger animals need to be clarified. It is feasible that the use of sedation and/or paralysis may have blunted the metabolic effect related to reduction in activity during H_2S–exposure, an effect which was observed in mice. Alternatively, the differences in response to H_2S may rely on the relation between body weight and basal metabolic rate. Small animals have a higher basal metabolic rate, a large portion of which is used for heat production, not for ATP generation. H_2S–induced lowering of metabolism in small animals may therefore not affect ATP–production. In mammals with a higher body mass, higher doses of the gas may be needed to induce hypo–metabolism. Indeed, inhalation of doses of H_2S that already induced hypo–metabolism in mice, were reported to have no apparent side effects in exercising men (105–107). Obviously, increasing the dose carries the risk of toxicity. However, also doses of 50 ppm of H_2S have been shown to affect cell metabolism by reducing cytochrome c oxidase activity (108). In addition, using doses that induce hibernation did not result in considerable toxicity in animal models, and even tended to decrease the inflammatory response. Dose–finding studies that induce hibernation–like states but do not increase toxicity are mandated in appropriately–sized animal models.

Even when inducing hypo–metabolism proves to be feasible, potential adverse effects of inhibiting metabolism should be elucidated, such as the effect on host defense. It can

be hypothesized that suspended animation hampers adequate immune response during bacterial infections, possibly leading to diminished clearance of bacteria. Indeed, the production of heat shock proteins has been suggested to enhance host defense against bacteria (109). This issue needs to be studied in animal models of bacterial infections. Another potential adverse effect may include the rebound effect of nitric oxide. After cessation of suspended animation, it is possible that excessive rebound NO production may result in reactive oxygen species, when recovery of mitochondria has not yet been established.

We have suggested that hypo–metabolism can be considered to restore the imbalance between oxygen demand and consumption in critically ill patients. In these patients, additional issues need to be studied, such as whether beneficial results of hypo–metabolism also apply to anesthetized patients. Indeed, most of the clinical applications we mentioned require intubation and respiratory support, and a number of these patients will be sedated. In addition, critically ill patients most often require different types of pharmacological support, including vasoactive drugs and antibiotics. Future experiments are needed to study possible interaction of drugs with H_2S.

Lastly, there are a number of practical hurdles. H_2S has the smell of rotten eggs. In a closed system of mechanical ventilation, exposure to the odor may be limited, but accidental disconnections from the ventilation may pose a risk to medical personnel. Also, corrosion of metal parts and tubes may result in shortening of the durability of the mechanical ventilator.

Conclusions

H_2S–induced suspended animation has resulted in a reduction of CO_2–production and O_2–consumption, and thus resistance to hypoxia in various experimental models. Inducing a hypo–metabolic state may be a beneficial therapy in various conditions of hypoxia–induced organ damage, in particular in critically ill patients, in whom bio–energetic failure is a hallmark of multiple organ damage. Restoring the imbalance between oxygen demand and consumption may provide a novel therapeutic approach towards critically ill patients. Future studies are required to address feasibility as well as issues of toxicity before clinical application can be considered.

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