disease. The NADPH oxidases are the only enzymes identified to date that have the primary function of producing superoxide ($O_2^{\bullet-}$) and are key contributors to the development of oxidative stress in cardiovascular disease, in particular the Nox2-containing NADPH oxidase isoform. We have *in vitro* data that show that NaHS both scavenges NADPH oxidase-derived $O_2^{\bullet-}$ and inhibits NADPH oxidase activity. Further, our recent data show that both elevation of vascular NADPH oxidase activity and endothelial dysfunction occur in several models of cardiovascular disease (atherosclerosis, hypertension and diabetes), and that intervention with NaHS treatment both reduces NADPH oxidase-derived $O_2^{\bullet-}$ production in the vasculature and improves endothelial function. In the most recent of these studies the aim was to investigate whether chronic treatment with H_2S via NaHS could elicit a vasoprotective effect in diabetes, where there is known to be increased vascular oxidative stress. Diabetes was induced in male C57 mice with streptozotocin (60 mg/kg daily, ip for 2 weeks) and confirmed by elevated blood glucose and HbA_{1C} levels. Following a further 2 weeks, mice were treated with NaHS (100 μmol/kg/day) for 4 weeks, then tissues collected. Myography was employed to examine endothelial and vascular smooth muscle cell function, as well as vascular NO bioavailability, and western blotting was used to assess vascular Nox2 and eNOS expression. ACh-mediated, endothelium-dependent vasorelaxation was significantly inhibited in diabetic aortae ($P < 0.05$), but NaHS treatment restored the ACh response. Vascular NO bioavailability was reduced ($P < 0.01$) in diabetes, but restored with NaHS treatment. In addition, vascular Nox2 expression was elevated ($P < 0.05$), and eNOS expression was reduced in diabetes; however these were reversed with the NaHS treatment. These data confirm that exogenous H_2S is an anti-oxidant that is useful in protecting endothelial function *in vivo* in these models of vascular disease. This anti-oxidant effect is consistent with NaHS preventing the increase in Nox2 expression and activity in these disease states.

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OP17
Mitochondria and sulfide

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Mitochondria are the powerhouse of the cell because they generate most of the cell's supply of adenosine triphosphate (ATP) used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in other tasks such as signaling and cellular differentiation. Recently, it has been demonstrated that mitochondria of mammalian organisms are able to oxidize sulfide due to the presence of a Sulfide Quinone Reductase (SQR) that is part of the Sulfide Oxidizing Unit (SOU) which in addition involves a dioxygenase and a sulfur transferase [1].

In the present report, we study isolated mitochondria from pig or human liver and we demonstrate that these mitochondria show high SQR activity and are able to neutralize quickly relatively high amounts of sulfide [2]. Additionally, we will present a direct evidence of ATP synthesis driven by hydrogen sulfide oxidation.

Moreover, we will show that some modifications should be introduced to the Seahorse technology in order to observe hydrogen sulfide oxidation by cells. These modifications are important to bypass the sulfide evaporation due to the plate remaining open to the oxygen atmosphere and the long time needed to obtain the first estimation of respiratory rate.

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OP18
Hydrogen sulfide maintains mitochondrial DNA replication via demethylation of TFAM

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Hydrogen sulfide (H_2S) exerts a wide range of actions in our body. The normal replication of mitochondrial DNA (mtDNA) is critical for cellular energy metabolism and mitochondrial biogenesis. The aim of this study was to investigate whether H_2S affects mtDNA replication and the underlying mechanisms. Here we demonstrated that deficiency of cystathionine gamma-lyase (CSE), a major H_2S-producing enzyme in the vascular system, reduces mtDNA copy number and the expressions of mitochondrial marker genes in both smooth muscle cells (SMCs) and aorta tissues from mice, while supply of exogenous H_2S stimulates mtDNA copy number and the expressions of mitochondrial marker genes. We further found that H_2S induces the mRNA and protein expressions of mitochondrial transcription factor A (TFAM), while knockout of CSE lowers the expression of TFAM in both SMCs and aorta. TFAM knockdown diminished H_2S-enhanced mtDNA copy number. In addition, CSE deficiency induced the expression of DNA methyltransferase 3a (Dnmt3a) and TFAM promoter DNA methylation, while H_2S repressed Dnmt3a expression and resulted in TFAM promoter demethylation. We further found that H_2S *S*-sulfhydrates transcription repressor interferon regulatory factor 1 (IRF-1) and enhances the binding of IRF-1 with Dnmt3a promoter following reduced Dnmt3a transcription. H_2S had little effects on the expressions of Dnmt1 and Dnmt3b as well as ten-eleven translocation methylcytosine dioxygenase 1, 2, and 3. These data suggest that a sufficient level of H_2S is able to inhibit TFAM promoter methylation and maintain mtDNA copy number. H_2S contributes to mtDNA replication and cellular bioenergetics and provides a novel therapeutic avenue for cardiovascular diseases.

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OP19
Hydrogen sulfide is a cytoprotective, rescue molecule in the GI tract

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Hydrogen sulfide is a potent, endogenous anti-inflammatory and cytoprotective factor. In the gastrointestinal (GI) tract, H_2S plays key roles in mediating mucosal defense and accelerating repair of injury.

Inhibition of H_2S synthesis renders the GI mucosa more susceptible to injury, results in mucosal inflammation and retards healing processes. These effects and roles have been demonstrated in the esophagus, stomach, small intestine and colon.

When injured, there is a rapid up-regulation of H_2S-synthesizing enzymes. There is also a marked decrease, at these same sites, of tissue oxidation of H_2S, and down-regulation of the main enzyme carrying out that task (SQR). Interestingly, these changes occur specifically at the site of injury (not in adjacent inflamed or normal tissue). The increased H_2S production drives repair and resolution of inflammation. The ulcer-healing effects of H2S are mediated largely through induction of HIF-1α.

H_2S modulates mucus production by the intestinal epithelium and can alter the types of bacteria residing in the lumen of the GI tract. These appear to be beneficial effects that contribute to repair of injury.

Together, these observations suggest a key role for H_2S in maintaining the integrity of the GI tract and, of particular importance, the epithelial barrier. There is also substantial evidence suggesting that H_2S can be exploited in rational drug design, so as to produce agents that are safe and that maintain mucosal defense and accelerate repair of injury.

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OP20
Hydrogen sulfide in hypoxic brain damage

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The overall survival rate of resuscitated patients after out-of-hospital cardiac arrest (CA) is 7–10% in Europe and the United States despite the advances in cardiopulmonary resuscitation (CPR) methods and post CA care. In addition, more than 50% of survivors have permanent neurological dysfunction of varying degrees. Mitochondrial dysfunction following ischemia/reperfusion (I/R) injury including CA and CPR is characterized by an impairment of electron transport, generation of reactive oxygen species and decreased mitochondrial membrane potential which leads to pro-apoptotic signaling and cell death. Targeted temperature management (TTM) is a promising therapeutic strategy in post CA syndrome, but no pharmacological agent has yet been found to improve clinical outcomes.

Hydrogen sulfide (H_2S) is a flammable and colorless gas with rotten egg odor. This gaseous signaling molecule mediates cytoprotective effects against I/R injury at least in part *via* preservation of mitochondrial integrity. We have previously reported that administration of sodium sulfide (Na_2S), a H_2S generating compound, 1 min before the initiation of CPR, but not 10 min after CPR, prevented neurological injury and markedly improved survival in mice subjected to CA and CPR. While the beneficial effects of H_2S after CA/CPR were later confirmed by several investigators, others failed to observe the protective effects of H_2S donor compounds. Although reasons for the conflicting results are undoubtedly multifactorial, at least a part of the discrepancy may relate to the use of Na_2S and sodium hydrosulfide (NaSH) as H_2S donor compounds in these studies. As these simple sulfide salts generate H_2S immediately in solution, concentrations of H_2S in prepared "H_2S donor solution" are often unstable and unreliable. Therefore, the H_2S concentrations in the target tissue (e.g., brain) are unpredictable after bolus or continuous infusion of sulfide salts. It is imperative to develop H_2S donor compounds that are targeted to certain tissues or cellular organelles and release H_2S in a more controlled manner to translate the unique cytoprotective effects of H_2S into a useful drug.

To translate the beneficial effects of H_2S to clinics, we have recently developed a number of novel H_2S donor compounds that exhibit enhanced neuroprotective properties in mice models of I/R injury. Furthermore, we recently observed unique effects of H_2S in hypoxia tolerance in mammals. The role of H_2S in brain protection after cardiac arrest and CPR, as well as during lethal hypoxia, will be reviewed.

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OP21
Hydrogen sulfide in renal disease and transplantation

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Hydrogen sulfide (H_2S), the third gasotransmitter, next to nitric oxide and carbon monoxide, is a key mediator in physiology and disease. It is involved in homeostatic functions, such as blood pressure control, electrolyte balance and apoptosis, and regulates pathological mechanisms, including oxidative stress and inflammation. Besides, it is believed to serve as an oxygen sensor under ischemic conditions. The kidney plays a decisive role in many of these processes, indicating an interplay between H_2S and renal (patho-)physiology. Hydrogen sulfide production can be measured by its end products thiosulfate and sulfate, although the latter also depends on intake. We measured these metabolites in various human renal conditions including dialysis and transplantation. We furthermore treated hypertensive proteinuric rats with NaHS or thiosulfate in order to study its therapeutic potential. In dialysate from dialysis patients thiosulfate was absent and sulfate reflected nutritional status. In a study in 707 renal transplant recipients (RTRs) and 110 controls (living donors) we showed urinary sulfate and thiosulfate to be associated with a favorable cardiovascular profile, improved graft survival and reduced all-cause mortality. These data were confirmed in a second analysis one year later. Also, urinary TS, but not sulfate, was higher in RTRs compared to controls. Since hydrogen sulfide exerts some of its actions through sulfhydration we also determined total protein SH in these patients. Protein SH was associated with an improved cardiovascular risk profile and patient and graft survival in RTR, which might be related to the significant negative correlation of SH with Nt-pro-BNP levels. Whether therapeutic SH-modification e.g. by treatment with H_2S metabolites or donors improves long-term outcome of renal transplantation needs to be explored. The alleged predictive value of sulfur metabolites and sulfhydration in the general population ($n > 7000$ individuals) is currently under investigation. In experimental animals, treatment with H_2S attenuates and protects against ischemia and against Ang II-associated functional and structural renal deterioration, suggesting that it might be a valuable addition to the already existing antihypertensive and renoprotective therapies. In conclusion, in addition to being a predictive biomarker in various conditions, hydrogen sulfide has exciting therapeutic potential.

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