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Hydrogen sulfide

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CHAPTER

GENERAL DISCUSSION
AND FUTURE PERSPECTIVES

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Hydrogen sulfide (H_2S), historically regarded as a poisonous gas, is generated endogenously in mammals. With its antioxidant, anti-inflammatory, vasodilating and many other functions, it has the potential to provide many health benefits. The interest in the field of H_2S research has grown markedly in recent years, with increased attention for the many physiological functions of H_2S and its protective role in various disease models. The present work on H_2S in diseases with underlying oxidative stress and inflammation was instigated after the discovery that H_2S induces a reversible hypometabolic state that resembles hibernation. This led to our general hypothesis that exogenous administration or endogenous activation of H_2S is protective in cardiac, renal and neurodegenerative disease.

Since it has become apparent that crosstalk between the three known gasotransmitters - nitric oxide (NO), carbon monoxide (CO) and H_2S - is important in cytoprotection, we reviewed the literature on gas-mediated cytoprotection in renal transplantation in **chapter 2**. H_2S has been examined extensively as a potential therapeutic agent in the setting of ischemia-reperfusion injury (IRI) in the heart, brain, lungs, and liver. The majority of in vitro and in vivo studies thus far have reported beneficial actions of H_2S administration. We show that H_2S has significant protective effects in the setting of predictable ischemia, where treatment can be instigated before the onset of hypoxia. We show this in **chapter 3**, where H_2S -induced hypometabolism reduces cardiac ischemic damage. Most of the studies investigating the protective effect of H_2S use soluble sulfide donors such as NaHS and Na_2S . The unique concept of our study in cardiac ischemia relates to the application of gaseous H_2S to induce a hypometabolic state. In the setting of cardiac IRI, the cytoprotective actions of H_2S are thought to result from its antiapoptotic, anti-inflammatory, antioxidant, and mitochondrial properties. The mechanisms behind these protective effects are likely a combination of various simultaneously occurring effects, such as modulation of metabolism and sustaining the balance between oxygen demand and oxygen availability during hypoxia. To gain more insight in the protective effect of H_2S in cardiac IRI, we compared hypometabolic and sub-hypometabolic concentrations. Our results indicate that H_2S -induced hypometabolism was associated with significantly more protection than sub-hypometabolic concentrations of H_2S . However, lower concentrations of gaseous H_2S did modulate several aspects of ischemic damage, indicating the protective effects of H_2S cannot be completely attributed to H_2S -induced hypometabolism. Furthermore, hypometabolism-inducing concentrations of H_2S may have hemodynamic effects that can be related to the reduction in damage by reducing cardiac load during ischemia. We have focused on the effects of H_2S on predictable ischemic damage, while others have also shown beneficial effects of H_2S administration after the ischemic event. The observation that post-treatment with H_2S is also advantageous significantly increases its value as a therapeutic agent, since there are many settings with unpredictable onset of ischemia.

Although many aspects of endogenously produced H_2S still have to be elucidated, several physiological functions have now been uncovered. The development of CBS and CSE deficient mice has tremendously contributed to our knowledge on the function

of H_2S produced by these enzymes. The development of $CSE^{-/-}$ mice has confirmed the vasodilator function of H_2S and has given us insight in the vasorelaxation-, signaling-, antioxidative- and angiogenic properties. The role of endogenously produced H_2S has been investigated in **chapter 4**, where genetic modulation of CSE expression is associated with the amount of oxidative stress. The absence of CSE resulted in increased renal damage after ischemia-reperfusion and eventually led to a higher mortality rate. Inversely, overexpression of CSE in vitro potently reduced the amount of reactive oxygen species in a model of oxidative stress. We show that H_2S produced by CSE is an endogenous modulator of oxidative stress. Furthermore, CSE expression prior to human renal transplantation is positively associated with renal function shortly after transplantation, which might indicate a role for CSE in human transplant related oxidative stress. Others have shown that cardiac specific overexpression of CSE is protective in the setting of myocardial infarction by protecting cardiac mitochondrial function. This suggests that pharmacological activation or upregulation of CSE might be beneficial in the transplant setting. Little is known about the regulation of CSE, but there are some substances that are able to influence CSE activity or transcription. There is evidence that myeloid zinc finger 1 and specificity protein 1 transcription factor affect the transcription of CSE. Furthermore, studies suggest that CSE can be upregulated by bacterial endotoxin and by NO. Last, S-adenosylmethionine and pyridoxal-5'-phosphate stimulate CSE activity to increase H_2S production.

Hypertension and IRI are both initiated by damaging processes like oxidative stress and inflammation. Because of the potent protective effects of H_2S in IRI we investigated its beneficial effects in hypertension related renal and cardiac disease in **chapter 5 and 6**. In these studies we used a unique approach by administering sodium thiosulfate (STS) as a donor of H_2S . Thiosulfate is an intermediate of sulfur metabolism from cysteine and a metabolite of H_2S that can also lead to the production of H_2S through the action of thiosulfate reductase. Since STS is a more steady substance than H_2S , its use in experimental- as well as clinical settings is simpler. STS was almost equally effective as NaSH in reducing cardiac and renal damage caused by angiotensin II-induced hypertension. Since thiosulfate is safely used to treat calciphylaxis in humans for decades, our results provide an interesting bridge between experimental research and clinical application. Recent literature more and more includes STS as an important player in H_2S related processes. Urinary thiosulfate excretion was found to associate with a favorable cardiovascular risk profile and even improved survival in renal transplant recipients. These results further strengthen the presumed beneficial effects of STS.

In our model of angiotensin-II-induced hypertension, it is difficult to discriminate between several contributing protective mechanisms. Since administration of H_2S causes hemodynamic changes, the protective effects might be attributed to the reduction in blood pressure. However, it seems likely that H_2S has secondary effects because of its wide range of actions. Unfortunately, there are no studies using antihypertensive compounds in combination with H_2S to discriminate between hemodynamic and other

protective effects. Another interesting approach would be using a compound that is able to only block the vasodilatory effects of H₂S. Recent findings of our group show a positive correlation between sulfhydration and survival in renal transplant recipients. These findings indicate that sulfhydration is one of the protective mechanisms of H₂S.

Until now we and several other groups have shown the protective effects of H₂S against the detrimental effects of oxidative stress in different in vitro and animal models. The known role of oxidative stress in aging related neurodegenerative diseases instigated a project leading to the results described in chapter 7. Overexpression of CSE was protective against neurodegeneration in a *Drosophila* model for spinocerebellar ataxia type 3 (SCA3). These effects are associated with a reduction in inflammation and oxidative stress. We found no effect of CSE overexpression on the formation of aggregates; therefore its protective effects are most likely downstream of these toxic entities. Since we observed reduced levels of CSE in brain tissue of SCA3 patients, CSE might be an interesting target in reducing the damaging effects of the SCA3 mutation, and thereby slowing the progression of the disease. Recently, it was demonstrated that CSE deficient mice show impaired locomotor functions, therefore it is possible that low levels of CSE negatively influence the development of neurodegenerative phenotypes in Huntington's disease and in SCA3. This is consistent with our findings that CSE overexpression is beneficial in the *Drosophila* SCA3 model. Treatment of SCA3 flies with STS resulted in similar protective effects to CSE overexpression. This leads to the possibility that the beneficial effects of CSE as demonstrated in the SCA3 model are related to increased levels of H₂S. Other studies also describe neuroprotective effects of H₂S in models for Parkinson's disease. The amount of research on H₂S has exploded the past few years with rising enthusiasm to develop therapeutic applications that can be used in human medicine.

FUTURE PERSPECTIVES

Given the widely described beneficial effects of H₂S in ischemia-reperfusion related injury, organ transplantation is a promising clinical setting for H₂S therapy. One of the advances in this setting is the predictable onset of ischemia, providing us with several options for administration of H₂S such as adding H₂S to preservation solutions to protect organs from ischemic injury. Another option is gaseous administration of H₂S to brain dead organ donors. Because these patients are intubated and mechanically ventilated by definition, the addition of H₂S to the gas mixture is relatively straightforward. These patients are strictly monitored in the intensive care unit, reducing the risks for negative side effects. Another promising setting for further development of H₂S treatment is neurodegeneration. For many neurodegenerative diseases, there is no cure that halts the devastating course of symptoms. To date, the first steps of H₂S treatment in this setting have been made and show very promising results.

A challenge for the H₂S field is the development of clinically relevant therapeutic agents. Our group has moved forward in investigating STS as a potential compound

to bridge the gap between experimental and clinical use of H₂S- based therapy. Treatment of hypertensive human patients with STS will learn us whether STS can be added to the already existing antihypertensive and reno-protective therapies. A randomized controlled trial that investigates STS as a treatment option in patients suffering from a myocardial infarction is currently initiated.

Aside from the recent development of a long-acting donor with controlled H₂S release, developing a drug that can specifically target an organ system would alleviate unwanted side effects. The mechanisms of site-specific delivery remain challenging; however, targeted H₂S delivery to myocardial microvasculature was achieved using ultrasound to release encapsulated H₂S from perfluorocarbon-filled microbubbles. In addition, mitochondria-targeted H₂S donors are in development and contain a mitochondria-targeting moiety aimed to mediate oxidative stress and cell injury. Use of these substances in cardiac ischemia might be promising.

The fear of toxicity is a major hurdle for acceptance of H₂S in the clinical setting. Therefore, it is critical to measure H₂S levels accurately in blood and tissue samples from patients and quantifying its bioavailability in vivo. Future research must focus on optimizing existing and developing new measurement techniques.

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More knowledge is required to develop effective therapeutics. Specifically, function and signaling relating to the enzymes responsible for the endogenous production of H₂S are worthy of further study. Understanding localization and activity of these enzymes in particular disease states would help direct gene therapy or localized drug delivery. Understanding these mechanisms could identify what tissues can be affected and what pathological conditions are most responsive to H₂S therapy. Mastering these issues would drastically advance H₂S research and further translate it into clinical relevance.

