Characterising hydrogen sulfide production and degradation pathways in murine kidney: effects of pregnancy and high fat diet

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Background: Renal haemodynamics are regulated by a range of endocrine, neural and locally acting paracrine signalling pathways. Our understanding of the latter has been informed by the discovery of nitric oxide (NO), carbon monoxide (CO), and more recently hydrogen sulfide (H_2S) as signalling molecules. These so-called "gasotransmitters" can act alone and in concert to exert a range of effects through (1) direct activation of cell signalling cascades, and (2) posttranslational modification of proteins and participation in a myriad of redox reactions. H_2S is produced by enzymes of the transsulfuration pathway; cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS). However, MPST, which is localized to the mitochondria, is also capable of producing H_2S. Unlike NO and CO, H_2S also undergoes enzymatic degradation, regulated by a suite of mitochondrial enzymes: *SQRDL, SUOX, TST, CDO1*, *TSTD1* and *ETHE1* which eventually produce sulfate. The contribution of the degradation pathway in regulating H_2S activity *in vivo* has been largely overlooked.

In the kidney, exogenous H_2S administration is associated with increased GFR, and reductions in sodium reabsorption due to inhibition of the renal sodium transporters NKA and NKCC (Cao and Bian, 2016). However, the ability of endogenous H_2S production to effect these changes and under what physiological conditions remains to be determined. During pregnancy, there are significant changes in renal haemodynamics, characterized by marked increases in renal blood flow and glomerular filtration rate. Despite the important role of H_2S in the non-pregnant kidney, its function in the context of pregnancy has not been investigated.

Methods: To test the hypothesis that enzymes associated with renal H_2S production and/or degradation are associated with the changes in renal function during pregnancy, real-time PCR was used to quantify expression of the H_2S production and degradation enzymes in pregnant Balb/c mice at day 17 of pregnancy (n=15) and in age-matched non-pregnant mice (n=3). As maternal obesity during pregnancy has been associated with altered renal haemodynamics, pregnant mice were allocated to receive either a high fat diet (44% lipids) or a control diet (14% lipids) to determine whether the renal H_2S pathway is sensitive to maternal dietary fat during pregnancy. The diet was commenced 3 weeks prior to mating.

Results and conclusion: We confirm earlier reports that H_2S enzymes (CBS/CSE) are highly expressed in renal tissues, with expression indices markedly higher than in other tissues. For the first time, we demonstrate the expression of MPST and its associated enzymes in the pregnant mouse kidney. We also demonstrate all of the components of the H_2S degradation pathway, which suggests a degree of negative feedback is possible. In this study, neither pregnancy nor HFD were associated with changes in renal expression of H_2S pathway genes ($P>0.05$ for all enzymes). Measurements of H_2S production, rates of enzyme activity, and protein posttranslational modification at different stages of gestation will be a focus of future work in our laboratory.

Cao X, Bian J. (2016) The Role of hydrogen sulfide in renal system. *Frontiers in Pharmacology* **7**: 385.