Cystathionine γ-lyase and hypoxia: Implicating hydrogen sulfide in the hypoxic stress response

Sean Bryan, HBSc MSc (MD candidate)
Northern Ontario School of Medicine
The arrival of gasotransmitter biology

Endogenous gaseous signaling transmitters, or “gasotransmitters.”

Exert fine, modulatory control over myriad cellular functions.

Distinctive features versus classic neurotransmitters:

- small molecules of gas
- membrane-permeable
- receptor-independent
- enzymatic production
H₂S: An unlikely hero

From notorious toxicant to critical physiological mediator?

Endogenously produced from L-cysteine by cystathionine γ-lyase (CSE) in mammalian cardiovascular tissues.

Intriguing biological effects throughout the body, from insulin secretion to memory formation to suspended animation!

Cardiovascular system effects include:

- potent cardio- and vasculoprotectant
- vasodilator • pro-angiogenic • anti-atherogenic
- pre/post-conditioning against I-R injury
**H₂S** is a physiologic vasodilator and regulator of blood pressure

First *in vivo* (mouse) model of targeted CSE gene deletion.

-/- mice featured absent CSE mRNA and protein in various tissues, and substantially decreased tissue (A) and serum (B) H₂S levels.

Mutant mice developed age-dependent hypertension (C).

CSE-deficient SMCs over-proliferate

Smooth muscle cell (SMC) hypertrophy/hyperplasia contributes to vascular remodeling in hypertension.

Previously, CSE over-expression was shown to cause increased H$_2$S that inhibited SMC proliferation.

We found that CSE-KO SMCs had absent CSE mRNA and protein (A), decreased H$_2$S (B), and increased proliferative rates in vivo and in vitro (C), likely contributing to the observed hypertension.

Project: CSE-deficient SMCs and hypoxia

**Rationale:** Hypoxia is a ubiquitous feature of cardiovascular diseases including hypertension and atherosclerosis, wherein H$_2$S has been shown to have important vasculoprotective effects.

There is growing interest in the relationship of H$_2$S to O$_2$ homeostasis stemming from recent evidence that H$_2$S may act as a novel O$_2$ sensor and may regulate hypoxia-inducible factor (HIF)-1.

**Hypothesis:** CSE-deficient SMCs will demonstrate an impaired hypoxic stress response due to the absence of endogenous H$_2$S’s cytoprotective effects.

- survival
- redox status
- inflammatory profile
- mitochondrial function
- HIF-1 activity
CSE-deficient SMCs are more susceptible to hypoxia

(1) Cell viability (MTT assay)  
(2) Apoptosis (Caspase 3/7 assay)

Hypoxic stress caused significantly decreased viability of CSE-KO but not WT cells (Figure 1), and significantly higher apoptosis of KO versus WT cells (Figure 2). These data indicate that CSE-deficient SMCs are more susceptible to hypoxia, suggesting an essential contribution of endogenous H$_2$S to some aspect(s) of the protective hypoxic stress response.
CSE-deficient SMCs feature an inherent redox imbalance

Hypoxia induced similarly increased SOD activity in both cell lines (Figure 3), but CSE-KO cells exhibited substantially greater ROS levels versus WT under both basal and hypoxic conditions (Figure 4). These data indicate that hypoxia exaggerates an inherent redox imbalance in CSE-deficient SMCs, suggesting that oxidative stress is a likely mechanism of their susceptibility to hypoxic stress.
CSE-deficient SMCs exhibit blunted HIF-1α mRNA expression during hypoxia

Hypoxia elicited marked HIF-1α mRNA expression in CSE-WT cells, but only a relatively modest change in KO cells versus control. These data suggest that CSE-deficient SMCs may have impaired HIF-1 activity, which could underlie their compromised hypoxic stress response.
Summary & Future Directions

These data indicate that endogenous CSE/H₂S pathway:

- Exerts homeostatic control of SMC proliferation and intracellular ROS levels.

- Contributes to maintenance of SMC redox balance and survival under hypoxic conditions.

- Influences hypoxia-induced expression of HIF-1α and possibly HIF-1-mediated signalling.

Immediate plans include clarification of the inflammatory picture, examination of mitochondrial function, and further investigation of the potential HIF-1 connection.
Thank you!

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Contact: sean.bryan@nosm.ca