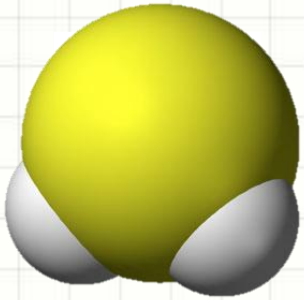
Cystathionine γ-lyase and hypoxia:
Implicating hydrogen sulfide in the hypoxic stress response



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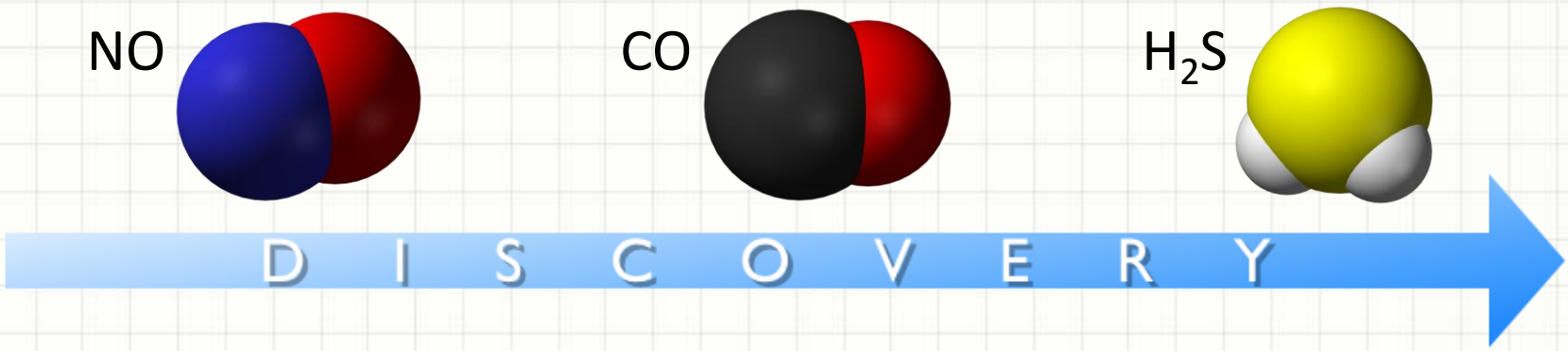
The arrival of gasotransmitter biology

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



H_2S: 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:

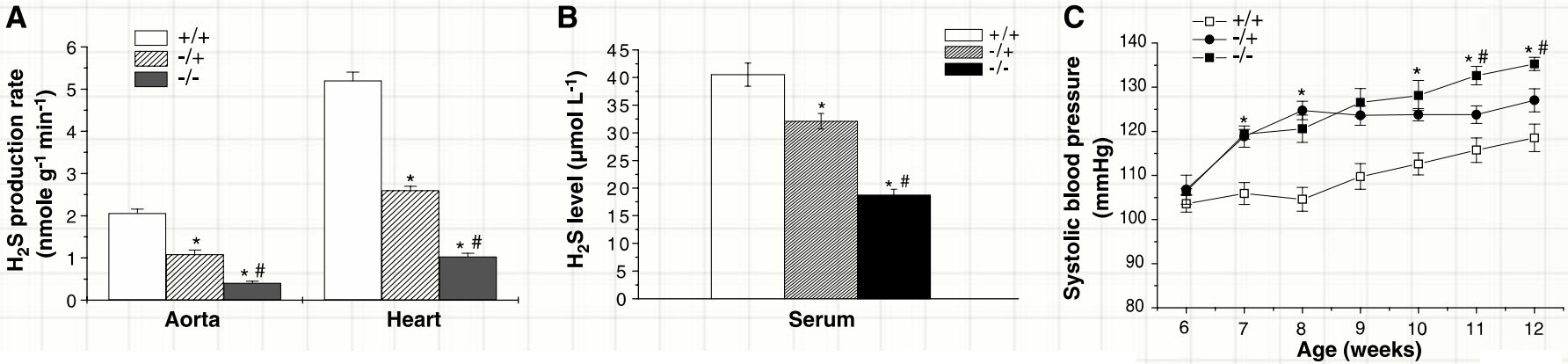
- potent cardio- and vasculoprotectant
- vasodilator • pro-angiogenic • anti-atherogenic
- pre/post-conditioning against I-R injury

H_2S 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**) H_2S levels.

Mutant mice developed age-dependent hypertension (**C**).



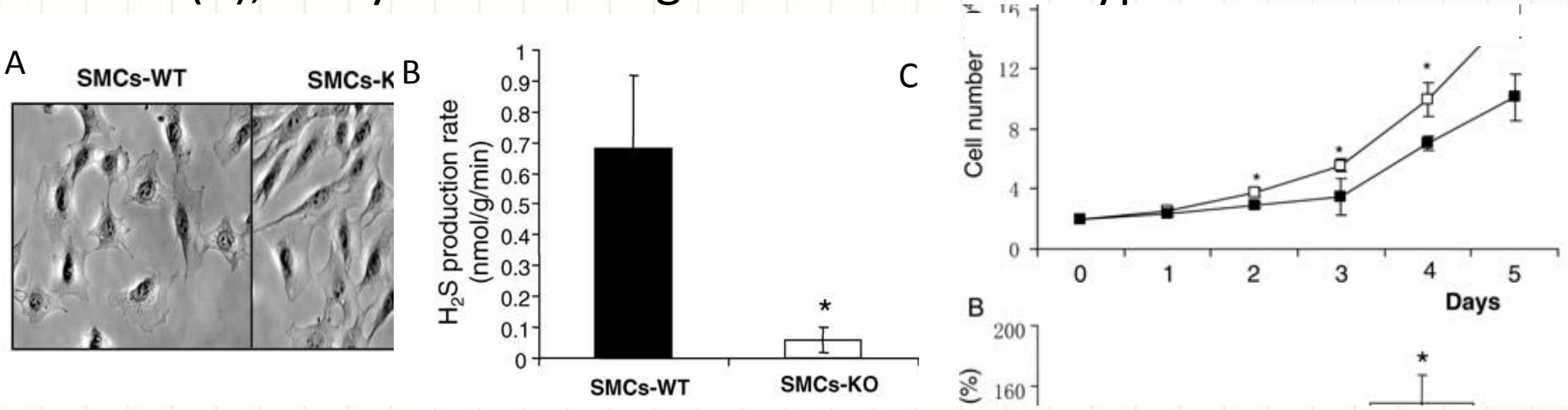
Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R. **H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase**. Science (2008) vol. 322 (5901) pp. 587-90

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_2S that inhibited SMC proliferation.

We found that CSE-KO SMCs had absent CSE mRNA and protein (**A**), decreased H_2S (**B**), and increased proliferative rates *in vivo* and *in vitro* (**C**), likely contributing to the observed hypertension.



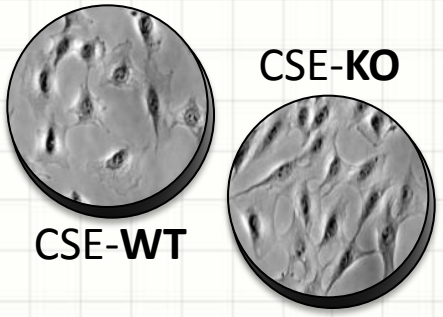
Yang G, Wu L, Bryan S, Khaper N, Mani S, Wang R. **Cystathionine gamma-lyase deficiency and over-proliferation of smooth muscle cells**. Cardiovasc Res (2010) vol. 86 (3) pp. 487-95.

Project: CSE-deficient SMCs and hypoxia

Rationale: Hypoxia is a ubiquitous feature of cardiovascular diseases including hypertension and atherosclerosis, wherein H_2S has been shown to have important vasculoprotective effects.

There is growing interest in the relationship of H_2S to O_2 homeostasis stemming from recent evidence that H_2S 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_2S's cytoprotective effects.



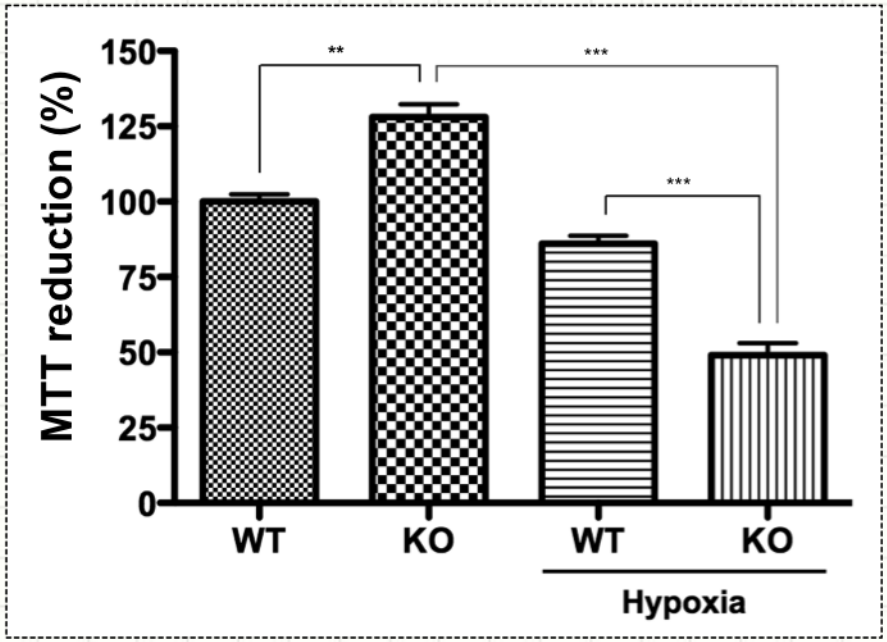
HYPOXIA

1% O_2 ◆ 12 h

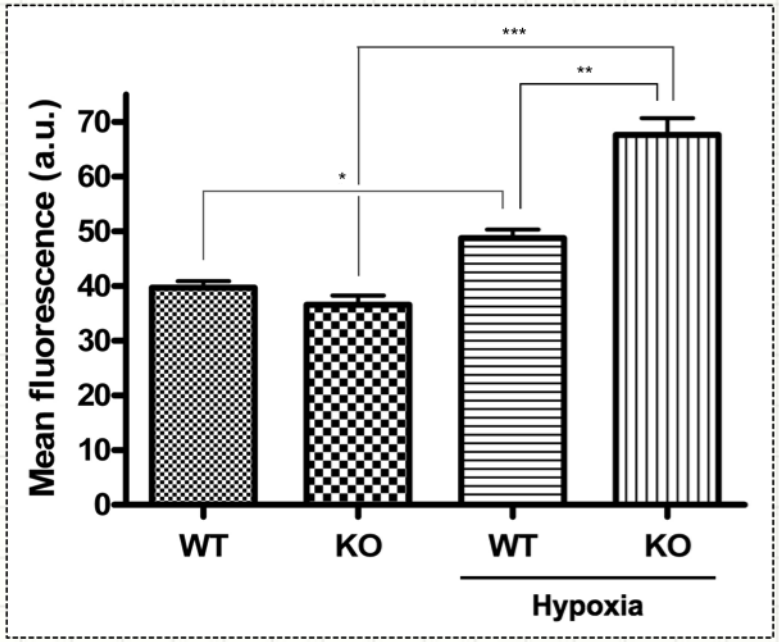
- survival
- redox status
- inflammatory profile
- mitochondrial function
- HIF-1 activity

CSE-deficient SMCs are more susceptible to hypoxia

(1) **Cell viability** (MTT assay)



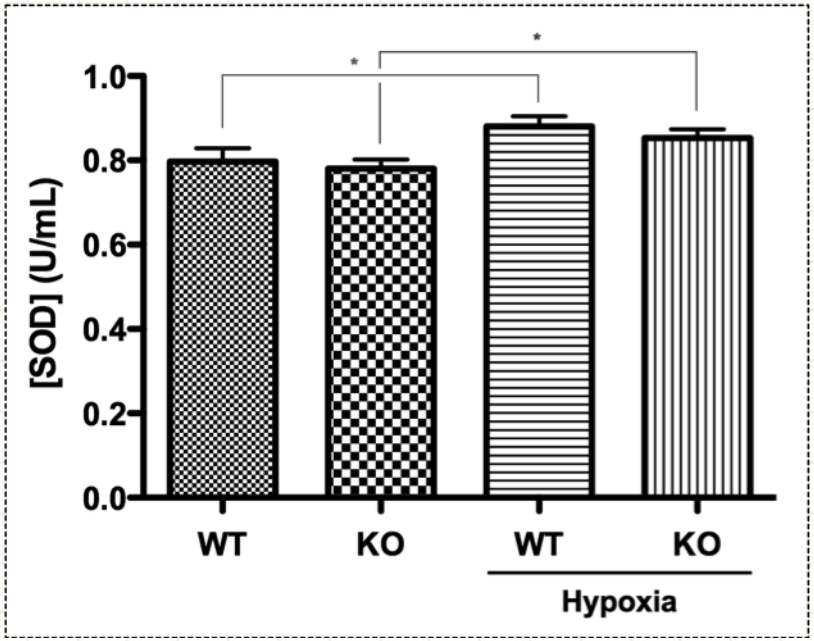
(2) **Apoptosis** (Caspase 3/7 assay)



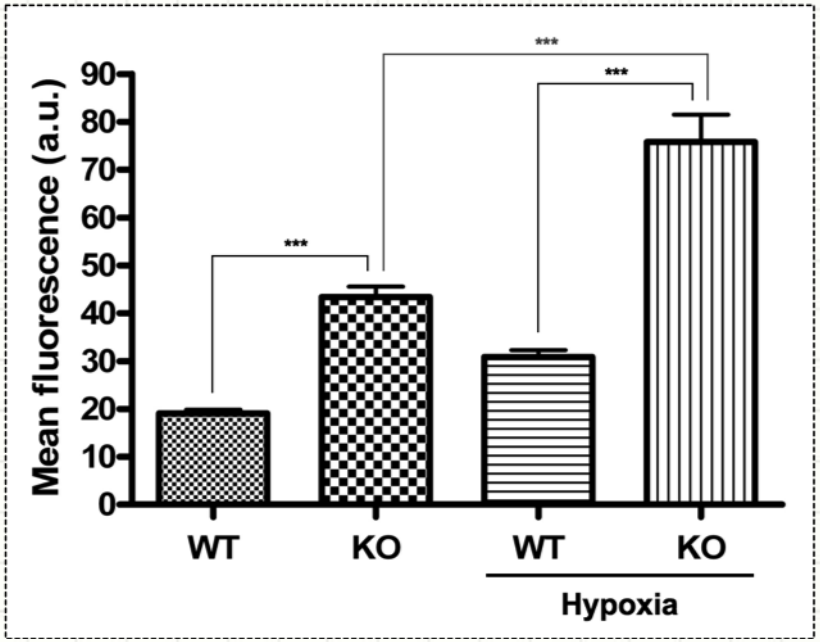
Hypoxic stress caused significantly decreased viability of CSE-KO but not WT cells (Figure 1), and significantly higher apoptosis of KO versus WT cells (Figure 2). **These data indicate that CSE-deficient SMCs are more susceptible to hypoxia, suggesting an essential contribution of endogenous H_2S to some aspect(s) of the protective hypoxic stress response.**

CSE-deficient SMCs feature an inherent redox imbalance

(3) **SOD activity** (colourimetric assay)



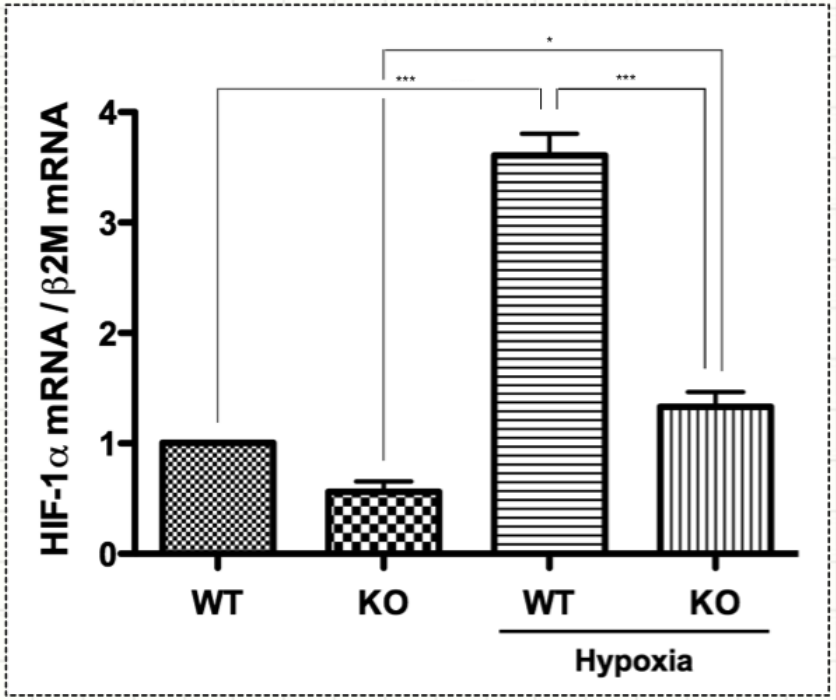
(4) **Intracellular ROS** (H_2DCFDA assay)



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

(5) HIF-1α expression (qPCR)



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/H_2S 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.

Hudson Bay sunrise, Fort Severn First Nation
NOSM 106 ICE placement - May 2011

Thank you!

- Dr. Neelam Khaper (NOSM)
- Dr. Rui Wang (LU)
- Dr. Guangdong Yang (LU)

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GREAT

Gasotransmitter REsearch And Training Program

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