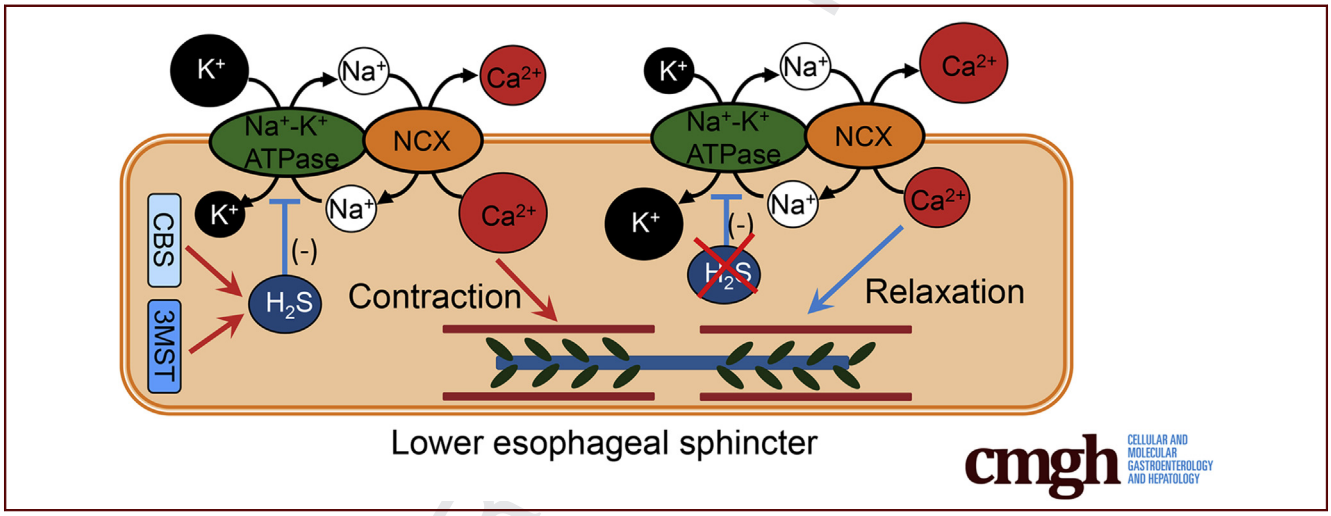
cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

# Endogenous Hydrogen Sulfide Contributes to Tone Generation in Porcine Lower Esophageal Sphincter Via $Na^+/Ca^{2+}$ Exchanger

Xiaopeng Bai,[1] Eikichi Ihara,[1] Katsuya Hirano,[2] Yoshimasa Tanaka,[1] Kayoko Nakano,[3] Satomi Kita,[4,5] Takahiro Iwamoto,[4] Haruei Ogino,[1] Mayumi Hirano,[6] Yoshinao Oda,[3] Kazuhiko Nakamura,[1] and Yoshihiro Ogawa[1,7]

*[1]Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Cardiovascular Physiology, Faculty of Medicine, Kagawa University, Kagawa Prefecture, Japan; [3]Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [4]Department of Pharmacology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan; [5]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan; [6]Division of Molecular Cardiology, Research Institute of Angiocardiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [7]Department of Molecular and Cellular Metabolism, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan*




## SUMMARY

Endogenous hydrogen sulfide is continuously produced by 2 hydrogen sulfide–generating enzymes, cystathionine-$\beta$-synthase and 3-mercaptopyruvate sulfurtransferase, in porcine lower esophageal sphincter smooth muscle. Endogenous hydrogen sulfide contributes to lower esophageal sphincter myogenic tone generation by maintaining cytosolic $Ca^{2+}$ concentration via the $Na^+/Ca^{2+}$ exchanger.

**BACKGROUND AND AIMS:** Hydrogen sulfide ($H_2S$) is a major physiologic gastrotransmitter. Its role in the regulation of the lower esophageal sphincter (LES) function remains unknown. The present study addresses this question.

**METHODS:** Isometric contraction was monitored in circular smooth muscle strips of porcine LES. Changes in cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and force were simultaneously monitored in fura-2-loaded strips with front-surface fluorometry. The contribution of endogenous $H_2S$ to LES contractility was investigated by examining the effects of inhibitors of $H_2S$-generating enzymes, including cystathionine-$\beta$-synthase, cystathionine-$\gamma$-lyase, and 3-mercaptopyruvate sulfurtransferase, on the LES function.

**RESULTS:** Porcine LES strips myogenically maintained a tetrodotoxin-resistant basal tone. Application of AOA (cystathionine-$\beta$-synthase inhibitor) or L-aspartic acid (L-Asp; 3-mercaptopyruvate sulfurtransferase inhibitor) but not DL-PAG (cystathionine-$\gamma$-lyase inhibitor), decreased this basal tone. The relaxant effects of AOA and L-Asp were additive. Maximum relaxation was obtained by combination of 1 mM AOA and 3 mM L-Asp. Immunohistochemical analyses revealed that cystathionine-$\beta$-synthase and 3-mercaptopyruvate sulfurtransferase, but not CBE, were expressed in porcine LES. AOA+L-Asp–induced relaxation was accompanied by a decrease in $[Ca^{2+}]_i$ and inversely correlated with the extracellular $Na^+$ concentration ($[Na^+]_o$) (25-137.4 mM),

indicating involvement of an $Na^+/Ca^{2+}$ exchanger. The reduction in the basal $[Ca^{2+}]_i$ level by AOA was significantly augmented in the antral smooth muscle sheets of $Na^+/Ca^{2+}$ exchanger transgenic mice compared with wild-type mice.

**CONCLUSIONS:** Endogenous $H_2S$ regulates the LES myogenic tone by maintaining the basal $[Ca^{2+}]_i$ via $Na^+/Ca^{2+}$ exchanger. $H_2S$-generating enzymes may be a potential therapeutic target for esophageal motility disorders, such as achalasia. *(Cell Mol Gastroenterol Hepatol 2017;■:■–■; https://doi.org/10.1016/j.jcmgh.2017.11.004)*

*Keywords:* Lower Esophageal Sphincter; Myogenic Tone Regulation; Hydrogen Sulfate; $Na^+/Ca^{2+}$ Exchanger.

The lower esophageal sphincter (LES) is a region of circular smooth muscle that possesses basal tone and functions as a barrier at the esophagogastric junction. The LES tone is primarily myogenic in origin and is regulated myogenically and neurogenically on stimulation.[1] Dysfunction of LES contractility underlies the pathogenesis of clinically important diseases, including gastroesophageal reflux disease and motility disorders represented by achalasia. Understanding the mechanisms controlling LES tone is crucial for gaining insight into the treatment of these diseases.

Control of cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and $Ca^{2+}$ sensitivity of the contractile apparatus play a key role in myogenic regulation of the LES tone. The activation of the L-type $Ca^{2+}$ channel and Rho-associated kinase contribute to maintaining the myogenic tone by increasing $[Ca^{2+}]_i$ and $Ca^{2+}$ sensitivity, respectively.[2,3] The LES tone is also neurogenically regulated by excitatory and inhibitory vagal pathways.[4,5] The excitatory vagal pathway is mediated by cholinergic neurons, whereas the inhibitory vagal pathway is mediated by the nonnoradrenergic/noncholinergic neurons. The gasotransmitters nitric oxide and carbon monoxide act as neurotransmitters in the inhibitory pathway.[6] Hydrogen sulfide ($H_2S$) has been identified as a third gasotransmitter[7,8]; however, its role in the regulation of the LES tone remains unclear.

$H_2S$ is synthesized endogenously by cystathionine-$\beta$-synthase (CBS), cystathionine-$\gamma$-lyase (CSE), or 3-mercaptopyruvate sulfurtransferase (3MST) in vertebrates. These 3 enzymes share a common substrate in L-cystein (L-Cys) for $H_2S$ generation; however, they differ in the dependency on 2-pyridoxal-50-phosphate and tissue distribution. CBS and CSE are 2-pyridoxal-50-phosphate-dependent, whereas 3MST is 2-pyridoxal-50-phosphate-independent. The expression of enzymes is regulated in a tissue-specific manner.[9–13] $H_2S$ carries out various physiologic functions in different tissues, including gastrointestinal smooth muscle. It opens ATP-sensitive $K^+$ channels ($K_{ATP}$ channels) to control gastrointestinal contractility.[14] $H_2S$ also stimulates transient receptor potential vanilloid 1 channels in rat duodenum, causing duodenal smooth muscle contraction via the local release of substance P.[15] The specific CBS inhibitor amino-oxyacetic acid (AOA) has been shown to inhibit the tonic contraction in rat duodenum and mouse antrum, suggesting the excitatory role of $H_2S$.[14,16] However, the role of $H_2S$ in the regulation of LES contractility in either physiological or pathologic situations remains largely unknown.

The present study examined the role of endogenous $H_2S$ in the regulation of the basal tone of LES. NaHS, a stable $H_2S$ donor, has been used to examine the effects of exogenous $H_2S$,[17,18] whereas inhibitors of $H_2S$-generating enzymes have been used to investigate the functional role of endogenous $H_2S$.[16,19] In the present study, the expression of 3 major $H_2S$-generating enzymes in porcine LES was determined and the effects of $H_2S$-generating enzyme inhibitors on the LES contractility were investigated.

## Methods

All protocols for animal experiments were approved by the Animal Use and Care Committee of Fukuoka University and Kyushu University. All authors had access to the study data and reviewed and approved the final manuscript.

### *Tissue Preparation of Porcine Lower Esophageal Sphincter Circular Muscle Strips*

A section of porcine esophagus containing the esophagogastric junction was freshly obtained from a local slaughterhouse and immediately transported to our laboratory in normal extracellular solution containing 137.4 mM NaCl (137-NES). The specimen was cut open in the longitudinal direction along the greater curvature of the stomach and pinned to the flat surface of a silicone rubber plate with the mucosal side up. After removing the mucosal and submucosal layer, the circular smooth muscle sheets of the LES were excised and cut into strips (5 × 2 mm) under a binocular microscope.

### *$Na^+/Ca^{2+}$ Exchanger Transgenic Mice and Tissue Preparation of Antral Smooth Muscle Sheets*

It has been shown that $H_2S$-generating enzyme CBS plays a role in myogenic tone generation in mouse antral smooth muscle.[16] $Na^+/Ca^{2+}$ exchanger (NCX) 1.3 transgenic (TG) mice were used to determine the association of endogenous $H_2S$ with NCX. NCX1.3 TG mice in a C57BL/6J background were generated as previously described.[20] TG mice were

***Abbreviations used in this paper:*** **AOA, amino-oxyacetic acid; $[Ca^{2+}]_i$, cytosolic $Ca^{2+}$ concentration; CBS, cystathionine-$\beta$-synthase; CCh, carbachol; CSE, cystathionine-$\gamma$-lyase; ES, extracellular solution; $H_2S$, hydrogen sulfide; $K_{ATP}$ channels, ATP-sensitive $K^+$ channels; KES, $K^+$ extracellular solution; L-Asp, L-aspartic acid; L-Cys, L-cysteine; LES, lower esophageal sphincter; L-NAME, $N^{\omega}$-nitro-L-arginine methyl ester; $[Na^+]_o$, extracellular $Na^+$ concentration; NCX, $Na^+/Ca^{2+}$ exchanger; NES, normal extracellular solution; 3MST, 3-mercaptopyruvate sulfurtransferase; PAG, propargylglycine; TEA, tetraethylammonium; TG, transgenic; TTX, tetrodotoxin.**



2352-345X
https://doi.org/10.1016/j.jcmgh.2017.11.004