Down-Regulation of Endogenous Hydrogen Sulfide Pathway in Pulmonary Hypertension and Pulmonary Vascular Structural Remodeling Induced by High Pulmonary Blood Flow in Rats

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Background The mechanisms responsible for the development of pulmonary hypertension (PH) and pulmonary vascular structural remodeling induced by high pulmonary blood flow are not fully understood. The present study was designed to explore the possible changes in endogenous hydrogen sulfide (H₂S), a novel gasotransmitter, on the pathogenesis of PH and pulmonary vascular structural remodeling induced by high pulmonary blood flow.

Methods and Results Twenty-two male Sprague-Dawley rats were randomly divided into a shunting group (n=11) and control group (n=11). Rats in the shunting group underwent an abdominal aorta-inferior cava vein shunting operation. After 11 weeks of shunting, the plasma level of H₂S and lung tissue H₂S producing rate were much lower than those of the control group (p<0.01). In situ hybridization analysis showed that the expression of cystathionine γ -lyase (CSE) mRNA was down-regulated in the pulmonary arteries of the shunting rats compared with the control group (p<0.01), and competitive quantitative reverse transcription-polymerase chain reaction showed that the relative amount of CSE mRNA in lung tissue was decreased significantly (p<0.01).

Conclusions The endogenous H₂S pathway is down-regulated in PH and pulmonary vascular structural remodeling is induced by high pulmonary blood flow. (Circ J 2005; 69: 1418–1424)

Key Words: High pulmonary blood flow; Hydrogen sulfide; Pulmonary hypertension; Pulmonary vascular structural remodeling

Pulmonary hypertension (PH) is a common complication of congenital heart disease with a left-to-right shunt characterized by high pulmonary blood flow. Pulmonary vascular structural remodeling is an important pathologic consequence of PH. To date, the mechanisms responsible for the induction of pulmonary vascular structural remodeling by increased pulmonary blood flow are still not fully understood. Some studies have demonstrated that high pulmonary blood flow produces high shear stress on pulmonary artery (PA) endothelial cells (ECs) and smooth muscle cells (SMCs), resulting in maladjustment of the maintenance of the structure and function of blood vessels, in which endogenous gasotransmitters, such as nitric oxide (NO) and carbon monoxide (CO), play important regulatory roles.^{1–5} However, the molecular mechanisms of pulmonary vascular structural remodeling are still poorly understood, and researchers are seeking novel endogenously

produced gasotransmitters to investigate their possible significance in the pathogenesis of pulmonary vascular structural remodeling. Recently, hydrogen sulfide (H₂S) was found to facilitate the induction of hippocampus long-time potentiation as a neuromodulator.⁶ More interestingly, a previous study of ours demonstrated that H₂S was endogenously produced in the heart as well as in vessels⁷ and could inhibit the proliferation of cultured vascular SMCs in vitro.^{8,9} Another of our studies also showed that the endogenous H₂S pathway was involved in the development of hypoxic PH (HPH) and that exogenous H₂S could attenuate the elevation of PA pressure and lessen pulmonary vascular structural remodeling during HPH.¹⁰ Based on those findings, the present study was designed to investigate the possible role of endogenous H₂S in the development of PH and pulmonary vascular structural remodeling induced by high pulmonary blood flow.

Methods

Rat Model of High Blood Flow

Experiments were conducted in accordance with the Guide to The Care And Use of Experimental Animals issued by the Ministry of Health of the People's Republic of China. Male Sprague-Dawley (SD) rats weighing 150–200g were provided by the Animal Research Centre of Peking University First Hospital. They were kept in a temperature-controlled room with a 12 h light–dark cycle. Tap water and rat chow were provided ad libitum. The animal

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model of high blood flow was established by an abdominal aorta-inferior vena cava shunting operation performed according to the description by Garcia and Diebold,¹¹ with some modifications! Briefly, 22 male SD rats were randomly divided into a shunting group (n=11) and control group (n=11). Rats in the shunting group were anesthetized using 0.25% pentobarbital sodium (40 mg/kg, ip). The abdominal aorta and inferior vena cava were exposed by opening the abdominal cavity via a midline incision. A bulldog vascular clamp was placed across the aorta caudal to the left renal artery. The aorta was punctured with an 18-gauge disposable needle at the union of the segment two-thirds caudal to the renal artery and one-third cephalic to the aortic bifurcation. The needle was advanced into the aorta at 45°, perforating its adjacent wall and penetrating the vena cava. Then the needle was slowly withdrawn and a 9-0 silk thread was used to suture the puncture. The clamp was removed 30 s later and the patency of the shunt was verified visually by the mixture of arterial and venous blood in the inferior vena cava. The peritoneal cavity was closed with silk thread and gentamycin was injected for the prevention of infection. In the control group, rats underwent the same experimental process but without the shunting operation.

Measurement of Hemodynamic Parameters and Sample Preparation

At the end of the experimental period, the animals were weighed and anesthetized with pentobarbital sodium (40 mg/kg, ip). A silicone catheter (0.9 mm in outer diameter) was introduced under fluoroscopic guidance into the right jugular vein via a venotomy and passed across the tricuspid valve and right ventricle (RV) into the PA. The other end of the catheter was connected to a YZ-05-1 transducer (Beijing, China). The mean PA pressure (mPAP), systolic PAP (sPAP) and diastolic PAP were simultaneously recorded. After the chest was opened, the right lung was removed and placed in liquid nitrogen for rapid freezing and then stored at -70°C, and the lower left lung was removed and placed in 10% paraformaldehyde for fixation. Then the heart was removed and the RV and the left ventricle plus the septum (LV+SP) were dissected free. These tissues were blotted and weighed using an electronic scale. The ratio of the wet weight of the RV to that of the LV+SP [RV/(LV+SP)] was calculated as an indicator of RV hypertrophy. Plasma was collected from the rats, centrifuged and stored at -70°C for the measurement of H₂S concentration.

Morphological Analysis of the PAs

The lung tissue fixed in 10% (w/v) paraformaldehyde was dehydrated, embedded in paraffin, and sectioned at 4 μm. The elastic fiber in the lung tissues was stained according to the modified Weigert's method and re-stained with van Gieson solution. Morphological analysis was performed using a video-linked microscope digitizing board system (Leica Q550CM, Germany). Only vessels showing clearly defined external and internal elastic lamina were included in the analysis. The relative medial thickness (RMT) and relative medial areas (RMA) were calculated according to Barth et al's method.¹²

A small section of lung tissue was cut from the upper part of the left lobe and quickly immersed in 3% glutaraldehyde, after which it was cut into pieces 1×1×1 mm in size and post-fixed in 1% phosphate-buffered osmium

tetroxide for 6 h. The samples were rinsed again (overnight) and dehydrated in a graded series of ethanol, before being infiltrated with propylene oxide and embedded with Epon 812. These procedures were all done at room temperature. Finally, the specimens were embedded in new batches of Epon 812 and polymerized at 40°C (24 h) and 60°C (48 h). From selected blocks, series of transverse or longitudinal sections were made. Semi-thin sections (1 μm) were stained with azur-II and methylene blue. Ultra-thin sections (60–90 nm) were cut with an ultra-microtome and mounted on formvar-coated copper grids (75 meshes), for staining with uranylacetate and lead citrate, and examination under a transmission electron microscope (JEM-100CX, JEOL, Japan).

Measurement of Plasma H₂S Concentration

A sample of plasma (0.1 ml) was added to a test tube containing 0.5 ml of 1% zinc acetate and 2.5 ml of distilled water, then 0.5 ml of 20 mmol/L N,N-dimethyl-p-phenylenediamine dihydrochloride in 7.2 mol/L HCl and 0.4 ml of 30 mmol/L FeCl₃ in 1.2 mol/L HCl were also added to the same test tube for 20 min of incubation at room temperature. The protein in the plasma was removed by adding 1 ml of 10% trichloroacetic acid to the solution and centrifuging it. The optical absorbance of the resulting solution at 670 nm was measured with a spectrometer (Shimadzu UV 2100, Japan). H₂S concentration in the solution was calculated against the calibration curve of the standard NaHS solution.

Measurement of Tissue H₂S Production Rate

Tissue H₂S production rate was measured using the method of chemical catalytic reaction.¹¹ The right lung was homogenized in ice-cold 50 mmol/L potassium phosphate buffer (pH 6.8). Reactions were performed in a 25 ml Erlenmeyer flask. The reaction mixture contained (mmol/L): 10 L-cysteine, 2 pyridoxal 5'-phosphate, 100 potassium phosphate buffer (pH 7.4), and 10% (w/v) homogenates. The total volume of the reaction mixture was 1 ml. A small piece of filter paper was put into the central well of the flask and 0.5 ml of 1% zinc acetate was also added to trap evolving H₂S in the mixture. The flask was then flushed with N₂ before being sealed with a double layer of parafilm. The catalytic reaction was initiated by transferring the flask from an ice bath to a 37°C shaking water bath. After 90 min, the reactions were stopped by injecting 0.5 ml of 50% trichloroacetic acid. The flask was incubated in the shaking water bath at 37°C for an additional hour to complete the trapping of H₂S. The content of the central well was transferred to test tubes and mixed with 3.5 ml of distilled water and 0.5 ml of 20 mmol/L N,N-dimethyl-p-phenylenediamine dihydrochloride in 7.2 mol/L HCl. To each tube, 0.4 ml of 30 mmol/L FeCl₃ in 1.2 mol/L HCl was added immediately. After 20 min of incubation at room temperature, the optical absorbance of the resulting solution at 670 nm was measured with a spectrometer (Shimadzu UV 2001). The H₂S concentration in the solution was calculated against the calibration curve of the standard NaHS solution for each sample and the measurement was done in duplicate. The H₂S producing rate was expressed as nmol/mg · wet · tissue/min.

Expression of Cystathionine -Lyase (CSE) mRNA in Lung Tissue by In Situ Hybridization

The upper part of the left lung fixed in 10% (w/v) par-

Table 1 Changes in the Hemodynamic Parameters and Right Ventricle (RV) Hypertrophy in Rats

Group	n	sPAP (mmHg)	dPAP (mmHg)	mPAP (mmHg)	RV/BW (g/kg)	RV/(LV + S) (g/g)
Control	11	25.02±3.31	9.15±3.02	15.86±1.92	0.48±0.08	0.22±0.01
Shunt	11	34.53±2.62*	10.13±3.41	23.64±2.21*	0.80±0.17*	0.27±0.02*

Data are mean ± SD.

sPAP, systolic pulmonary artery pressure; dPAP, diastolic pulmonary artery pressure; mPAP, mean pulmonary artery pressure; RV/BW, wet weight of right ventricle/basal weight; RV/(LV + SP), wet weight of right ventricle/left ventricle + septum.

* $p < 0.01$ vs control group.

A **B**

Fig 1. (A) Pulmonary muscular artery (MA) in a control rat (Hart's elastic fibers staining+VG staining, ×400). (B) Pulmonary MA in a rat from the shunt group, showing the thickened medial layer (Hart's elastic fibers staining+VG staining, ×400). VG, van Gieson.

aformaldehyde was dehydrated, embedded in paraffin, and sectioned at 5 μm. After being dewaxed and hydrated routinely, the sections were blocked in H₂O₂ for 10 min at room temperature, and digested in 3% citrate solution for 10–15 min at 37°C. Then they were washed in 0.5 phosphate-buffered saline (PBS) for 5 min 3 times and in distilled water once. Each section had 20 μl of labeled probe with oligonucleotide added, and was then covered with film for hybridization for 120 min at 37–40°C. Later, the film was removed, and the section was washed for 5 min 3 times in 2× standard saline citrate (SSC) solution at 30–37°C. The sections were stabilized with hybridization probe for 20 min at 37–40°C and were washed for 5 min 3 times in 2×SSC

solution. After the sections were blocked in blocking solution for 20 min at 37°C, and rabbit-anti-DIG was added for 60 min at 37°C, they were washed for 2 min 3 times in 0.5 mol/L PBS. Again, goat-anti-rabbit-biotin IgG was added to sections for 20 min at 37°C, after which the sections were washed for 2 min 3 times in 0.5 mol/L PBS solution. Finally, SABC was added to the sections for 20 min at 37°C, after which they were washed for 5 min 4 times in 0.5 mol/L PBS. The color was developed using DAB-H₂O₂ for 20 min before the sections were stained with hematoxylin.

Endogenous H₂S is generated from thio-containing amino acids in a reaction catalyzed by cystathionine-γ-synthase (CBS) and CSE. In the present study, brown granules in the cell nucleus was defined as the expression of CSE mRNA, the intensity of which was determined semi-quantitatively: negative (–) if there was no positive signal in the PA cells; (+) if 1–50% of cells showed a positive signal; (++) if 51–100% of cells were positive. At least 10 medial and small PAs were examined, respectively, for each slide. For the convenience of interpretation and statistical analysis, the CSE mRNA content of PAs was expressed as integral scores: the percentage of PAs with a certain extent of reaction intensity was multiplied by the weighted values of their reaction intensity. The weighted value of the reaction intensity was 0, 0.5 and 1.0, if the reaction intensity was –, + and ++, respectively.¹³

Measurement of CSE mRNA in Lung Tissue by Competitive Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from rat lung tissue using Trizol reagent. The cDNA was synthesized using an oligo (dT)₁₅ primer and M-MLV reverse transcriptase. The polymerase chain reaction (PCR) primers used to amplify the fragment of CSE cDNA were 5'-TCCGG ATGGA GAAAC ACTTC for CSE-S and 5'-GCTGC CTTTA AAGCT TGACC for CSE-A.

With these primers PCR yielded a 400 bp fragment of wild-type rat CSE cDNA. The competitive internal standard for the measurement of CSE cDNA had the same sequences as the 400 bp fragment of wild-type rat CSE cDNA, except that a 39 bp fragment at the downstream site of the CSE-S primer was deleted according to the methods described by Siebert and Carrick¹³ and Sambrook and Russell.¹⁴ Wild-type rat CSE cDNA in the sample and the internal standard were competitively amplified in the same PCR system. Quantitative PCR was performed in a 0.2 ml PCR tube containing 1 μl of rat lung cDNA, 1 μl of 3.9 fmol/L competitive internal standard, 1 μl of 5 μmol/L of each CSE-S and CSE-A mixture, 1 μmol/L of each dNTP polymerase, in a total volume of 25 μl. PCR products were separated on 2% agarose gel and stained with ethid-

Table 2 Percentage of 3 Types of Small Pulmonary Blood Vessels, and the RMT and RMA of the Pulmonary Arteries in Rats

	<i>n</i>	<i>Small pulmonary blood vessels (%)</i>			<i>RMT</i>		<i>RMA</i>	
		<i>MA</i>	<i>PMA</i>	<i>NMA</i>	<i>MPA</i>	<i>SPA</i>	<i>MPA</i>	<i>SPA</i>
<i>Control</i>	11	13.62±1.82	14.73±1.72	71.76±2.63	5.72±0.33	7.54±0.75	9.32±1.73	13.53±1.21
<i>Shunt</i>	11	23.28±3.13*	23.95±3.23	52.85±6.02*	7.76±0.62*	11.78±1.02*	14.56±1.12*	22.26±2.13*

Data are mean±SD.

RMT, relative medial thickness; RMA, relative medial area; MA, muscular artery; PMA, partly muscular artery; NMA, non-muscular artery; MPA, medial pulmonary artery; SPA, small pulmonary artery.

**p*<0.01 vs control group.

Fig 2. Ultrastructure of a pulmonary artery in (A) control rat showing the flat endothelial cells (ECs), regular internal elastic lamina and cytoplasm of the smooth muscle cells full of myofilaments and dense bodies. (B) Rat from the shunt group showing swollen and hypertrophic ECs, irregular internal elastic lamina and increased rough endoplasmic reticulum and free ribosomes in the cytoplasm of smooth muscle cells (A, B: ×10,000).

ium bromide. The ratio of the optical density of the 2 DNA bands was measured using a Gel Image Analysis System (AlphaImager, Alpha Innotech, CA, USA) under UV light. A standard curve of the ratio was drawn under the same conditions as described except that rat lung cDNA was changed for a series of dilutions of a plasmid containing the 400bp wild-type CSE cDNA fragment. The relative amount of CSE cDNA in the samples was then obtained from the standard curve. To calibrate the amount of sample loaded into the PCR mixture, β -actin cDNA was measured using the same method: 3 μ l of PCR product was amplified again using 2 rat β -actin primers (β -actin-S: 5'-ATCTG GCACC ACACC TTC; β -actin-A: 5'-AGCCA GGTCC AGACG CA). The relative amount of β -actin cDNA in the loaded sample was then obtained from an established standard curve of β -actin cDNA measurement and the standard relative amount of CSE cDNA was used for further analysis.

Chemicals and Reagents

NaHS solution was freshly prepared by mixing stock solutions of sodium sulfide and hydrochloric acid. Phosphate 5'-pyridoxal was purchased from Sigma Corporation (St Louis, MO, USA); Trizol reagent from Invitrogen (San Diego, CA, USA), and oligo (dT)₁₅ primer, dNTP, and M-MLV reverse transcriptase from Beijing Chemical Reagents. Hybridization kits were purchased from Zhongshan Biological Technique Ltd (Beijing, China).

Data Analysis

The results were expressed as mean±SD. SPSS 10.0

statistical analysis software was used to compare the difference between 2 groups (t-test), and a value of *p*<0.05 was considered statistically significant.

Results

Hemodynamic Parameters

After 11 weeks of shunting, the sPAP and mPAP increased significantly compared with the control group (*p*<0.01) (Table 1). The ratios of the wet weight of the RV to basal weight (RV/BW) and the wet weight of RV/LV+SP, indicators of RV hypertrophy, also increased markedly in the rats of shunting group compared with those of the control group (*p*<0.01) (Table 1).

Morphological Analysis of the PAs

Compared with the control rats, the RMT and RMA, which were defined as parameters of pulmonary vascular structural remodeling, were increased significantly in medial and small PAs, together with the development of PH in the shunting rats (*p*<0.01) (Fig 1). In addition, among the small pulmonary blood vessels in the shunting rats the percentage of muscular artery (MA) and partly MA (PMA) increased significantly and the percentage of non-MA (NMA) decreased significantly (*p*<0.01) (Table 2). Furthermore, the ECs were swollen and hypertrophic, the internal elastic lamina appeared irregular, and rough endoplasmic reticulum and free ribosomes increased in cytoplasm of SMCs in these rats (Fig 2).

Table 3 Changes in the Plasma Level of H₂S, Lung Tissue H₂S Production Rate, and the Amount of CSE mRNA in the Lung Tissues of Rats

Group	n	Plasma level of H ₂ S (μmol/L)	H ₂ S production rate (nmol/mg wet tissue min)	CSE mRNA (10 ⁻³ fmol/L)
Control	11	50.83±4.01	0.26±0.02	6.84±2.11
Shunt	11	36.42±3.12*	0.13±0.02*	2.30±1.07*

Data are mean ± SD.

H₂S, hydrogen sulfide; CSE, cystathionine γ -lyase.

* $p < 0.01$ vs control group.

Table 4 Integral Scores of CSE mRNA Expression by Pulmonary Artery Smooth Muscle Cells in Rats

Group	Medial pulmonary arteries	Small pulmonary arteries
Control	40.95±4.93	35.05±7.72
Shunting	25.92±4.95*	20.92±4.93*

CSE, cystathionine γ -lyase.

* $p < 0.01$ vs control group.

Measurement of Plasma H₂S Concentration

The plasma level of H₂S and the H₂S production rate in the shunting group were both much lower than those of the rats in the control group ($p < 0.01$) (Table 3).

Expression of CSE mRNA in Lung Tissue by In Situ Hybridization

CSE mRNA expression was obviously down-regulated in

the SMCs of the PAs of shunt rats as compared with those of control rats (Table 4, Fig 3).

Measurement of CSE mRNA in Lung Tissue by Competitive Quantitative RT-PCR

In contrast to the control group, the relative amount of CSE mRNA in the lung tissue of the shunting group was decreased significantly ($p < 0.01$) (Fig 4).

Discussion

PH is an important pathophysiological process in a variety of cardiovascular and pulmonary diseases, and it directly affects the development of the diseases and their prognosis. Pulmonary vascular structural remodeling is a remarkable pathologic consequence of PH^{15,16} but the cellular and molecular mechanisms are not fully understood.

The results of the present study showed that abdominal aorta-cava shunting for 11 weeks successfully induced PH

Fig 3. In situ hybridization showing (A) positive CSE mRNA expression (++) localized in smooth muscle cells of small pulmonary muscular arteries and minimal expression in pulmonary vascular endothelial cells (ECs) in a control rat. (B) Negative CSE mRNA expression (-) in both smooth muscle cells and ECs of a small pulmonary muscular artery (MA) in a rat from the shunt group. (C) Positive CSE mRNA expression (++) localized in the smooth muscle cells of a medial pulmonary MA from a control rat (DAB, ×250). (D) Positive CSE mRNA expression (+) in the smooth muscle cells and ECs of a medial pulmonary MA in a rat from the shunt group. CSE, cystathionine γ -lyase (A–D: DAB, ×250).

and pulmonary vascular structural remodeling. The sPAP and mPAP of shunt rats increased significantly compared with those in the control group. The ratios of RV/BW and RV/LV+SP, indicators of RV hypertrophy, also increased in rats with high pulmonary blood flow. In this study, we defined medial PAs as those with an outer diameter ranging from 50 to 150 μ m, and small PAs as those with an outer diameter ranging from 15 to 50 μ m. The results demonstrated that after 11 weeks of shunting, the percentage of MA and PMA increased and the percentage of NMA decreased significantly in small pulmonary vessels of shunting rats. Furthermore, we observed that the RMT and RMA of MA increased significantly in medial and small PAs with the development of PH. Ultrastructural examination showed that ECs were swollen and hypertrophic, the internal elastic lamina appeared irregular, and rough endoplasmic reticulum and free ribosomes increased in the cytoplasm of SMCs. These findings indicate that PH and pulmonary vascular structural remodeling developed in rats after 11 weeks of aorta-caval shunting, which was in accordance with our previous study!

Although numerous humoral factors in the development of PH and pulmonary vascular structural remodeling in left-to-right shunt congenital heart diseases have been investigated, the mechanisms responsible for pulmonary vascular structural remodeling induced by high pulmonary blood flow are still not fully understood. The discovery of endogenous gasotransmitters, such as NO and CO, has moved research into these conditions to a very new phase!^{7,18} As gas molecules, NO and CO are characterized by continuous generation, fast transmission and easy passage through biological membrane under pathophysiological or physiological conditions.

H₂S has been known for decades as a toxic gas in water pollution and industrial air pollution with a strong odor of rotten eggs, but it was only recently that researchers came to understand H₂S as a novel gasotransmitter playing an important biological role, especially in the cardiovascular system!^{9–22} In rats, endogenous H₂S is generated from thio-containing amino acids in a reaction catalyzed by CBS and CSE. CBS is highly expressed in the brain, and CSE is rich in the wall of the PAs, aorta, tail arteries, mesenteric arteries and portal vein. In mammalian tissues H₂S may be a physiological regulator in the nervous system and cardiovascular system;^{23,24} with its vascular effect possibly mediated by the opening of the KATP channel of vascular tissues.²⁵ Importantly, we recently reported that H₂S could inhibit SMC proliferation⁹ and it has also been reported that the H₂S concentration of rat serum was 45.6 $\pm$ 14.2 μ mol/L, which exerted an important biological effect in a dose-dependent manner.²³ Our research group first reported that the endogenous H₂S synthase/H₂S pathway participated in a variety of vascular diseases including in the pathogenesis of spontaneous hypertension, HPH and pulmonary vascular structural remodeling, which suggests that H₂S is an important regulatory factor in cardiovascular physiological and pathophysiological processes.^{9,10,26}

In the present study of the possible significance of endogenous H₂S in left-to-right shunt rats with PH and pulmonary vascular structural remodeling induced by high pulmonary blood flow, the plasma level of H₂S and H₂S production rate in the shunting group were much lower than those of the control rats. Furthermore, the expression of CSE mRNA in the lung tissues of the shunt rats was decreased significantly, which suggests that CSE expres-

Fig 4. Electrophoregram of CSE mRNA in the pulmonary tissue of each group. 1–3, control group; 4–6 shunting group. Every lane has 4 bands, of which the 2 former bands are the CSE cDNA (400bp) sample and CSE internal control (361 bp), and the 2 latter bands are -actin cDNA and the -actin internal control. CSE, cystathionine - lyase.

sion is downregulated in PH with pulmonary vascular structural remodeling. We used in situ hybridization for CSE localization and CSE mRNA expression was obviously downregulated in the SMCs of the pulmonary vasculature of shunt rats as compared with control rats. We also found that CSE mRNA was expressed mainly by the SMCs of the PAs, not by the ECs. Summarizing these findings, we found that there was downregulation of the H₂S/CSE pathway in the PAs of rats with PH and pulmonary vascular structural remodeling induced by the high pulmonary blood flow.

Taken together, the evidence to date suggests that downregulation of the endogenous H₂S/CSE pathway might be significant in the development of PH and pulmonary vascular structural remodeling. However, further studies using propargylglycine, an inhibitor of CSE, are needed to clarify the possible role of H₂S/CSE pathway in the pathogenesis of this condition.

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