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Hydrogen Sulfide Up-Regulates Substance P in Polymicrobial Sepsis-Associated Lung Injury

Huili Zhang,* Akhil Hegde,* Siaw Wei Ng,* Sharmila Adhikari,† Shabbir M. Moochhala,‡ and Madhav Bhatia*†

Hydrogen sulfide (H2S) has been shown to induce the activation of neurogenic inflammation especially in normal airways and urinary bladder. However, whether endogenous H2S would regulate sepsis-associated lung inflammation via substance P (SP) and its receptors remains unknown. Therefore, the aim of the study was to investigate the effect of H2S on the pulmonary level of SP in cecal ligation and puncture (CLP)-induced sepsis and its relevance to lung injury. Male Swiss mice or male preprotachykinin-A gene knockout (PPT-A−/−) mice and their wild-type (PPT-A+/+) mice were subjected to CLP-induced sepsis. DL-propargylglycine (50 mg/kg i.p.), an inhibitor of H2S formation was administered either 1 h before or 1 h after the induction of sepsis, while NaHS, an H2S donor, was given at the same time as CLP. L703606, an inhibitor of the neurokinin-1 receptor was given 30 min before CLP. DL-propargylglycine pretreatment or posttreatment significantly decreased the PPT-A gene expression and the production of SP in lung whereas administration of NaHS resulted in a further rise in the pulmonary level of SP in sepsis. PPT-A gene deletion and pretreatment with L703606 prevented H2S from aggravating lung inflammation. In addition, septic mice genetically deficient in PPT-A gene or pretreated with L703606 did not exhibit further increase in lung permeability after injection of NaHS. The present findings show for the first time that in sepsis, H2S up-regulates the generation of SP, which contributes to lung inflammation and lung injury mainly via activation of the neurokinin-1 receptor. *The Journal of Immunology, 2007, 179: 4153–4160.

Neuropeptides, such as substance P (SP),3 which are expressed in and released from airway sensory nerves, contribute to the event of neurogenic inflammation in the respiratory tract (1). Although SP has been described as a peptide of neuronal origin, studies in rodents have demonstrated its production by inflammatory cells such as macrophages, eosinophils, lymphocytes, and dendritic cells (1, 2). Subsequent to its release, SP binds to a family of ubiquitous G protein-coupled receptors, of which the neurokinin-1 receptor (NK1R) has the highest affinity for SP. Activation of NK1R by SP on effector cells elicits local vasodilation and increases microvascular permeability and plasma extravasation, thereby enhancing the delivery and accumulation of leukocytes to injured tissue (1, 2). SP has also been implicated in inducing the release of proinflammatory mediators, enhancing lymphocyte proliferation, and stimulating the chemotaxis of lymphocytes, monocytes, and neutrophils (1, 2). Thus, by promoting vasodilation, extravasation, leukocyte chemotaxis, and cytokine release, SP acts as an important proinflammatory mediator via activation of NK1R in many inflammatory diseases of respiratory, gastrointestinal, and musculoskeletal systems (1–3). For example, it has been shown that blockade of NK1R with CP96345 or genetic deletion of NK1R-protected mice against acute pancreatitis and associated lung injury (4, 5). These data are further substantiated by the findings that deletion of the preprotachykinin-A (PPT-A) gene, the precursor gene for SP, resulted in a remarkable reduction in the severity of acute pancreatitis-associated lung injury (6).

Various clinical cases of sepsis as well as preclinical animal models have been investigated to study the importance of SP in sepsis. The circulating level of SP, which was related to the lethal outcome of sepsis, was significantly elevated in patients with postoperative sepsis (7). Our previous study in mice has shown that the plasma and pulmonary level of SP was significantly increased after induction of sepsis and that genetic deletion of SP greatly attenuated inflammation and damage in the lung (8). Similarly, endotoxin-induced airway inflammation was found to stimulate the release of SP from sensorineuronal terminals (9). These data suggest that SP plays a crucial role in sepsis and associated lung injury. Although an elevated SP level has been found in sepsis-associated lung injury, to date, the underlying mechanism by which the release and production of SP is regulated remains unknown.

Hydrogen sulfide (H2S) has been known for several decades as a toxic gas with the smell of rotten eggs. However, it is also generated endogenously during cysteine metabolism in many types of mammalian cells. This reaction is catalyzed by cystathionine β-synthase (EC4.2.1.22) and cystathionine γ-lyase (CSE; EC4.4.1.1) (10–12). Recent studies have implicated that H2S plays an important role in many physiological and pathological processes. By acting on ATP-dependent K+ channel, endogenous H2S can hyperpolarize cell membranes, relax smooth muscle cells, and therefore regulate blood pressure (11–14). Moreover, by enhancing the sensitivity of N-methyl-D-aspartate receptors to glutamate, H2S can promote hippocampal long-term potentiation and play a

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*Department of Pharmacology, National University of Singapore, Singapore, Singapore; and †Centre for Biomedical Science, Defence Medical and Environmental Research Institute, Defence Science Organization, Singapore, Singapore

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2 Address correspondence and reprint requests to Dr. Madhav Bhatia, Cardiovascular Biology Research Programme, Department of Pharmacology, Centre for Life Sciences, National University of Singapore, 28 Medical Drive, No. 03-02, Singapore 117456. E-mail address: mbhatia@nus.edu.sg

3 Abbreviations used in this paper: SP, substance P; NK1R, neurokinin-1 receptor; H2S, hydrogen sulfide; CSE, cystathionine γ-lyase; CLP, cecal ligation and puncture; PAG, DL-propargylglycine; NK2R, neurokinin-2 receptor; MPO, myeloperoxidase; TRPV1, transient receptor potential vanilloid receptor 1.

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role in neurodegenerative diseases (11, 12, 15). In addition, as a potent vasodilator and atypical neurotransmitter, H2S has also been demonstrated to play a proinflammatory role in various animal models of hind paw edema (16), acute pancreatitis (17), LPS-induced endotoxemia (18), as well as cecal ligation and puncture (CLP)-induced sepsis (19, 20).

Intriguingly, several studies have suggested that H2S may participate in regulating the release of SP. An early study revealed that pulmonary defense against the effect of inhaled toxic gas, such as H2S, was modified by pretreatment with capsaicin, which is known to deplete SP in local sensory nerve terminals (21). Recently, it was shown that neuropeptides including SP were the final mediators of H2S-induced excitatory effects in rat bladders in vitro (22, 23). In another in vitro study, H2S has been reported to induce the tachykinin-mediated neurogenic inflammatory response in guinea pig airways (24). However, all these studies merely investigated tachykinin-mediated neurogenic inflammatory response in guinea pigs (25). In another in vivo study, H2S has been demonstrated to play a proinflammatory role in various animal models of hind paw edema (16), acute pancreatitis (17), LPS-induced sepsis (19, 20).

Measurement of SP levels
Lung samples were homogenized in 1 ml of ice-cold SP assay buffer for 20 s (Bachem; Peninsula Laboratories). The homogenates were centrifuged (13,000 × g, 20 min, 4°C) and the supernatants were collected. They were adsorbed on C18 cartridge columns (Bachem) as described (5, 8). The adsorbed peptide was eluted with 1.5 ml of 75% v/v acetonitrile. The samples were freeze-dried and reconstituted in the SP assay buffer (Bachem; Peninsula Laboratories). SP content in the sample was then determined with an ELISA kit (Bachem; Peninsula Laboratories) according to the manufacturer’s instructions and expressed as nanograms per milliliter. Results were then corrected for the DNA content of the tissue samples (31) and were expressed as nanograms per microgram of DNA.

Semi-quantitative RT-PCR analysis of lung PPT-A, NK1R, and neurokinin-2 receptor (NK2R) mRNA
Total RNA from lung was extracted with TRizol reagent (Invitrogen Life Technologies) according to the manufacturer’s protocol. One microgram of RNA was reverse transcribed using the iScript cDNA Synthesis kit (Bio-Rad). The reaction mixture was first subjected to 95°C for 3 min, followed by an optimal cycle of amplifications, consisting of 95°C for 30 s, optimal annealing temperature, optional cycles, and product sizes were as shown in Table I. PCR amplification was conducted in MyCycler (Bio-Rad). The primer sequences for detection of PPT-A, NK1R, NK2R, and 18S, optimal annealing temperature, optional cycles, and product sizes were as shown in Table I. PCR amplification was conducted in MyCycler (Bio-Rad). The reaction mixture was first subjected to 95°C for 3 min, followed by an optimal cycle of amplifications, consisting of 95°C for 30 s, optimal annealing temperature (Table I) for 30 s and 72°C for 30 s. Final extension was at 72°C for 10 min. PCR products were analyzed on 1.5% w/v agarose gels containing 0.5 μg/ml ethidium bromide.

Measurement of plasma H2S
Aliquots (120 μl) of plasma were mixed with distilled water (100 μl), trichloroacetic acid (10% w/v, 120 μl), zinc acetate (1% w/v, 60 μl), N,N-dimethyl-l-phenylendiamine sulfate (20 μM; 40 μl) in 7.2 M HCl and FeCl3 (30 μM; 40 μl) in 1.2 M HCl in 96-well plates. The absorbance of the resulting solution was measured 10 min thereafter at 670 nm (13, 32, 33). All samples were assayed in duplicate and H2S was calculated against a calibration curve of NaHS (3,125–100 μM). Results were expressed as plasma H2S concentration in micromoles per liter.

Assay of liver H2S-synthesizing activity
Liver H2S-synthesizing activity in liver homogenates was measured essentially as described elsewhere (18). Briefly, liver tissue was homogenized in 100 mM ice-cold potassium phosphate buffer (pH 7.4). The assay mixture contained 100 mM potassium phosphate buffer (pH 7.4), l-cysteine (20 μl, 20 mM), pyridoxal 5'-phosphate (20 μl, 2 mM), salmine (30 μl), and 4.5% w/v tissue homogenate (430 μl). The reaction was performed in tightly sealed microcentrifuge tubes and initiated by transferring the tubes from ice to a water bath at 37°C. After incubation for 30 min, 250 μl of zinc acetate (1%
RESULTS

H₂S affects the pulmonary level of SP in sepsis-associated lung injury

Induction of sepsis by CLP resulted in a significant increase in the pulmonary level of SP as compared with sham operation (Fig. 1, p < 0.01). Next, we examined whether H₂S would have an effect on the level of SP in sepsis-associated lung injury. As shown in Fig. 1, inhibition of endogenous H₂S formation by PAG pre- or posttreatment significantly decreased the pulmonary level of SP whereas administration of NaHS, an H₂S donor, resulted in a further rise in the pulmonary level of SP 8 h after induction of sepsis (p < 0.05).

We also investigated the mRNA level of PPT-A, NK1R, and NK2R in lung by RT-PCR. Densitometric analysis of PCR products on agarose gel showed that pulmonary PPT-A mRNA and NK1R mRNA expression significantly elevated during sepsis (Fig. 2, p < 0.05). However, the NK2R mRNA expression remained unchanged 8 h after CLP (data not shown). Administration of PAG 1 h before or after CLP significantly suppressed the PPT-A mRNA expression in lung (Fig. 2A, p < 0.05), but had no effect on the pulmonary level of NK1R (Fig. 2B, p > 0.05) and NK2R mRNA (data not shown). In contrast, administration of NaHS significantly up-regulated pulmonary gene expression for SP (Fig. 2C, p < 0.05), but not NK1R (Fig. 2D, p > 0.05) and NK2R (data not shown).

PPT-A gene deletion has no effect on endogenous generation of H₂S in sepsis

It is known that CSE (EC 4.4.1.1) is the main H₂S-forming enzyme in the cardiovascular system (11, 12). Therefore, we tested the hepatic activity of CSE and plasma level of H₂S in PPT-A⁻/⁻ mice to determine whether SP would affect the endogenous synthesis of H₂S in sepsis. As shown in Fig. 3, CLP-induced sepsis significantly increased liver CSE activity and plasma level of H₂S in both PPT-A⁻/⁻ and PPT-A⁻/-/⁻ mice (p < 0.01). Furthermore, we noticed that hepatic CSE activity and plasma H₂S level in septic PPT-A⁻/⁻ mice were comparable to those in septic PPT-A⁻/-/⁻ mice. Endogenous synthesis of H₂S after sham operation was also similar in PPT-A⁻/⁻ and PPT-A⁻/-/⁻ mice. These findings suggested that PPT-A gene deletion does not change the CSE activity in mice with or without CLP and that SP has no effect on the production of endogenous H₂S in sepsis.

PPT-A gene deletion prevents H₂S from aggravating lung inflammation in sepsis

PPT-A⁻/-/⁻ mice exhibited an alleviated lung inflammation in sepsis, as characterized by a significant reduction in pulmonary levels of cytokines (TNF-α, IL-1β, IL-6) and chemokines (MIP-1α and MIP-2) as well as lung MPO activity (Fig. 4, p < 0.05, compared
with PPT-A+/+ mice with CLP). This observation is consistent with other studies, suggesting a key role of PPT-A gene in sepsis-associated lung injury (8).

Administration of exogenous H2S in the form of NaHS further increased neutrophil infiltration (MPO activity) and pulmonary levels of TNF-α, IL-1β, IL-6, MIP-1α, and MIP-2 in wild-type mice with CLP operation. However, NaHS intervention failed to aggravate lung inflammation in septic PPT-A−/− mice as the levels of TNF-α, IL-1β, IL-6, MIP-1α, and MIP-2 and MPO activity in lung were comparable to those without NaHS intervention. Therefore, the data indicate that PPT-A gene deletion not only alleviates sepsis-induced lung inflammation but also prevents H2S from aggravating lung inflammation in sepsis.

Exacerbation of lung inflammation by administration of NaHS in sepsis is reversed by pretreatment with NK1R antagonist

Neuropeptide SP binds preferentially to NK1R and elicits inflammatory response at the inflamed sites. Therefore, we examined the effect of NK1R antagonist on sepsis-associated lung injury. Pretreatment with L703606, an NK1R antagonist, reduced the pulmonary levels of proinflammatory mediators (TNF-α, IL-1β, IL-6, MIP-1α, and MIP-2) and lung MPO activity in a dose-dependent manner (Table II). Because L703606 administered at a dose of 4 mg/kg had maximal effect with minimal toxicity, it was selected as the optimal dose.

Pretreatment with L703606 (4 mg/kg) significantly suppressed lung inflammation in CLP-induced sepsis (Fig. 5, p < 0.05). The
treatment also reduced the aggravation of lung inflammation caused by NaHS in sepsis. As shown in Fig. 5, the pulmonary levels of TNF-α, IL-1β, IL-6, MIP-1α, and MIP-2 and lung MPO activity in septic mice with both L703606 pretreatment and NaHS intervention were not significantly different to those with L703606 pretreatment alone.

![FIGURE 4. Alterations in lung MPO activity (A) and pulmonary levels of TNF-α (B), IL-1β (C), IL-6 (D), MIP-1α (E), and MIP-2 (F) in septic PPT-A−/− mice. PPT-A−/− mice and their wild-type PPT-A+/+ mice were randomly given NaHS (10 mg/kg, i.p.) or saline at the same time of CLP operation. Sham-operated mice served as controls. Eight hours after CLP or sham operation, MPO activity and levels of TNF-α, IL-1β, IL-6, MIP-1α, and MIP-2 in lung were measured as described in Materials and Methods. Results shown are the mean ± SEM (n = 10 animals in each group). *, Significant difference (p < 0.05). **, Significant difference (p < 0.01). □, Wild-type mice; ■, PPT-A−/− mice.](http://www.jimmunol.org/)

<table>
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<th>CLP plus L703606</th>
<th>1 mg/kg</th>
<th>2 mg/kg</th>
<th>4 mg/kg</th>
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<td><strong>MPO</strong></td>
<td>1.00 ± 0.11</td>
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<td><strong>TNF-α</strong></td>
<td>925.46 ± 94.76</td>
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<td>3,579.25 ± 191.26</td>
<td>2,949.42 ± 133.30</td>
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<td><strong>IL-1β</strong></td>
<td>754.35 ± 108.98</td>
<td>6,839.70 ± 610.64</td>
<td>5,922.80 ± 636.13</td>
<td>5,806.32 ± 291.89</td>
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<tr>
<td><strong>IL-6</strong></td>
<td>833.09 ± 81.46</td>
<td>7,013.07 ± 708.23</td>
<td>4,720.42 ± 801.67</td>
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<td><strong>MIP-1α</strong></td>
<td>522.25 ± 54.10</td>
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<td>1,412.15 ± 164.06</td>
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<tr>
<td><strong>MIP-2</strong></td>
<td>1,479.11 ± 164.65</td>
<td>11,333.22 ± 827.08</td>
<td>9,316.80 ± 932.67</td>
<td>8,870.59 ± 464.24</td>
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Table II. Effect of L703606 on CLP-induced sepsis

* Mice were randomly given L703606 at doses of 1, 2, 4, 8 mg/kg (i.p.) or vehicle (saline) 30 min before CLP operation. Sham-operated mice served as controls. Eight hours after CLP or sham operation, MPO activity (fold increase over sham group) and levels of TNF-α (picograms per microgram of DNA), IL-1β (picograms per microgram of DNA), IL-6 (picograms per microgram of DNA), MIP-1α (picograms per microgram of DNA), and MIP-2 (picograms per microgram of DNA) in lung were measured as described in Materials and Methods. Results shown are the mean ± SEM (n = 10 animals in each group).

* Value of p < 0.01, CLP vs sham.

* Value of p < 0.01, CLP vs CLP plus L703606 4 mg/kg.

* Value of p < 0.01, CLP vs CLP plus L703606 8 mg/kg.

* Value of p < 0.01, CLP vs CLP plus L703606 2 mg/kg.

* Value of p < 0.05, CLP vs CLP plus L703606 1 mg/kg.

* Value of p < 0.05, CLP vs CLP plus L703606 2 mg/kg.
Genetic deletion of the PPT-A gene and pretreatment with NK1R antagonist alleviates H2S-induced lung injury in sepsis

The clinical pathology of acute lung injury includes increased microvascular permeability and edema with a marked influx of polymorphonuclear leukocytes. Therefore, we used lung microvascular permeability to test the severity of lung injury in sepsis. We found that lung microvascular permeability was significantly increased 8 h after CLP, indicating some tissue damage in the lung (Figs. 6 and 7, *p < 0.05, compared with sham operation). Although CLP resulted in an elevation in lung permeability in both PPT-A+/− and PPT-A−/− mice, an obvious reduction in lung permeability was observed in septic PPT-A−/− mice compared with the wild-type mice (Fig. 6, *p < 0.05). In addition, pretreatment with L703606 (4 mg/kg) significantly decreased lung permeability in sepsis (Fig. 7, **p < 0.01).

NaHS administration caused a further increase in pulmonary permeability in wild-type mice with CLP (Fig. 6, *p < 0.05). However, pulmonary permeability in septic PPT-A−/− mice treated with NaHS was similar to those administered with saline (Fig. 6). Similarly, pretreatment with L703606 prevented NaHS from exacerbating the lung permeability in sepsis. Septic mice pretreated with L703606 did not exhibit further increase in lung permeability after injection of NaHS (Fig. 7, *p < 0.05).
FIGURE 7. Effect of L703606 pretreatment on lung microvascular permeability in sepsis. Mice were randomly given NaHS (10 mg/kg, i.p.) or saline at the time of CLP operation. L703606 (i.p., 4 mg/kg) or vehicle (saline) were randomly given to mice 30 min before CLP. Sham-operated mice served as controls. Eight hours after CLP or sham operation, lung permeability was measured as described in Materials and Methods. Results shown are the mean ± SEM (n = 6 animals in each group). *, Significant difference (p < 0.05). **, Significant difference (p < 0.01).

Discussion

Our previous time-course study of CLP in mice demonstrated that neutrophil infiltration in the lung reached its peak 8 h after the onset of sepsis (34). At that time point, mouse lung sections exhibited characteristic signs of lung injury, such as interstitial edema, alveolar thickening, and severe leukocyte infiltration in the interstitium and alveoli (19). Therefore, the alteration of SP levels in sepsis was evaluated 8 h after CLP. SP has been proposed to be a key mediator in regulating the severity of sepsis and sepsis-associated lung injury in some studies (7, 8). In the present study, we found an obvious increase in pulmonary SP after CLP-induced sepsis and that genetic deletion of the PPT-A gene or pretreatment with an NK1R blocker was able to protect mice against sepsis-associated lung injury. Our findings are consistent with the earlier observations and reinforce the essential role of SP in sepsis-associated lung inflammation and injury. In addition, CLP-induced sepsis is, of course, multifactorial and numerous mediators other than SP are involved. Deletion of PPT-A gene or blockage of NK1R only moderately decreased the pulmonary levels of cytokines and chemokines in sepsis and therefore partially reversed the pathological progression of sepsis.

In contrast, recent studies have shown that synthesis of endogenous H2S dramatically increased in CLP-induced sepsis and endotoxemia (18, 19, 20). The gas appears to play an important role in regulating the severity of systemic inflammation and sepsis-associated multiple organ damage (18–20, 35). Thus, it seems of interest to determine whether endogenous H2S would be correlated to SP in sepsis. In isolated guinea pig airways, NaHS not only provoked the release of SP but also produced a concentration-dependent contractile response. NaHS-induced contractile effects were totally suppressed by the desensitization of capsaicin-sensitive primary afferent neurons by pretreatment with a high concentration of capsaicin or a combination of tachykinin NK1R and NK2R antagonists (24). We have previously reported that i.p. administration of NaHS in normal mice caused a significant rise in the circulatory level of SP in a dose-dependent manner, coupled with obvious lung inflammation (25). Similarly, in rat bladder, H2S at a physiological concentration was capable of stimulating capsaicin-sensitive primary afferent neurons with a consequent release of tachykinin, in turn leading to the contractile response of the smooth muscles (22, 23).

Consistently in the present study, we found that inhibition of H2S formation by PAG pre- or posttreatment significantly decreased the level of PPT-A gene expression and SP in the lung whereas exogenous H2S magnified the pulmonary level of SP in CLP-induced sepsis. In contrast, synthesis of H2S in mice genetically deficient in PPT-A gene was similar to that in wild-type mice, suggesting that SP has no effect on the level of H2S. These data suggest a possibility that H2S is located upstream of SP and plays an important role in regulating the production and release of SP in sepsis. However, it is to be noted that inhibition of H2S formation did not restore the pulmonary level of SP in septic mice to that in sham-operated animals, indicating that the etiology of CLP-induced sepsis is complex and involves the overproduction of SP induced by various mediators other than H2S. In addition, we tested the relation between H2S and NK1R, which was also elevated in sepsis. Unfortunately, we did not see any effect of H2S on NK1R mRNA. Because NK3R is undetectable in lung, we did not test its mRNA level (4).

To further ascertain the role of H2S in neurogenic inflammation in CLP-induced sepsis, we used two different and complementary approaches: PPT-A−/− mice, which are genetically deficient in SP, and L703606, an NK1R antagonist. Both genetic deletion of SP and pretreatment with an NK1R antagonist prevented H2S from further aggravating lung inflammation in sepsis, as evidenced by a nonsignificant alteration in the pulmonary levels of TNF-α, IL-1β, IL-6, MIP-1α, and MIP-2 and lung MPO activity. These data also indicate that the impact of H2S on lung inflammation via SP in lung is mainly mediated by NK1R. In addition, it is to be noted that neither deletion of PPT-A gene nor pretreatment with L703606 could completely abolish the exacerbation of lung inflammation induced by exogenous H2S in sepsis. This observation suggests that in addition to SP, H2S may provoke lung inflammation via other unknown mediators. The possible cascade by which H2S evokes lung inflammation by SP in sepsis is summarized in Fig. 8.

Our findings indicate that overproduced H2S in sepsis seems to up-regulate the pulmonary expression of the PPT-A gene and thereby led to a substantial rise in the production of SP in lung. Although the present study offers the possibility that H2S may
modulate the production of SP at the gene level, the site at which H2S induces the transcription of PPT-A and the transcriptional factors which may participate in this process remain unclear. Two earlier studies have suggested that H2S may regulate the release of SP in guinea pig airways and rat urinary bladder via the transient receptor potential vanilloid receptor 1 (TRPV1) on sensory nerve endings (22–24). Activation of TRPV1 thereby results in an influx of Ca2+ and induces depolarization of nerves and SP release from nerve terminals (36). Similarly, our previous study has also shown that ablation of sensory nerves with capsaicin or pretreatment with capsaazepine, a TRPV1 antagonist, protected mice from H2S-induced lung inflammation (25). In light of the data obtained from the present study and earlier studies, we propose that under different conditions, H2S may regulate the release or production of SP by different pathways, including up-regulating the PPT-A gene and stimulating TRPV1. For example, when the airways and urinary bladder are stimulated with NaHS for a short time, H2S may induce the activation of TRPV-1 (22–25). If H2S persistently exists in an already inflamed site, such as sepsis-induced lung injury, the gas may participate in provoking PPT-A gene expression. Clearly, the precise mechanism by which H2S elicits its effect on SP and thereby contributes to sepsis-associated lung injury remains to be investigated.

In addition, our data also showed that depletion of SP by genetically knocking out the PPT-A gene and blocking the effect of SP by pretreatment with the NK1R inhibitor not only alleviated lung damage caused by sepsis but also decreased lung microvascular permeability impaired by exogenous H2S. This is consistent with the reduced levels of proinflammatory mediators we observed and thus provides more evidence to the veracity of our hypothesis. In conclusion, the present findings show for the first time that H2S up-regulates the generation of SP, which orchestrates the inflammatory response mainly via activation of NK1R, and consequently contributes to lung inflammation and injury in sepsis. The precise pathway involved in this process will be the subject of future study.

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Disclosures
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