Hydrogen Sulfide Causes Relaxation in Mouse Bronchial Smooth Muscle

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Abstract. We investigated the effects of NaHS, a hydrogen sulfide (H₂S) donor, on the tension of isolated mouse and guinea-pig bronchial rings. NaHS at 0.01 – 10 mM had no effect on the tone of those preparations without precontraction. When the preparation was precontracted with carbachol, NaHS at 0.1 – 3 mM strongly relaxed the mouse rings, but produced only slight relaxation in the guinea-pig rings. The NaHS-induced relaxation in the mouse bronchus was resistant to inhibitors of ATP-sensitive K⁺ channels, soluble guanylyl cyclase, cyclooxygenase (COX)-1 or COX-2, and antagonists of tachykinin receptors. Thus, NaHS evokes bronchodilation in mice, suggesting a possible role for H₂S in the respiratory system.

Keywords: hydrogen sulfide (H₂S), smooth muscle, bronchodilation

Hydrogen sulfide (H₂S) is now considered as the third endogenous signaling gasotransmitter, besides nitric oxide and carbon monoxide. Endogenous H₂S is formed from L-cysteine by cystathionine-β-synthase (CBS) and/or cystathionine-γ-lyase (CSE) (1). Apart from its important roles in the neuronal systems (1, 2), one of the main functions of H₂S in the mammalian peripheral tissues is to modulate smooth muscle tone (1). H₂S at relatively high concentrations relaxes vascular smooth muscle, an effect involving both ATP-sensitive K⁺ (K⁺ATP) channel-dependent and -independent components (3 – 5). In contrast, H₂S at low concentrations enhances the vascular tension caused by phenylephrine or high KCl possibly through direct inhibition of endothelial nitric oxide (NO) synthase (5) and/or reaction with NO followed by formation of nitrosothiol (6). H₂S also reduces contractility of the intestinal smooth muscle and vas deferens (7). In contrast, H₂S contracts rat urinary bladder through activation of NK₁ and NK₂ tachykinin receptors by stimulating capsaicin-sensitive primary afferent neurons (8). Similarly, it has recently been reported that H₂S provokes tachykinin-mediated bronchoconstriction accompanied by neurogenic inflammatory responses in the guinea-pig airway (9). However, little is known about roles for H₂S in modulation of airway smooth muscle in other species. In the present study, we therefore investigated the effect of NaHS, known as a donor for H₂S, on the tension of ring preparations of murine bronchial tissues in comparison with guinea-pig bronchial rings.

NaHS, carbachol, L-cysteine hydrochloride, capsaicin, (2S,3S)-3-((3,5-bis(trifluoromethyl) phenyl) methoxy)-2-phenylpiperidine hydrochloride (L-733060), and glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Na₂SO₃ was obtained from Wako Pure Chemicals (Osaka), and Boc-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH₂ (GR83074) was from BACHEM (Bubendorf, Switzerland). 1H-(1,2,4) oxadiazolo-(4,3a) quinoxalin-1-one (ODQ) was provided from Tocris Bioscience (Avonmouth, UK), and 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole (SC-560) and N-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonamide (NS-398) were obtained from Cayman Chemical (Ann Arbor, MI, USA). Capsaicin, glibenclamide, ODQ, and L-733060 were dissolved in DMSO, and all other chemicals were dissolved in distilled water.

Male ddY mice (25 – 35 g) and male Hartley guinea pigs (330 – 350 g) were obtained from Japan SLC Inc. (Shizuoka). All animals were used with approval by the Kinki University School of Pharmacy’s Committee for the Care and Use of Laboratory Animals. The animals were sacrificed by abdominal exsanguination under urethane (1.5 g/kg, i.p.) anesthesia, and the airway tissues were removed. As described previously (10), ring
segments of main bronchi (2 mm in length) were prepared in an ice-cold Krebs-Henseleit solution (composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, and 10 mM glucose, pH 7.4). The ring segments were suspended in micro (mice) or standard (guinea pigs) organ baths containing 2 ml of the Krebs-Henseleit solution maintained at 37°C and gassed with 95% O₂/5% CO₂. The bronchial segments were allowed to equilibrate for 60 min under a resting tension of 2.5 mN (mice) or 15 mN (guinea pigs), and isometric tension was recorded through a force-displacement transducer (UL-10GR; Minebea, Tokyo). The integrity of the ring segment was first monitored by measuring the contractile response to carbachol at 1 µM. Effects of cumulatively applied NaHS, Na₂SO₃, or L-cysteine were observed in the resting preparations and in the preparations precontracted with carbachol at 1 M. Capsaicin at 10 µM was added to the bath three times in total: at 60, 30, and 0 min before precontraction with carbachol or before application of NaHS in resting preparations, in order to desensitize C-fiber sensory neurons. L-733060 (an inhibitor of NK₁ tachykinin receptors, at 10 µM) or GR83074 (an inhibitor of NK₂ tachykinin receptors, at 10 µM) and glibenclamide (an inhibitor of K⁺ATP channels, at 10 µM) were added 20 and 30 min, respectively, before precontraction with carbachol. ODQ (an inhibitor of soluble guanylyl cyclase, at 10 µM) and SC-560 [an inhibitor of cyclooxygenase (COX)-1, at 0.3 µM] or NS-398 (an inhibitor of COX-2, at 10 µM) were applied to the bath solution 5 and 15 min, respectively, before addition of carbachol. The contractile responses to NaHS or Na₂SO₃ in the tissues without precontraction are expressed as a percentage of the contraction induced by carbachol at 1 µM. The relaxant activity of NaHS, Na₂SO₃, or L-cysteine in the preparations precontracted with carbachol is represented as a percentage of the initial tension before addition of NaHS, Na₂SO₃, or L-cysteine. Data indicate the mean ± S.E.M. Statistical analysis was performed by Student’s t-test for unpaired data or by ANOVA followed by Tukey’s test, when applicable. Significance was set at a P<0.05 level.

In the guinea-pig bronchial rings without precontraction, the H₂S donor NaHS, when applied cumulatively in a range of 0.01 – 10 mM, did not cause any contractile responses [Fig. 1: a (top) and b], being inconsistent with the recent report (9). Of note is that application of capsaicin caused contractile responses in the same resting preparations, suggesting the integrity of the endings of capsaicin-sensitive sensory neurons in these tissues (data not shown). In the guinea-pig preparations precontracted with carbachol at 1 µM, cumulative application of NaHS at 0.1 – 10 mM caused slight relaxation responses in a concentration-dependent manner [Fig. 1: a (bottom) and c]. Desensitization of C-fiber sensory neurons by repeated application of capsaicin did not alter the responses to NaHS in the bronchial preparations (Fig. 1: b and c).

In the resting mouse bronchial rings, NaHS produced no contractile activity [Fig. 2: a (left) and b]. However, when the preparations were precontracted with carbachol at 1 µM, NaHS at 0.1 – 3 mM exhibited marked relaxant activity in a concentration-dependent manner.
On the other hand, sulfite salt as Na$_2$SO$_3$, a metabolite of H$_2$S in the mammalian body (11), caused neither contractile nor relaxation responses in the mouse bronchi (Fig. 2: d, e). L-Cysteine, a source of endogenous H$_2$S, did not relax the bronchial preparations, either (Fig. 2e).

The concentration-response curve for the relaxant effect of NaHS in the mouse bronchus was not notably affected by the NK$_1$ tachykinin-receptor antagonist L-733060 at 10 µM, NK$_2$ tachykinin-receptor antagonist GR83074 at 10 µM, K$_{ATP}$-channel inhibitor glibenclamide at 10 µM, soluble guanylyl cyclase inhibitor ODQ at 10 µM, COX-1-selective inhibitor SC-560 at 0.3 µM, and COX-2-selective inhibitor NS-398 at 10 µM (Fig. 3).

The present study, for the first time to our best knowledge, provides evidence that NaHS, an H$_2$S donor, causes strong relaxation in the isolated mouse main bronchus, although the mechanisms underlying the evoked relaxation have yet to be clarified. In contrast, our data show that NaHS only slightly relaxes guinea-pig preparations, suggesting species differences.

To our surprise, NaHS in the concentration range of 0.01 – 10 mM did not cause contractile responses in the guinea-pig main bronchus, while Trevisani et al. (9) most recently reported that NaHS in the same concentration range caused concentration-dependent constriction in the main bronchus of the same species, an effect being abolished by desensitization of sensory neurons with capsaicin and by pretreatment with the combination of NK$_1$ tachykinin- and NK$_2$ tachykinin-receptor antagonists. They also showed that NaHS produced small relaxation responses in the precontracted guinea-pig bronchus after desensitization of sensory neurons with capsaicin (9), which was similar to the small relaxant effect of NaHS in either the control or capsaicin-treated preparation, as shown in our study (see Fig. 1c). Thus, the inconsistency in the present and previous studies is the absence of the effect of NaHS on sensory neurons in our study. Some differences in experimental conditions might be responsible for this discrepancy, for example, strain differences (‘Hartley strain of guinea pigs in our experiments).

Fig. 2. Effect of NaHS and related compounds on tension in mouse bronchial rings. a: Typical recordings of modulation of bronchial tension by NaHS in resting and precontracted preparations. CCh, carbachol; Papa, papaverine. b and c: Contractile or relaxant activity of NaHS in resting (b) and precontracted (c) preparations. d and e: Lack of effect of Na$_2$SO$_3$ (d, e) or L-cysteine (e) on tension in resting (d) and precontracted (e) preparations. It is of note that L-cysteine at 10 mM was inactive in resting preparations (not shown). Data show the mean ± S.E.M. from 4 – 9 experiments.
study’ vs ‘no indication in the previous study’), use of anesthesia (‘urethane in our study for ethical reason’ vs ‘no indication in the previous study’), etc. However, the discrepancy is still open to question.

Reported concentrations of H$_2$S in the mammalian tissues and blood range from 1 to 160 $\mu$M (12, 13). In neutral solution, one-third of NaHS is considered to exist as H$_2$S and the remaining two-thirds are present as HS$^-$ (14). Therefore, effective concentrations of H$_2$S derived from NaHS, as a relaxant, can be roughly estimated to range from 30 – 1000 $\mu$M in mouse bronchus, suggesting physiological and/or pathological roles for endogenous H$_2$S in the mouse tissues.

There is evidence that NaHS might stimulate sensory neurons through transient receptor potential vanilloid-1 (TRPV1)-dependent and/or -independent mechanisms (8, 9). Most recently, we have shown that peripherally applied NaHS directly sensitizes T-type Ca$^{2+}$ channels, leading to hyperalgesia and subsequent excitation of spinal nociceptive neurons (2). In the mouse main bronchus, stimulation of sensory neurons with capsaicin and application of substance P or synthetic NK$_1$-receptor

Fig. 3. Effect of various inhibitors on the relaxation caused by NaHS in precontracted mouse bronchus. NaHS was applied to the preparations precontracted with carbachol at 1 $\mu$M in the presence or absence of each inhibitor. The following inhibitors were employed: the NK$_1$-receptor antagonist L-733060 at 10 $\mu$M (a), NK$_2$-receptor antagonist GR83074 at 10 $\mu$M (b), K$_{ATP}$-channel inhibitor glibenclamide at 10 $\mu$M (c), soluble guanylyl cyclase inhibitor ODQ at 10 $\mu$M (d), and COX-1-selective inhibitor SC-560 at 0.3 $\mu$M and COX-2-selective inhibitor NS-398 at 10 $\mu$M (e). Data show the mean ± S.E.M. from 4 – 21 experiments. *$P<0.05$ vs the control.
agonists are capable of causing bronchodilation through generation of prostanoids (15). Nonetheless, our data from inhibition experiments indicate that the relaxant effects of NaHS were independent of NK₁ or NK₂ tachykinin receptors and of COX-1 or COX-2. Although there is also evidence for involvement of K⁺_{ATP} channels or the NO-cGMP pathway in modulation of smooth muscle tension by H₂S in blood vessels (1, 5, 6), our data also demonstrate that NaHS-evoked relaxation in the mouse bronchial preparations does not involve those two pathways. More in-depth analyses to elucidate the mechanisms for bronchodilation triggered by H₂S are now in progress in our laboratory.

In conclusion, our data suggest that H₂S acts as a bronchodilator in mice.

**References**