Short Communication

Hydrogen Sulfide Causes Relaxation in Mouse Bronchial Smooth Muscle

Satoko Kubo[1], Ichiko Doe[1], Yuko Kurokawa[1], and Atsufumi Kawabata[1,*]

[1]*Division of Pharmacology and Pathophysiology, Kinki University School of Pharmacy,*
3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan

Abstract. We investigated the effects of NaHS, a hydrogen sulfide (H_2S) 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 H_2S in the respiratory system.

Keywords: hydrogen sulfide (H_2S), smooth muscle, bronchodilation

Hydrogen sulfide (H_2S) is now considered as the third endogenous signaling gasotransmitter, besides nitric oxide and carbon monoxide. Endogenous H_2S 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 H_2S in the mammalian peripheral tissues is to modulate smooth muscle tone (1). H_2S 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, H_2S 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). H_2S also reduces contractility of the intestinal smooth muscle and vas deferens (7). In contrast, H_2S contracts rat urinary bladder through activation of NK_1 and NK_2 tachykinin receptors by stimulating capsaicin-sensitive primary afferent neurons (8). Similarly, it has recently been reported that H_2S provokes tachykinin-mediated bronchoconstriction accompanied by neurogenic inflammatory responses in the guinea-pig airway (9). However, little is known about roles for H_2S 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 H_2S, on the tension of ring preparations of murine bronchial tissues in comparison with guinea-pig bronchial rings.

NaHS, carbachol, L-cysteine hydrochloride, capsaicin, (2*S*,3*S*)-3-((3,5-bis(trifluoromethyl) phenyl) methoxy)-2-phenylpiperidine hydrochloride (L-733060), and glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Na_2SO_3 was obtained from Wako Pure Chemicals (Osaka), and Boc-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (GR83074) was from BACHEM (Bubendorf, Switzerland). 1*H*-(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
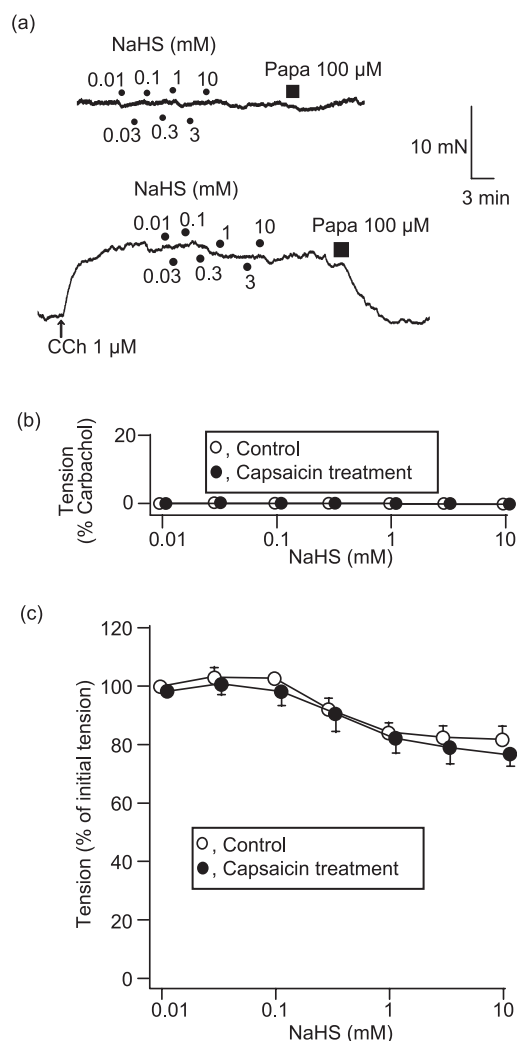

Fig. 1. Effect of NaHS on tension in guinea-pig bronchial rings. a: Typical recordings of modulation of bronchial tension by NaHS in resting and precontracted preparations. CCh, carbachol; Papa, papaverine. b and c: Contractile and relaxant activity of NaHS in preparations after repeated application of capsaicin or vehicle (control). NaHS was applied cumulatively to the preparations without precontraction (b) or precontracted with carbachol at 1 μM (c). Capsaicin at 10 μM was added to the bath three times, at 60, 30, and 0 min, before NaHS (b) or CCh (c). Data show the mean ± S.E.M. from 4 – 9 experiments.

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
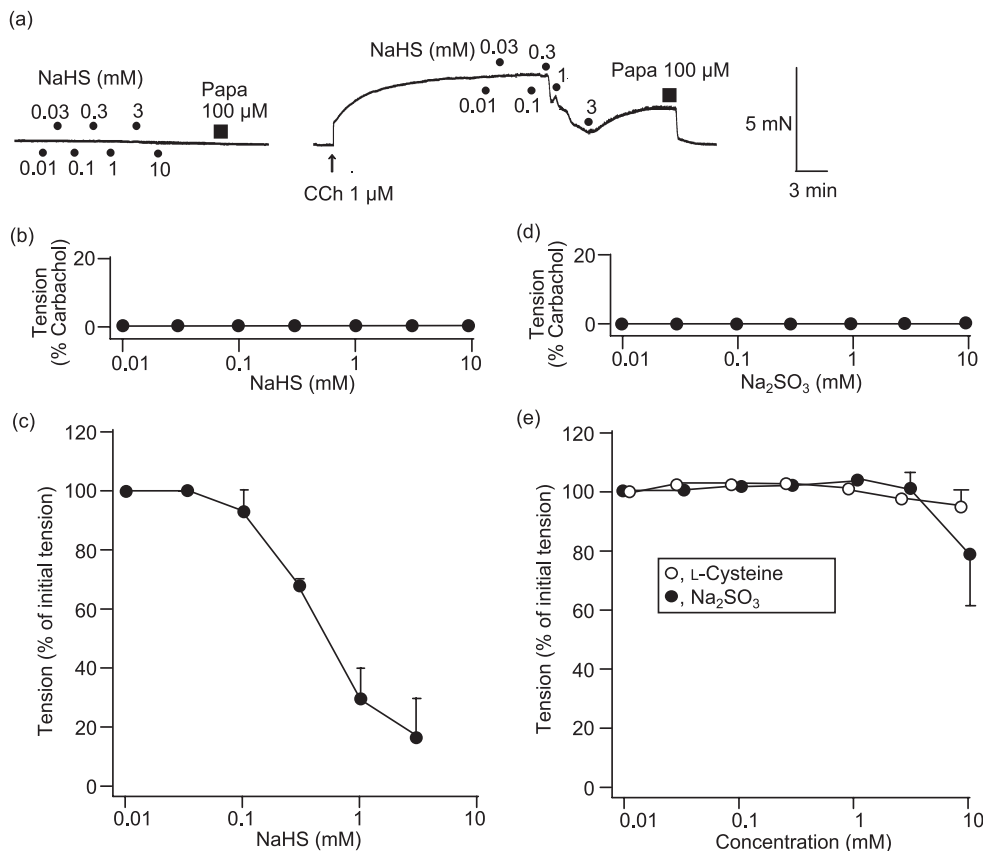
Fig. 2. Effect of NaHS and related compounds on tension in mouse bronchial rings. a: Typical recordings of modulation of bronchial tension by NaHS in resting and precontracted preparations. CCh, carbachol; Papa, papaverine. b and c: Contractile or relaxant activity of NaHS in resting (b) and precontracted (c) preparations. d and e: Lack of effect of Na_2SO_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.

[Fig. 2: a (right) and c]. On the other hand, sulfite salt as Na_2SO_3, a metabolite of H_2S 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_2S, 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_2S 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
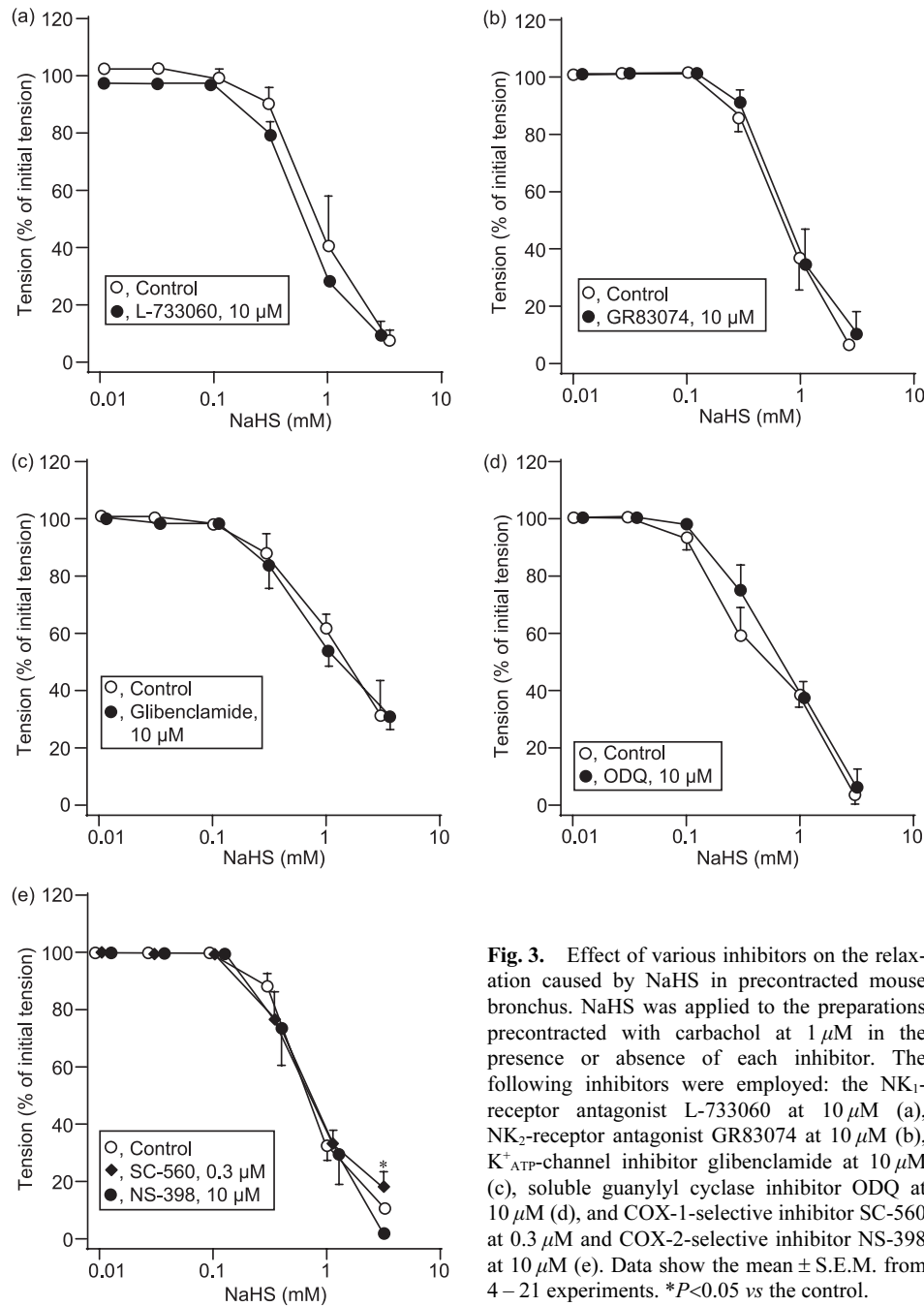
Fig. 3. Effect of various inhibitors on the relaxation caused by NaHS in precontracted mouse bronchus. NaHS was applied to the preparations precontracted with carbachol at $1\,\mu M$ in the presence or absence of each inhibitor. The following inhibitors were employed: the NK_1-receptor antagonist L-733060 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 $\pm$ S.E.M. from $4-21$ experiments. $*P<0.05$ *vs* the control.

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_2S 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_2S and the remaining two-thirds are present as HS^- (14). Therefore, effective concentrations of H_2S derived from NaHS, as a relaxant, can be roughly estimated to range from $30-1000\,\mu M$ in mouse bronchus, suggest-

ing physiological and/or pathological roles for endogenous H_2S 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

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 NK_1 or NK_2 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 H_2S 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 H_2S are now in progress in our laboratory.

In conclusion, our data suggest that H_2S acts as a bronchodilator in mice.

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