INTRODUCTION

Hydrogen sulfide (H\textsubscript{2}S) is enzymatically produced from L-cysteine by certain enzymes including cystathionine-\gamma-lyase (CSE) and cystathionine-\beta-synthase (CBS), and causes excitation of nociceptors mainly via activation of Ca\textsubscript{3.2} T-type Ca\textsuperscript{2+} channels in the peripheral tissue, facilitating somatic and colonic pain. Here, we investigated whether sensory nerves and Ca\textsubscript{3.2} are involved in the H\textsubscript{2}S-induced mucosal cytoprotection against 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats. Colitis was evaluated 3 days after intracolonic (i.c.) TNBS in the rat. Phosphorylation of ERK in the spinal dorsal horn was detected by immunohistochemistry. Protein expression of Ca\textsubscript{3.2} in the dorsal root ganglia (DRG) and of CSE and CBS in the colon was determined by Western blotting. Repeated i.c. NaHS significantly suppressed the TNBS-induced colitis in rats, an effect prevented by ablation of sensory nerves with repeated administration of capsaicin. Repeated pretreatment with T-type Ca\textsuperscript{2+} channel blockers including ethosuximide significantly reduced the protective effects of repeated i.c. NaHS in the rats with TNBS-induced colitis. A single i.c. administration of NaHS induced ethosuximide-sensitive prompt phosphorylation of ERK in the spinal dorsal horn at T13 and L6-S1 levels in the rats 1 day or 3 days after TNBS treatment, but not in naive rats. Ca\textsubscript{3.2} protein was upregulated in DRG 1 day after i.c. TNBS in rats, while CSE, but not CBS, protein was downregulated in the colon. Our findings suggest that luminal H\textsubscript{2}S causes excitation of sensory nerves most probably by activating Ca\textsubscript{3.2} T-type Ca\textsuperscript{2+} channels that are upregulated in the early stage of colitis, leading to colonic mucosal cytoprotection in rats.

Key words: colitis, cytoprotection, hydrogen sulfide, sensory nerve, T-type calcium channel

MATERIALS AND METHODS

Experimental animals

Male Wistar rats weighing 200-400 g were purchased from Japan SLC (Shizuoka, Japan) or Kiwa Laboratory Animals (Wakayama, Japan) and housed in a temperature-controlled (about 24\degree C) room under 12-hour day/night cycles, and had free access to food and water. All experimental protocols were approved by the Committee for the Care and Use of Laboratory Animals at Kinki University and were in accordance with the Guide for the Care and Use of Laboratory Animals Published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).
Creation of 2,4,6-trinitrobenzenesulfonic acid-induced colitis in rats and intracolonic administration of NaHS

As described previously (17), rats fasted for 18 h and were water-deprived for the last 5 h, before intracolonic (i.c.) administration of TNBS. The rats were anesthetized with i.p. injection of sodium pentobarbital (Dainippon Sumitomo Pharmaceutical, Osaka, Japan) at 45 mg/kg, and TNBS at 45 mg/ml in 0.5 ml of 40% ethanol was administered into the colon from the anus through a disposable polytetrafluoroethylene gavage tube with a silicon ball tip (Cat No. 7210, Fuchigami-Kikai, Kyoto, Japan). 110 mm long tube, and 2.1 and 2.9 mm in the external diameter of the tube and silicon ball, respectively) that was inserted 8 cm deep. Using the same gavage tube, NaHS, an H2S donor, at 30 µmol/kg or vehicle in a volume of 1 ml/kg, was administered repeatedly into the colon of unanesthetized rats (8 cm deep), 1 h and 7 h after i.c. TNBS for the first day, and twice a day for the next 3 days (8 i.c. applications for 4 days in total).

Assessment of ulcer and inflammation in rats with 2,4,6-trinitrobenzenesulfonic acid-evoked colitis

The rats were euthanized by decapitation 2 hours after the final dose of NaHS. The colon was excised and incised, and the severity of colitis was evaluated by determining scores of ulcer and inflammation ranging from 0 to 10, as described previously (21). The colonic tissue was cut into 3 pieces, the proximal, middle (the most damaged region) and distal colonic parts, and the thickness of each piece was measured with a caliper. For morphological observation, the colonic tissues were fixed, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Immunohistochemical analysis of phosphorylated ERK in the spinal dorsal horn in rats after intracolonic administration of NaHS

Under anesthesia with sodium pentobarbital (45 mg/kg, i.p.), the rat colon was cannulated, as described above, in the naive rats or in the rats 1 or 3 days after TNBS treatment, and the immunohistochemical analysis of phosphorylation of ERK in the spinal dorsal horn after i.c. administration of NaHS was performed, as reported previously (6, 22). After a 5 min-resting period, NaHS at 30 µmol/kg was administered into the colon. Exactly 5 min after i.c. NaHS, the rats were quickly perfused transcardially with 300 ml of physiological saline. The bilateral dorsal root ganglia (DRG) at T12-L1 and L5-S1 spinal levels, and the middle part of the colon (the most damaged area by TNBS) were excised and frozen in liquid nitrogen. Each sample was homogenized and sonicated in a radioimmunoprecipitation assay (RIPA) buffer (PBS, 1% Igepal CA-630, 0.5% sodium deoxycholate and 0.1% SDS) containing 0.1 mg/ml phenyl methyl sulfonyl fluoride, 0.15 U/ml aprotinin and 1 mM sodium orthovanadate. After centrifugation, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was identified around 43-47 kDa, and 3-mercaptoprotothiol, 10% SDS, 1 M Tris-HCl (pH 6.7) and bromophenol blue were added to the supernatant, and then denatured at 95-100°C for 5 min. The proteins were separated by electrophoresis on a 12.5% gel (for the detection of cystathionine-γ-synthase (CSE), cystathionine-β-synthase (CBS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) or 7.5% (for Ca3.2) sodium dodecylsulfate (SDS)-polyacrylamide gel (Wako Pure Chemicals, Osaka, Japan) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore, Billerica, MA). The membrane was blocked with a blocking solution containing 5% skim milk, 137 mM NaCl, 0.1% Tween 20 and 20 mM Tris-HCl (pH 7.6). After a wash, the membrane was incubated overnight at 4°C with an anti-CSE rabbit polyclonal antibody (1:1000) (custom preparation by Sigma-Aldrich Japan, Ishihara, Japan), an anti-CBS rabbit polyclonal antibody (1:5000) (Abnova, Taipei city, Taiwan), an anti-Ca3.2 rabbit polyclonal antibody (1:1000) (custom preparation by Sigma-Aldrich Japan) or an anti-GAPDH rabbit polyclonal antibody (1:5000) (Santa Cruz Biotechnology, Santa Cruz, CA). After another wash, the membrane was incubated with a horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (1:5000) (Cell Signaling Technology, Beverly, MA) (for the detection of CSE or Ca3.2) or anti-mouse antibody (1:5000) (Cell Signaling Technology) (for CBS), Positive bands for CSE, CBS, Ca3.2 or GAPDH were identified around 43-47, 60, 230 or 37 kDa, respectively, by enhanced chemiluminescence staining (Nacalai Tesque, Inc., Kyoto, Japan). The resulting films were scanned and quantified using densitometric software (Win ROOF, Mitani corporation, Fuku, Japan).

Ablation of sensory nerves by capsaicin and administration of T-type Ca2+ channel blockers

Ablation of C-fiber sensory nerves was performed by administration of capsaicin as described previously (23). Under pentobarbital anesthesia, capsaicin at 25, 50, and 50 mg/kg was administered s.c. to the rat three times, at 0, 6, and 32 hours, respectively (125 mg/kg in total), and the rat was used for experiments 10 days after the last dose. Ethosuximide, a T-type Ca2+ channel blocker, at 50 mg/kg was administered i.p. repeatedly, 45 min before the first dose of i.c. NaHS and 60 min before each of the subsequent administrations. NNC 55-0396, another T-type Ca2+ channel blocker, at 10 mg/kg was administered i.p. 30 min before each dose of i.c. NaHS administrations. In the experiments for immunohistochemical sulfate, and 0.035% hydrogen peroxide for 10 min at 24°C. The sections were then rinsed three times with the Tris-HCl buffer for 10 min. In 5 sections from each spinal segment (T13, L3-L4 or L6-S1) of a rat, the number of pERK-positive cells was determined bilaterally in distinct layers, laminae I-II, III-IV, V-VI, VII-IX and X of the spinal segments at different levels.

Determination of protein levels of major H3-forming enzymes and Ca3.2 T-type Ca2+ channels by Western blotting

The naive rats and the rats 1 day or 3 days after i.c. TNBS were anesthetized with i.p. administration of 1.35 g/kg urethane (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), and were perfused transcardially with 300 ml of physiological saline. The bilateral dorsal root ganglia (DRG) at T12-L1 and L5-S1 spinal levels, and the middle part of the colon (the most damaged area by TNBS) were excised and frozen in liquid nitrogen. Each sample was homogenized and sonicated in a radioimmunoprecipitation assay (RIPA) buffer (PBS, 1% Igepal CA-630, 0.5% sodium deoxycholate and 0.1% SDS) containing 0.1 mg/ml phenyl methyl sulfonyl fluoride, 0.15 U/ml aprotinin and 1 mM sodium orthovanadate. After centrifugation, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was identified around 43-47 kDa, and 3-mercaptoprotothiol, 10% SDS, 1 M Tris-HCl (pH 6.7) and bromophenol blue were added to the supernatant, and then denatured at 95-100°C for 5 min. The proteins were separated by electrophoresis on a 12.5% gel (for the detection of cystathionine-γ-synthase (CSE), cystathionine-β-synthase (CBS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) or 7.5% (for Ca3.2) sodium dodecylsulfate (SDS)-polyacrylamide gel (Wako Pure Chemicals, Osaka, Japan) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore, Billerica, MA). The membrane was blocked with a blocking solution containing 5% skim milk, 137 mM NaCl, 0.1% Tween 20 and 20 mM Tris-HCl (pH 7.6). After a wash, the membrane was incubated overnight at 4°C with an anti-CSE rabbit polyclonal antibody (1:1000) (custom preparation by Sigma-Aldrich Japan, Ishihara, Japan), an anti-CBS rabbit polyclonal antibody (1:5000) (Abnova, Taipei city, Taiwan), an anti-Ca3.2 rabbit polyclonal antibody (1:1000) (custom preparation by Sigma-Aldrich Japan) or an anti-GAPDH rabbit polyclonal antibody (1:5000) (Santa Cruz Biotechnology, Santa Cruz, CA). After another wash, the membrane was incubated with a horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (1:5000) (Cell Signaling Technology, Beverly, MA) (for the detection of CSE or Ca3.2) or anti-mouse antibody (1:5000) (Cell Signaling Technology) (for CBS), Positive bands for CSE, CBS, Ca3.2 or GAPDH were identified around 43-47, 60, 230 or 37 kDa, respectively, by enhanced chemiluminescence staining (Nacalai Tesque, Inc., Kyoto, Japan). The resulting films were scanned and quantified using densitometric software (Win ROOF, Mitani corporation, Fuku, Japan).
detection of p-ERK, ethosuximide at 50 mg/kg was administered i.p. once 60 min before i.c. NaHS.

**Major chemicals**

TNBS was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and NaHS was from Kishida Chemical Co., Ltd., (Osaka, Japan). Capsaicin, ethosuximide and NNC 55-0396 were obtained from Sigma-Aldrich (St. Louis, MO). Capsaicin was dissolved in a saline solution containing 10% Tween 80 and 10% ethanol, while NaHS, ethosuximide and NNC 55-0396 were dissolved in saline.

**Statistical analysis**

Data are presented as the mean with S.E.M. Statistical significance for parametric data was analyzed by Student's t test for comparisons between two groups and by analysis of variance followed by Tukey's test for multiple comparisons. Non-parametric data were analyzed by the Kruskal-Wallis H test followed by a least significant difference (LSD)-type test for multiple comparisons. Significance was set at a level of \( P < 0.05 \).

**RESULTS**

The colonic mucosal cytoprotection caused by intracolonic administration of NaHS is dependent on capsaicin-sensitive sensory nerves in rats with 2,4,6-trinitrobenzenesulfonylic acid-evoked colitis

Treatment with TNBS caused obvious colonic mucosal injury including ulcer, leukocyte infiltration and edema in the mucosa and submucosa in rats, which was attenuated by repeated i.c. administration of NaHS (Figs. 1A, 1B). The increased damage scores and thickness of the colonic wall were significantly reduced by repeated i.c. NaHS (Figs. 1C, 1D). The effect of NaHS on the increased wall thickness was the greatest in the middle part of the colon (Fig. 1D).

Ablation of sensory nerves with repeated administration of capsaicin reduced the protective effect of repeated doses of NaHS on the TNBS-induced colonic mucosal injury, although capsaicin treatment itself did not affect the extent of the colitis (Fig. 2A). The suppressive effects of NaHS on the increased damage score and colonic wall thickness were significantly attenuated by capsaicin treatment (Figs. 2B, 2C).

**Effects of T-type Ca\(^{2+}\) channel blockers on the colonic mucosal cytoprotection caused by intracolonic NaHS in rats with 2,4,6-trinitrobenzenesulfonylic acid-evoked colitis**

Repeated i.p. preadministration of ethosuximide, a T-type Ca\(^{2+}\) channel blocker, at 50 mg/kg, markedly counteracted the protective effects of NaHS (Fig. 3A). The suppressive effects of NaHS on the increased damage score and colonic wall thickness were significantly reduced by ethosuximide (Figs. 3B, 3C). Similarly, NNC 55-0396, another T-type Ca\(^{2+}\) channel blocker known to be highly selective, that inhibits both Cav3.1 and Cav3.2 (5, 24), when repeatedly administered i.p. at 10 mg/kg, significantly \( P < 0.01 \) reduced the suppressive effect of NaHS on the increased colonic wall thickness: the mean±S.E.M (mm) was 0.11±0.02 in the non-TNBS-treated (control) rats receiving i.p. vehicle plus vehicle, and 0.22±0.02, 0.15±0.01 and 0.24±0.02 in the TNBS-treated rats receiving i.p. vehicle plus i.c. vehicle, vehicle plus NaHS, and NNC 55-0396 plus NaHS, respectively (n=6). NNC 55-0396 also tended to counteract the suppressive effect of NaHS.
Fig. 2. Effect of ablation of sensory nerves on the colonic protection caused by NaHS in rats with TNBS-evoked colitis. Ablation of the sensory neurons was achieved by repeated doses of capsaicin (Caps). The rats pretreated with capsaicin or vehicle received NaHS at 30 µmol/kg or vehicle twice daily for 4 days after i.c. TNBS. A: Typical photographs of the colon. B and C: Visible damage of the colon was scored on 0-10 scale (B), and the wall thickness of the middle part of the colon was measured with calipers (C). Data show the mean with S.E.M. for 10-18 rats.

Fig. 3. Effect of ethosuximide, a T-type Ca\(^{2+}\) channel blocker, on the colonic protection caused by NaHS in rats with TNBS-evoked colitis. The rats received NaHS at 30 µmol/kg or vehicle twice daily for 4 days after TNBS. Ethosuximide at 50 mg/kg or vehicle was repeatedly administered i.p. to rats 45-60 min before each dose of i.c. NaHS or vehicle. A: Typical photographs of the colon. B and C: Visible damage of the colon was scored on 0-10 scale (B), and the wall thickness of the middle part of the colon was measured with calipers (C). Data show the mean with S.E.M. for 6-16 rats.

Fig. 4. Typical microphotographs for p-ERK immunostaining in the spinal dorsal horn following i.c. NaHS in naive and TNBS-treated rats. The spinal cord at T13 (A-F), L3-L4 (G-L) or L6-S1 (M-R) levels was perfused, fixed and excised 5 min following i.c. NaHS at 30 µmol/kg in naive rats (A, B, G, H, M and N) and in the rats 1 day (C, D, I, J, O and P) or 3 days (E, F, K, L, Q and R) after TNBS treatment. Scale bars indicate 100 µm.
effect of NaHS on the increased damage score: the mean±S.E.M was 0±0 in the non-TNBS-treated (control) rats receiving i.p. vehicle plus i.c. vehicle, and 6.8±0.6, 4.7±0.4 and 5.7±0.5 in the TNBS-treated rats receiving i.p. vehicle plus i.c. vehicle, vehicle plus NaHS, and NNC 55-0396 plus NaHS, respectively (n=6).

Intracolonic NaHS causes phosphorylation of ERK in the spinal dorsal horn neurons via activation of T-type Ca²⁺ channels in rats with 2,4,6-trinitrobenzenesulfonic acid-evoked colitis

To ask whether colonic luminal H₂S evokes excitation of sensory nerves in rats, we detected p-ERK in the rat spinal dorsal horn following i.c. administration of NaHS. In naive rats, NaHS did not cause phosphorylation of ERK in the spinal dorsal horn at T13, L3-L4 or L6-S1 levels (Figs. 4A, 4B, 4G, 4H, 4M, and 4N), and there was no significant increase in the number of p-ERK-positive cells in any layer of each level of the bilateral spinal dorsal horn in the NaHS-treated rats (data not shown). In the rats 1 day or 3 days after TNBS, however, NaHS caused prompt phosphorylation of ERK in the spinal dorsal horn, particularly at T13 and L6-S1 levels (Figs. 4C, 4D, 4E, 4F, 4O, 4P, 4Q, and 4R). The p-ERK-positive cell number significantly increased mainly in the superficial layers, laminae I-II and/or V-VI, of the bilateral T13, and L6-S1 spinal sections in the rats treated with TNBS.

**Fig. 5.** The number of p-ERK-positive cells in the spinal cord following NaHS in TNBS-treated rats. The spinal cord was transcardially perfused and fixed 5 min following NaHS at 30 µmol/kg in the rats 1 day or 3 days after TNBS treatment. The p-ERK-positive cells were counted in laminae I-II, III-IV, V-VI, VII-IX and X of the bilateral spinal cord at T13, L3-L4 and L6-S1 levels, and the total number in laminae I-X was calculated. Data indicate the mean with S.E.M. for 20-25 slices from 4-5 rats.

**Fig. 6.** Effects of ethosuximide on the NaHS-induced increase in the number of p-ERK-positive cells in the spinal cord of the TNBS-treated rats. Ethosuximide (Etho) at 50 mg/kg or vehicle (V) was administered i.p. 1 h before i.c. NaHS at 30 µmol/kg or vehicle (V) in rats 1 day after TNBS treatment. The spinal cord was transcardially perfused and fixed 5 min after i.c. NaHS. Data indicate the mean with S.E.M. for 30-40 slices from 6-8 rats.
Our data suggest that the colonic mucosal cytoprotection caused by luminal H$_2$S in rats with TNBS-induced colitis involves excitation of capsaicin-sensitive sensory nerves and activation of T-type Ca$^{2+}$ channels in rats, being consistent the previous evidence that colonic luminal H$_2$S stimulates nociceptors and causes visceral pain through activation of Cav3.2 T-type Ca$^{2+}$ channels in mice (6, 25). The T-type Ca$^{2+}$-channel-dependent neural activation by luminal H$_2$S in the early stage of colitis is supported by the T-type Ca$^{2+}$-channel-sensitive phosphorylation of ERK in the spinal dorsal horn following i.c. NaHS. The upregulation of Cav3.2 protein in DRG in the early stage of colitis is in agreement with the finding that the luminal NaHS caused spinal ERK phosphorylation in the TNBS-treated rats, but not naive rats. Together, we propose that luminal H$_2$S causes excitation of sensory nerves most probably by activating Cav3.2 T-type Ca$^{2+}$ channels that are upregulated in the early stage of colitis, leading to colonic mucosal cytoprotection against colitis.

Wallace et al. (17) have suggested that the protective effect of NaHS involves K$_{ATP}$ channels, since glibenclamide, a K$_{ATP}$ channel inhibitor, increased the mortality in TNBS-treated rats. Nonetheless, the involvement of K$_{ATP}$ channels is still controversial, because their study has not demonstrated if glibenclamide inhibits the protective effect of NaHS. Given that pinacidil, a K$_{ATP}$ channel agonist, did not suppress the severity of TNBS-evoked colitis, in their study, it is likely that endogenous and exogenous H$_2$S might target K$_{ATP}$ channels and Cav3.2 T-type Ca$^{2+}$ channels, respectively, contributing to colonic mucosal cytoprotection. It is still open to question whether TRPA1, another target for H$_2$S, expressed in sensory nerves contributes to the protective effect of NaHS in the TNBS-treated rats. In particular, it is interesting to ask if inhibition of TRPA1 reduces the NaHS-induced phosphorylation of ERK in the spinal cord at L6-S1 levels in TNBS-treated rats, which was resistant to inhibition of T-type Ca$^{2+}$ channels in the present study (Fig. 6). However, it is to be noted that sensitization of TRPA1 channels rather accelerates the TNBS-induced colitis in mice (26). Apart from the luminal H$_2$S-induced neuronal excitation in the rats with colitis, NaHS failed to cause phosphorylation of ERK in the spinal dorsal horn of the naive rats (see Fig. 4), differing from our previous study in where NaHS caused prompt phosphorylation of spinal ERK in naive mice (6, 25). It is thus likely that Cav3.2 expressed in the sensory nerve ending in the naive rat colon may not be as abundant as that in the naive mouse colon.

The downregulation of CSE, but not CBS, protein in the colon 1 day after TNBS treatment (Fig. 7) may suggest decreased production of endogenous H$_2$S. This finding may be associated with the pathogenesis of inflammatory bowel diseases in humans, and therefore, it would be important to examine if CSE is actually downregulated in the colonic biopsy from patients with ulcerative colitis and/or Crohn's disease. Nonetheless, Wallace et al. (17) have shown that the generation of H$_2$S by CSE and/or CBS, greatly increase in the tissue homogenate of the colon isolated from rats with TNBS-colitis. Thus, the physiological significance of the downregulation of CSE in the rat colon after TNBS treatment observed in the present study remains to be investigated.

There is a growing evidence for the protective role of capsaicin-sensitive sensory nerves in the gastrointestinal tract

### DISCUSSION

**Fig. 7.** Effects of TNBS treatment on the expression levels of Cav3.2 in rat DRG and of H$_2$S-forming enzymes in rat colon. Expression of Cav3.2 protein in bilateral DRG at T12-L1 or L5-S1 levels (A) or of CSE and CBS protein in the middle part of the colon (B) from the rats 1 day after TNBS treatment or the control rats. Typical photographs of Western blotting are shown in the top, and the quantified data by densitometry are in the bottom. Data show the mean with S.E.M. for 4-5 rats.

**Fig. 5.** NaHS-induced increases in the total number of p-ERK-positive cells over all layers were significant in both T13 and L6-S1 spinal sections 1 day after TNBS and in only T13 sections 3 days after TNBS (Fig. 5). Ethosuximide, a T-type Ca$^{2+}$ channel blocker, preadministered i.p. at 50 mg/kg, significantly prevented the NaHS-induced increase in the p-ERK-positive cell numbers in laminae I-II, III-IV and V-VI, of the bilateral spinal dorsal horn at T13 levels in the rats 1 day after TNBS treatment (Fig. 6). Nonetheless, ethosuximide did not reduce the increases in the number of spinal p-ERK-positive cells at L6-S1 levels following NaHS in the TNBS-treated rats (Fig. 6).

**Protein expression levels of Cav3.2 T-type Ca$^{2+}$ channels in the DRG and major H$_2$S-forming enzymes in the colonic tissue obtained from 2,4,6-trinitrobenzenesulfonic acid-treated rats**

Expression of Cav3.2 protein in DRG at T12-L1 and L5-S1 levels were upregulated in the rats 1 day after TNBS treatment (**Fig. 7A**). In contrast, expression of CSE, but not CBS, protein in the colonic tissue, was downregulated in the TNBS-treated rats (**Fig. 7B**).

**Table 1.**

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<thead>
<tr>
<th>Condition</th>
<th>DRG T12-L1</th>
<th>DRG L5-S1</th>
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<td>Control TNBS</td>
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### Protein expression levels of Cav3.2 T-type Ca$^{2+}$ channels in the DRG and major H$_2$S-forming enzymes in the colonic tissue obtained from 2,4,6-trinitrobenzenesulfonic acid-treated rats

Expression of Cav3.2 protein in DRG at T12-L1 and L5-S1 levels were upregulated in the rats 1 day after TNBS treatment

(Fig. 7A). In contrast, expression of CSE, but not CBS, protein in the colonic tissue, was downregulated in the TNBS-treated rats (Fig. 7B).
(18, 19, 23, 27). Several reports have shown that calcitonin gene-related peptide (CGRP) released from the capsaicin-sensitive sensory nerves may play a protective role during the gastrointestinal inflammation including TNBS-induced colitis (23, 27, 28). The released CGRP may increase mucus secretion and mucosal blood flow in the gastrointestinal tract, contributing to the mucosal cytoprotection (23, 29, 30, 31). The involvement of neuropeptides including CGRP in the protective effect of NaHS has yet to be investigated.

Our study thus implies that Ca3.2 T-type Ca2+ channel-dependent excitation of sensory nerves mediates the protective effects of luminal H2S in the colon during inflammation. A similar mechanism may also be involved in the protective or anti-inflammatory role played by H2S in various organs other than the colon (32).

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Conflict of interests: None declared.

REFERENCES

