Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats

Youqin Cheng, Joseph Fomusi Ndisang, Guanghua Tang, Kun Cao, and Rui Wang

Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5E5

Submitted 6 April 2004; accepted in final form 4 June 2004

Cheng, Youqin, Joseph Fomusi Ndisang, Guanghua Tang, Kun Cao, and Rui Wang. Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. Am J Physiol Heart Circ Physiol 287: H2316–H2323, 2004. First published June 10, 2004; doi:10.1152/ajpheart.00331.2004.—Hydrogen sulfide (H2S) has been shown recently to function as an important gasotransmitter. The present study investigated the vascular effects of H2S, both exogenously applied and endogenously generated, on resistance mesenteric arteries of rats and the underlying mechanisms. Both H2S and NaHS evoked concentration-dependent relaxation of in vitro perfused rat mesenteric artery beds (MAB). The sensitivity of MAB to H2S (EC50, 25.2 ± 3.6 μM) was about fivefold higher than that of rat aortic tissues. Removal of endothelium or coapplication of charybdotoxin and apamin to endothelium-intact MAB significantly reduced the vasorelaxation effects of H2S. The H2S-induced relaxation of MAB was partially mediated by ATP-sensitive K+ (KATP) channel activity in vascular smooth muscle cells. Pinacidil (EC50, 1.7 ± 0.1 μM, n = 6) mimicked, but glibenclamide (10 μM, n = 6) suppressed, the vasorelaxant effect of H2S. KATP channel currents in isolated mesenteric artery smooth muscle cells were significantly augmented by H2S. L-Cysteine, a substrate of cystathionine-γ-lyase (CSE), at 1 mM increased endogenous H2S production by sixfold in rat mesenteric artery tissues and decreased contractility of MAB. dl-Proparglycine (a blocker of CSE) at 10 μM abolished l-cysteine-dependent increase in H2S production and relaxation of MAB. Our results demonstrated a tissue-specific relaxant response of resistance arteries to H2S. The stimulation of KATP channels in vascular smooth muscle cells and charybdotoxin/apamin-sensitive K+ channels in vascular endothelium by H2S represents important cellular mechanisms for H2S effect on MAB. Our study also demonstrated that endogenous CSE can generate sufficient H2S from exogenous l-cysteine to cause vasodilatation. Future studies are merited to investigate direct contribution of endogenous H2S to regulation of vascular tone.

HYDROGEN SULFIDE (H2S) has traditionally been known for its toxic effects on living organisms for more than 200 years (21). This dogma has been challenged recently by accumulating evidence that H2S is a gasotransmitter endowed with biological and physiological functions (29). Mammalian cells possess two pyridoxal-5′-phosphate-dependent enzymes that catalyze the generation of H2S from l-cysteine (3, 24, 25). The expression of these two enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), is tissue type specific (29). The expression of CSE mRNA and endogenous production of H2S in vascular tissues have been shown previously (9, 33). Relaxation of rat portal vein and aortic tissues by NaHS has also been documented (9, 32, 33). The vascular effect of H2S is dependent on extracellular calcium entry but independent of the activation of cGMP pathway in aorta tissues (32). The opening of ATP-sensitive K+ (KATP) channels in vascular smooth muscle cells (SMCs) by H2S potentially underlies H2S-induced vasorelaxation (29, 32, 33). Zhao et al. (33) have previously demonstrated that intravenous bolus injection of H2S transiently decreased blood pressure of rats by 12–30 mmHg, an effect antagonized by the KATP channel blocker glibenclamide. This hypotensive effect of H2S may not be readily linked to the H2S-induced relaxation of the aorta (33) or portal vein (9) as it is peripheral resistance arteries that determine peripheral resistance to blood flow and thus to blood pressure. To date, the effect of H2S on peripheral resistance arteries has been unclear. Interestingly, it has been reported that H2S-induced vasorelaxation is much more potent in the portal vein than in the aorta. NaHS at 1 mM relaxes rat aortic tissue by 30%, whereas at the same concentration it evokes 100% relaxation of the portal vein (9). In our preliminary unpublished study, a significant endothelium-dependent effect of H2S on the rat mesenteric artery was observed. In contrast, relaxation of the rat aorta induced by H2S was only weakly mediated by endothelium (32, 33). These observations, together with the known heterogeneity of vascular tissues regarding their structure, functions, and pharmacological sensitivities, promoted us to further investigate the effect of H2S on the contractility of peripheral resistance arteries, such as the rat mesenteric artery bed (MAB), as well as the underlying mechanisms. Whether endogenous H2S relaxes vascular tissues as exogenous H2S does has been unknown. The vasorelaxant effect of endogenous H2S was therefore also examined in rat MAB in the present study.

MATERIALS AND METHODS

Preparation of in vitro perfused MAB. Male Sprague-Dawley (SD) rats (8–12 wk) weighing 250–400 g were anesthetized with pentobarbital sodium (60 mg/kg) intraperitoneally. In total, 87 rats were used in this study. The Animal Care and Use Committee of the University of Saskatchewan approved the animal use protocol for this study.

Through an abdominal midline incision, the superior mesenteric artery was cannulated near its origin in the aorta. The ileocolonic, colic, and pancreaticoduodenal branches of the superior mesenteric artery were tied off, and the gut was removed. The isolated MAB was mounted to a perfusion apparatus and perfused with Krebs bicarbonate solution containing (in mM) 115.0 NaCl, 5.4 KCl, 1.2 MgSO4, 1.2 NaH2PO4, 25.0 NaHCO3, 1.8 CaCl2, and 11.0 glucose (30). The MAB perfusate “flowed out from the cut ends of the vessels on the intestinal margin of the mesentery but some flowed out from the cut end of the superior mesenteric vein” (14). Flow rate was maintained at 4 ml/min by a peristaltic pump (model 77120-62, MASTER FLEX; Barrington, IL). Krebs solution was bubbled with a 5% CO2-95% O2 gas mixture (pH 7.4) and maintained at a temperature of 37°C. The perfusion pressure was continuously monitored by means...
of a pressure transducer coupled to a Biopac System (Biopac Systems). A period of 60 min to stabilize baseline perfusion pressure was allowed for all experiments. The dose-dependent effect of phenylephrine (PE) under the present experimental conditions was examined first, which indicated that at 100 μM PE induced submaximal contraction of the perfused MAB. Thus MAB was stimulated with PE at 100 μM three times, each lasting for 1 min, to sensitize tissues. MAB was then preconstricted with PE (100 μM) to maintain a stable perfusion pressure ranging from 100 to 130 mmHg. In different experiments, different compounds, such as glibenclamide, charybdotoxin (ChTX), apamin, or N-acetylcycteine (NAC), were included in the perfusion solution together with PE to pretreat MAB. The changes in perfusion pressure were evaluated, taking the stable level of perfusion pressure in the presence of PE (100 μM) as 100%. In the experiments with endothelium-free MAB preparations, endothelium removal was achieved by perfusing MAB with 0.5% saponin for 40 s (15, 19) using a syringe pump (74900 series, Cole-Palmer). Thereafter, the preparation was allowed to equilibrate for 2–3 h to return to the original baseline. Endothelium removal was verified by the lack of relaxant effect of 1 μM acetylcholine. Saponin treatment did not alter the PE-induced vasconstriction or sodium nitroprusside (SNP; 100 μM)-induced relaxation of MAB.

Measurement of endogenous H2S production in MAB pretreated with PE. The experiment consisted of three groups, with each group having five homogenized MAB preparations. MAB in group A were incubated with l-cysteine (1 mM). MAB in group B were treated with both dl-propargylglycine (PPG; 10 μM) and l-cysteine (1 mM). As a control, MAB in group C were incubated with Krebs solution. The incubation period for all tissues was 90 min in a 37°C water bath. The measurement of H2S production rate in these three groups of MAB followed the established protocol (24), which has been routinely used in our laboratory (31–33). In brief, isolated rat mesenteric artery tissues were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 6.8), and the whole homogenate was added to the center wells, which each contained 0.5 ml of 1% zinc acetylthioacetate as trapping solution. The flashes containing reaction mixture and center wells were flushed with N2 before being sealed. The reaction was initiated by transferring the flashes from ice to a 37°C shaking water bath. After incubation at 37°C for 90 min, 0.5 ml of 50% trichloroacetic acid were added into the reaction mixture to stop the reaction. The contents of the center tubes were finally transferred to test tubes, which each contained 3.5 ml of water. Then, 0.5 ml of 20 mM N,N-dimethyl-p-phenylenediamine sulphate in 7.2 M HCl and 0.4 ml of 30 mM FeCl3 in 1.2 M HCl were added. The absorbance of the resulting solution at 670 nm was measured with a spectrophotometer.

SMC preparation. Single mesenteric artery SMCs were isolated from male SD rats according to our previously published method (11, 33). Briefly, small mesenteric arteries below the second branch off the main mesenteric artery were dissected. The freshly isolated tissues were cut into 5 mm long pieces and then digested with 0.2 mg/ml collagenase and 0.8 mg/ml hyaluronidase. Single cells released by gentle triturating through a Pasteur pipette exhibited a long and spindle shape. Cells were stored at 4°C and used within the same day of isolation.

Recording of whole cell KATP channel currents. The whole cell patch-clamp technique was used to record KATP channel currents (26, 33). Briefly, two to three drops of cell suspension were added to the perfusion chamber inside a petri dish that was mounted on the stage of an inverted phase-contrast microscope (Olympus IX70). Cells were left to stick to the glass coverslip in the experimental chamber for 5–10 min before an experiment was started. Pipettes were pulled from soft microhemocrit capillary tubes (Fisher; Nepean, Ontario, Canada) with a tip resistance of 2–4 MΩ when filled with the pipette solution. Whole cell currents were recorded with an Axopatch 200-B amplifier (Axon Instruments) controlled by a Digidata 1200 interface and pCLAMP software (version 7, Axon Instruments). Membrane currents were filtered at 1 kHz with a four-pole Bessel filter, digitized, and stored. At the beginning of each experiment, junction potential between the pipette and bath solutions was electronically adjusted to zero. Test pulses were made with a 10-mV increment from −80 to +70 mV with a holding potential of −60 mV. A 600-ms test pulse to different membrane potential was applied every 10 s. All experiments were conducted at room temperature (20–22°C).

The bath solution for recording KATP current contained (in mM) 140 NaCl, 5.4 KCl, 1.2 MgCl2, 10 HEPES, 1 EGTA, and 10 glucose (pH adjusted to 7.4 with NaOH). The pipette solution was composed of (in mM) 140 KCl, 1 MgCl2, 10 EGTA, 10 Hepes, 5 glucose, 0.3 Na3-ATP, and 1 GDP (pH adjusted to 7.2 with KOH). Na3-ATP and GDP were directly dissolved in the pipette solution to achieve the desired concentrations on the day of experiments. The cells were perfused continuously with the bath solution at a rate of about 2 ml/min. A complete solution change in the recording chamber was accomplished within 30 s. The absence of Ca2+ in the bath and pipette solutions and the inclusion of 1 and 10 mM EGTA in the bath and pipette solutions, respectively, minimized the involvement of Ca2+-activated K+ (KCa) currents.

Protein preparations and Western blotting. Various tissues (300 mg each) from SD rats were homogenized using a Polytron homogenizer with 0.5 ml of Tris-EDTA solution containing EDTA (0.5 M), Tris-Cl (1 M, pH 7.4), sucrose (0.3 M), antipain hydrochloride (1 μg/ml), benzamidine hydrochloride hydrate (1 mmol/ml), leupeptin hemisulfate (1 μg/ml), pepstatin A (1 μg/ml), 1,10-phenanthroline monohydrate (1 mmol/ml), phenylmethylsulfonyl fluoride (0.1 mmol/ml), and iodoacetamide (1 μg/ml). Supernatants containing crude cellular proteins were collected by centrifugation at 12,000 rpm for 15 min and used for Western blotting studies. Briefly, samples were denatured in a 4X denaturing buffer at 95°C for 5 min before electrophoresis on a 10% SDS-polyacrylamide gel. Protein samples separated by electrophoresis were transferred onto a nitrocellulose membrane. The membrane was blocked with 3% milk powder solution at 4°C overnight and rinsed three times with PBS before being incubated with a monoclonal anti-CSE antibody (1:500), which was provided by Dr. N. Nishi at Kagawa Medical School, at room temperature on a shaker for 2 h (17, 18). After incubation, the membrane was washed three times with blocking solution (5 min/4°C) and then incubated with a secondary goat anti-mouse IgG antibody (1:5,000, Sigma) for 2 h at room temperature on a shaker. Finally, the membrane was washed consecutively with blocking solution and PBS three times followed by an enhanced chemiluminescence assay (Perkin Elmer) to detect positive bands. Anti-β-actin monoclonal antibody (Sigma) was used as a housekeeping control.

Chemical treatments and statistical analysis. The H2S-saturated solution (0.09 M) was made by bubbling pure H2S gas (Praxair; Mississauga, Ontario, Canada) into Krebs solution at 30°C for 40 min as described previously (32, 33). Both the H2S gas-saturated solution and NaHS solution (Sigma) were freshly prepared on the day of the experiment. At 37°C and pH 7.4, the concentration of H2S solution was relatively stable (33). The H2S concentration was calculated according to the calibration curve of the standard H2S solutions.

Pinacidil, GDP, and ATP were purchased from Sigma. Glibenclamide was from Research Biochemicals (Natick, MA). PE stock solutions (1 M) were made in distilled water and stored at −20°C until use. Pinacidil and glibenclamide were initially dissolved in DMSO. The final concentration of DMSO in the Krebs solution was 0.01% (vol/vol), which did not influence vascular responses (8). NAC was dissolved in distilled water and then diluted with Krebs solution to achieve the desired final concentration.

All concentration-effect curves were fitted with nonlinear regression using a computerized curve-fitting software (Microcal Origin, version 5.0, Microical Software; Northampton, MA) to obtain EC50. Data are expressed as means ± SE. A paired Student’s t-test was used to compare changes in the same MAB preparation before and after different treatments. Multiple comparisons were made with one-way
ANOVA followed by a post hoc analysis (Tukey test). Statistical significance was set at $P < 0.05$.

RESULTS

Characterization of H$_2$S- and NaHS-induced vasorelaxation of PE-precontracted MAB. The effects of H$_2$S or NaHS on PE-precontracted MAB were examined in seven concentration increments from 0.01 $\mu$M to 10 mM. Between each application of H$_2$S or NaHS at different concentrations was a H$_2$S- or NaHS-free perfusion interval of 8–10 min. Our previous study has demonstrated that the concentrations of H$_2$S in H$_2$S gas-bubbled solution and NaHS solution are similar (Fig. 1 in Ref. 31). In the present study, it was found that the vasorelaxant effects of H$_2$S and NaHS were similar at concentrations lower than 1 $\mu$M (Fig. 1A). Relaxation of MAB by H$_2$S manifested itself in two different phases. Low concentrations of H$_2$S (below 1 $\mu$M) and NaHS (below 30 $\mu$M) relaxed MAB about 20%. Once the concentrations of H$_2$S and NaHS increased to 10–100 $\mu$M, a greater extent of relaxation (20–90%) was observed (second phase reaction). On the basis of this second phase of MAB reaction to H$_2$S, an EC$_{50}$ of 25.2 ± 3.6 $\mu$M for the H$_2$S effect was derived ($n = 6$), which was significantly lower than the vasorelaxant effect of H$_2$S on PE-precontracted rat aortic tissues (EC$_{50}$ 125 ± 14 $\mu$M, $P < 0.05$) (3). NaHS also relaxed the precontracted MAB but with a significant lower potency (EC$_{50}$ 103.7 ± 10.0 $\mu$M, $n = 6$) than that of H$_2$S (Fig. 1B).

To test whether the vascular effect of H$_2$S was mediated by endothelium, endothelium was removed from the isolated MAB. In the absence of a functional endothelium, vasorelaxation induced by H$_2$S was significantly weakened (Fig. 2A). For example, at the concentration of 100 $\mu$M, H$_2$S relaxed the precontracted MAB in the presence or absence of endothelium by 84.1 ± 6.5 or 39.3 ± 4.0% ($P < 0.01$). The endothelium dependence of the H$_2$S effect was not apparent at lower concentration of H$_2$S (<10 $\mu$M). The H$_2$S concentration-response curve shifted to the right in the absence of endothelium with EC$_{50}$ changed from 25.2 ± 3.6 $\mu$M in the presence of endothelium to 160.8 ± 8.6 $\mu$M ($n = 6$, $P < 0.01$).

The possibility that H$_2$S stimulated the release of EDHF from endothelium was tested by pretreating endothelium-intact...
H2S-INDUCED RELAXATION OF MESENTERIC ARTERY

MAB with ChTX and apamin for 30 min. This treatment significantly decreased the vasorelaxant effects of H2S with EC50 changed from 22.5 ± 1.1 to 141.9 ± 21.5 μM, similar to the change induced by endothelium removal (n = 4; Fig. 2B). However, once endothelium was removed, the vasorelaxant effect of H2S on endothelium-free MAB was not altered by the coapplication of ChTX and apamin (n = 3, P > 0.05; not shown).

Mediation by KATP channels of H2S-induced vasorelaxation of MAB. Because the relaxant effect of H2S on rat aortic tissues was mainly mediated by KATP channels (32, 33), we compared the effect of pinacidil, a KATP channel opener, on the PE-precontracted MAB with that of H2S. The concentration-response curve of pinacidil was similar to that of H2S. However, the potency of pinacidil (EC50 1.7 ± 0.1 μM, n = 6) was significantly greater than that of H2S (P < 0.05). Glibenclamide, an inhibitor of KATP channels, was used to pretreat MAB for 30 min. In the presence of glibenclamide (10 μM) (6), the pinacidil-induced vasorelaxation was significantly reduced with EC50 changed to 12.3 ± 0.2 μM (n = 6). Pretreatment of MAB with glibenclamide (10 μM) also shifted the H2S concentration-response curve to the right compared with that of H2S in the absence of glibenclamide. The EC50 of the vasorelaxant effects of H2S on endothelium-intact MAB was changed after pretreatment with 10 μM glibenclamide from 25.3 ± 10.4 to 54.2 ± 9.6 μM (n = 4; Fig. 3A). However, glibenclamide (10 μM) did not alter the acetylcholine-induced relaxation of the PE-preconstricted endothelium-intact MAB. For example, acetylcholine at 0.1 μM evoked 94.5 ± 2.5% (n = 6) or 93.2 ± 3.1% (n = 6) relaxations of endothelium-intact MAB in the absence or presence of glibenclamide, respectively. In the absence of endothelium, the vasorelaxant effect of H2S at concentrations lower than 1 mM was abolished by glibenclamide pretreatment (Fig. 3B). Glibenclamide treatment did not affect the vasorelaxant effect of H2S on endothelium-free MAB at concentrations higher than 1 mM.

Effect of NAC on MAB relaxation induced by H2S. After MAB was pretreated with NAC (0.6 mM) for 30 min, the vasorelaxant effect of H2S was tested again. NAC treatment did not cause a significant shift of the concentration-response curve of H2S (Fig. 4A) with an EC50 of 13.0 ± 10.3 μM (n = 6, P > 0.05 vs. that of H2S in the absence of NAC).

Vasorelaxant effect of endogenous H2S on MAB. l-Cysteine is a substrate and inducer of CSE that is responsible for producing H2S in mesenteric artery SMCs. l-Cysteine (0.01 μM to 10 mM) was applied to the PE-precontracted MAB for 30 min at each concentration (n = 6). l-Cysteine induced insignificant 2–6% relaxation at low concentrations from 0.01 to 100 μM (Fig. 4B). When the concentrations of l-cysteine were increased to 1 and 10 mM, 30% and 80.1% vasorelaxations of MAB, respectively, were achieved (EC50 1.2 mM). To verify whether the vasorelaxant effect of l-cysteine was due to increased endogenous H2S production, MAB was treated with PPG, an irreversible inhibitor of CSE (31). Coapplication of PPG (10 μM) with l-cysteine completely abolished the vasorelaxation induced by l-cysteine alone (1 mM, n = 6, P < 0.01). In fact, coapplication of PPG with l-cysteine even induced a 6.0 ± 0.4% contraction of the precontracted MAB. On the other hand, coapplication of PPG with SNP did not reduce the SNP-induced relaxation of MAB. SNP (10 μM) induced similar relaxations of the PE-precontracted MAB (75.2 ± 3.6% vs. 78.4 ± 6.2%, P > 0.05, n = 6) in the absence and presence of PPG (10 μM). The vasorelaxant potency of pinacidil was also not changed before (EC50 1.7 ± 0.1 μM, n = 6) and after pretreatment of MAB with 10 μM PPG (EC50 1.6 ± 0.5 μM, n = 4, P > 0.05). These results indicated that the inhibition of the l-cysteine-induced vasorelaxation by PPG is unlikely due to the nonselective effects of PPG on vascular reactivity.

Endogenous production of H2S and its modulation in MAB. In the rat mesenteric artery and other vascular tissues, CSE is the only H2S-generating enzyme that has been identified, cloned, and sequenced (33). The expression of CSE at the protein level has not been shown in vascular tissues before and was investigated here. A Western blot study demonstrated the expression of CSE proteins (a 43-kDa band) in different rat vascular tissues (Fig. 5A). The expression level of CSE pro-
teins, normalized with β-actin control, was similar in the aorta (1.28 ± 0.02), mesenteric artery (0.93 ± 0.02), tail artery (0.99 ± 0.02), and pulmonary artery (1.01 ± 0.05).

The endogenous production of H₂S from vascular tissues was examined. Unstimulated mesenteric artery tissue produced detectable H₂S (Fig. 5B). Incubation of rat mesenteric artery tissues with L-cysteine (1 mM) for 90 min increased H₂S production by about sevenfold (P < 0.01). After treatment of MAB with PPG (10 μM) and L-cysteine (1 mM), the L-cysteine-induced increase in H₂S production was abolished, and the production of H₂S was even 51.3 ± 7.1% lower than control (n = 5, P < 0.01).

**Effect of H₂S on K<sub>ATP</sub> channel currents.** K<sub>ATP</sub> channel currents were recorded continuously before and after cells were perfused with H₂S-containing bath solution. In single vascular SMCs, the K<sub>ATP</sub> channel was maintained in the open state by low concentration of ATP (0.3 mM) and GDP (1 mM) in the pipette solution (Fig. 6). The recorded currents were not sensitive to iberiotoxin, a selective KCa channel blocker, excluding the possibility of the contamination of KCa currents (data not shown). K<sub>ATP</sub> channel currents were enhanced significantly by H₂S at 50 μM (from 340 ± 45 to 592 ± 102 pA, P < 0.01, n = 6; Fig. 6). A stable effect of H₂S was observed within 1–3 min of H₂S application. Glibenclamide (10 μM) not only inhibited basal K<sub>ATP</sub> currents (from 340 ± 40 to 203 ± 26 pA, P < 0.01, n = 4) but also abolished the stimulation of K<sub>ATP</sub> currents by H₂S (n = 4; Fig. 6). Basal K<sub>ATP</sub> currents were also stimulated by 30 μM pinacidil, a K<sub>ATP</sub> channel opener, from 301 ± 38 to 540 ± 85 pA (P < 0.01, n = 4; Fig. 6). In the presence of glibenclamide (10 μM), the stimulatory effect of pinacidil (30 μM) was abolished, and K<sub>ATP</sub> channel currents were reduced to 225 ± 31 pA at a membrane potential of +50 mV (P < 0.01, n = 4).

**DISCUSSION**

The hemodynamic effect of H₂S on living animals was firstly realized by Zhao et al. (33). In their study, intravenous bolus injection of H₂S transiently decreased blood pressure of rats by 12–30 mmHg (33). This blood pressure drop could be explained by reduced cardiac output and/or reduced peripheral resistance. Interestingly, Zhao et al. (33) did not find any change in heart beat after bolus H₂S injection. A reduced peripheral resistance by H₂S was thus speculated from this study and from the observation that rat aortic tissues were concentration dependently relaxed in vitro by H₂S (32, 33).
Our present study provides direct evidence that H2S reduces the contractility of MAB. As a representative peripheral resistance vessel, MAB makes more important contributions to peripheral resistance and hence blood pressure. We have found that H2S relaxes MAB in two different phases. Below 1 μM, H2S relaxed MAB about 20%. Above 10 μM, H2S induces a greater extent of relaxation up to 90%. This second phase of relaxation of MAB is similar to the steep relationship between H2S concentrations and relaxation of aortic tissues (33). The steepness of the concentration-response curve at relatively higher concentrations of H2S indicates that both conduit and resistance vascular tissues are sensitive to H2S within a narrow concentration range (32). Under resting conditions, the low endogenous H2S level may lead to a weak tonic relaxation of resistance mesenteric arteries and arterioles. In contrast, H2S at low concentration does not exert a tonic relaxant influence on conduit arteries such as the aorta. Upon physiological stimulations, the H2S level may be significantly elevated (33), and both resistance arteries and conduit arteries may relax to a much greater extent as a strong phasic response. The mechanism(s) for vascular type-selective sensitivities to H2S is not clear at the moment. Differences in distributions of H2S targets, KATP channel densities, sensitivities of voltage-dependent Ca2+ channels to membrane potential change, and sensitivities of contractile proteins to intracellular calcium levels between conduit and resistance arteries may be the mechanisms to be further explored.

Another difference in tissue-specific vascular response to H2S is the endothelium dependence of the H2S effect. Removal of a functional endothelium significantly reduced the H2S-induced relaxation of MAB by 6.4-fold. The EC50 of H2S changed from 25 μM in the presence of endothelium to 161 μM in the absence of endothelium (P < 0.05). The same treatment only induced a twofold decrease in the H2S-induced relaxation of rat aortic tissues (32). In the aorta, the EC50 of H2S changed from 136 μM in the presence of endothelium to 273 μM in the absence of endothelium (32). In fact, this endothelium-dependent reduction in the aorta was only obvious at one H2S concentration (around 200 μM). An analogy can be drawn between the tissue-selective endothelium-dependent effect of H2S and tissue-selective release of EDHF. The smaller the size of arteries, the greater the contribution of EDHF to the endothelium-dependent vasorelaxation. Is it possible that H2S released from vascular SMCs (33) stimulates endothelium of small peripheral resistance arteries to release EDHF? It has been known that EDHF action can be specifically blocked by coapplication of ChTX and apamin. In our study, the coapplication of ChTX and apamin significantly reduced the vasorelaxant effect of H2S on endothelium-intact MAB but not on endothelium-free MAB. These results suggest that H2S may have two targets: KATP channels in vascular SMCs (33) and ChTX/apamin-sensitive KCa channels in vascular endothelial cells, the target of EDHF. The activation of these two types of channels by H2S would compound to hyperpolarize SMCs, leading to vasorelaxation. Future studies on the effect of H2S on electrophysiological properties of endothelial cells from resistance arteries, using the patch-clamp technique, would help better understand the relationship of H2S and EDHF.

KATP channels are present in many tissues, including vascular smooth muscles (23). By affecting the contractility of vascular smooth muscles, KATP channels play an important role in the modulation of blood pressure (5, 7, 16, 20). Therefore, KATP channel blockers have potential to cushion abnormally increased vascular contractility and lower blood pressure. Zhao et al. (33) have reported that H2S relaxed rat
aortic tissues in vitro in a K_ATP channel-dependent manner. In isolated aorta SMCs, H2S directly increased K_ATP channel currents and hyperpolarized membrane. To elucidate whether K_ATP channels were the target of H2S in the mesenteric artery, the interaction of H2S with a known K_ATP channel opener (pinacidal) and blocker (glibenclamide) was examined in the present study. Pinacidal mimicked the vasorelaxation effect of H2S on MAB with a stronger potency. The relaxation of endothelium-free MAB induced by H2S at concentrations lower than 1 mM was virtually abolished by glibenclamide, which is in line with the notion that K_ATP channels in vascular smooth muscles are the main target of H2S. The reasons for the failure of glibenclamide to affect the vasorelaxant effect of H2S on endothelium-free MAB at concentrations higher than 1 mM are not clear. It is speculated that H2S at high concentration range may act on certain K_ATP channel-independent mechanisms to relax vascular tissues, possibly a consequence of cellular toxicity. The H2S-induced relaxation of endothelium-intact MAB can only be partially inhibited by glibenclamide, which supports that the endothelium-dependent effect of H2S is not mediated by K_ATP channels. Together, we believe that in mesenteric artery SMCs, K_ATP channels are the target, albeit not necessarily the sole target, of H2S as in aortic SMCs. The patch-clamp study provided direct evidence that H2S at a physiologically relevant concentration stimulated K_ATP channels in isolated mesenteric artery SMCs.

In physiological solution at neutral pH, about one-third of H2S exists in the nondissociated form (H2S) and the remaining two-thirds as HS- at equilibrium with H2S. HS- is a free radical. The H2S-induced relaxation of MAB may involve a HS- free radical mechanism. NAC is an intracellular free radical scavenger (22). It has been widely used as an antioxidant in vivo and in vitro. In the present study, 600 μM NAC did not attenuate the response to H2S of MAB. In fact, NAC slightly potentiated H2S effect by inducing a left shift of the H2S concentration-relaxation curve, although the difference was not significant (P > 0.05). It is unlikely, according to our results, that the vasorelaxation effect of H2S on the mesenteric artery is mediated by a free radical mechanism. It has to be noticed meanwhile that NAC has a wide spectrum of biological activities, including scavenging free radicals and providing intracellular thiols and sulphhydril (1, 2, 13). The involvement of a free radical-mediated mechanism in biological effects of H2S cannot be conclusively determined based only on the results with NAC.

The physiological relevance of the H2S-induced relaxation of MAB can be appreciated from both the concentrations of H2S used in the present study and the changes in vascular tone after endogenous H2S production was manipulated. Physiological levels of H2S in blood were ~10 μM in Wistar rats (12) and ~50 μM in SD rats (33). Physiological concentrations of H2S in brain tissue have been reported to be ~50–160 μM. In our study, H2S relaxed MAB at concentrations as low as 1 μM, which is physiologically relevant. At the vascular tissue level, significant production of H2S has been detected in rat MAB (32, 33). It should be noticed that the results shown in Fig. 5B are the endogenous production rate of H2S in MAB. This H2S production rate cannot be directly translated into the endogenous stable concentrations of H2S in MAB because the catabolism rate of H2S in this tissue has not been determined and because MAB contractility will also be affected by H2S in circulatory blood (about 10–100 μM). The exact endogenous concentrations of H2S in the mesenteric artery interstitial space are difficult to determine. Our study further shows that incubation of MAB with L-cysteine to increase the endogenous production of H2S relaxed the preconstricted MAB (EC50 1.2 mM). This effect of L-cysteine was significantly reduced by pretreatment of MAB with PPG to inhibit CSE. L-cysteine increased the H2S production rate of MAB by sevenfold, and the presence of PPG canceled this stimulatory effect of L-cysteine. Physiological concentrations of L-cysteine are ~0.5–2 mM in the liver and kidney (24, 28). L-Cysteine content in vascular smooth cells is unknown, but the concentrations of L-cysteine used in the present study (around 1 mM) appear quite physiologically relevant.

Our novel observations can be summarized as the following. First, both H2S and NaHS evoke a concentration-dependent vasorelaxation of the isolated rat MAB. The sensitivity of MAB to H2S is about five times higher than that of the rat aorta. Second, the H2S-induced vasorelaxation of MAB is partially and significantly mediated by endothelium via an EDHF-related mechanism. Third, the H2S-induced vasorelaxation of MAB is partially due to the activation of K_ATP channels in vascular SMCs. Fourth, enhancement of endogenous production of H2S with exogenous L-cysteine reduces the contraction force of MAB, which is suppressed after the H2S-generating enzyme is inhibited. Fifth, the expression of CSE at the protein level is demonstrated for the first time in vascular tissues, including mesenteric arteries. Sixth, K_ATP channel currents in isolated mesenteric artery SMCs were significantly augmented by H2S. Our results demonstrated a tissue-type specific relaxant response of resistance arteries to H2S. The stimulation of K_ATP channels in vascular SMCs and the release of EDHF from endothelial cells by H2S represent important cellular mechanisms for H2S effect. Finally, although we have provided evidence that endogenous CSE can generate sufficient H2S from exogenous L-cysteine to cause vasodilation, whether endogenous H2S can directly contribute to regulation of vascular tone still awaits to be confirmed.

GRANTS

This study was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada. J. F. Ndisang was supported by a fellowship from the Saskatchewan Health Research Foundation. G. Tang is supported by a studentship from the Heart and Stroke Foundation of Canada. K. Cao is supported by a fellowship from the Canadian Institutes of Health Research (CIHR)/Canadian Hypertension Society. R. Wang is supported by an investigator award from CIHR.

REFERENCES

H2S-INDUCED RELAXATION OF MESENTERIC ARTERY  


