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Hydrogen sulfide-induced relaxation of the bladder is attenuated in spontaneously hypertensive rats

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Abstract

Purpose To compare hydrogen sulfide (H_2S)-induced relaxation on the bladder between normotensive and spontaneously hypertensive rat (SHR), we evaluated the effects of H_2S donors (GYY4137 and NaHS) on the micturition reflex and on the contractility of bladder tissues. We also investigated the content of H_2S and the expression levels of enzymes related to H_2S biosynthesis [cystathionine β-synthase (CBS), 3-mercaptopyruvate sulfurtransferase (MPST), and cysteine aminotransferase (CAT)] in the bladder.

Methods Eighteen-week-old male normotensive Wistar rats and SHRs were used. Under urethane anesthesia, the effects of intravesically instilled GYY4137 (10^{-8}, 10^{-7} and 10^{-6} M) on the micturition reflex were evaluated by cystometry. The effects of NaHS (1×10^{-8}–3×10^{-4} M) were evaluated on carbachol (10^{-5} M)-induced pre-contracted bladder strips. Tissue H_2S content was measured by the methylene blue method. The expression levels of these enzymes were investigated by Western blot.

Results GYY4137 significantly prolonged intercontraction intervals in Wistar rats, but not in SHRs. NaHS-induced relaxation on pre-contracted bladder strips was significantly attenuated in SHRs compared with Wistar rats. The H_2S content in the bladder of SHRs was significantly higher than that of Wistar rats. CBS, MPST and CAT were detected in the bladder of Wistar rats and SHRs. The expression levels of MPST in the SHR bladder were significantly higher than those in the Wistar rat bladder.

Conclusion H_2S-induced bladder relaxation in SHRs is impaired, thereby resulting in a compensatory increase of the H_2S content in the SHR bladder.

Keywords Hydrogen sulfide · 3-Mercaptopyruvate sulfurtransferase · Spontaneously hypertensive rat · Bladder · Detrusor overactivity

Abbreviations

BBF	Bladder blood flow
BBR	Bladder body weight ratio
BL-D	Bladder dome
BL-T	Bladder trigone
CAT	Cysteine aminotransferase
CBS	Cystathionine β-synthase
CSE	Cystathionine γ-lyase
DO	Detrusor overactivity
IACUC	Institutional Animal Care and Use Committees
ICI	Intercontraction intervals
K_{ATP} channel	ATP-sensitive potassium channel
LUTS	Lower urinary tract symptoms
MPST	3-Mercaptopyruvate sulfurtransferase
MVP	Maximal voiding pressure
NO	Nitric oxide
OAB	Overactive bladder
SHR	Spontaneously hypertensive rat

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Introduction

Hydrogen sulfide (H_2S), a recently characterized gasotransmitter, is endogenously produced by three enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MPST). CBS and CSE produce H_2S from L-cysteine, while MPST produces H_2S from 3-mercaptopyruvate, which is synthesized from L-cysteine by cysteine aminotransferase CAT (CAT/MPST pathway) [1, 2]. H_2S can participate

in a wide range of physiological processes, including neuromodulation, vasorelaxation, and cytoprotection [3–6]. In the bladder, previous reports showed exogenous H_2S-induced contraction of the rat and guinea pig detrusor [7, 8] as well as relaxation of the pig bladder neck and human bladder tissues [9, 10], indicating that physiological roles of H_2S in the bladder remain controversial. Recently, we reported that exogenous H_2S induced relaxation of pre-contracted rat bladder dome (BL-D) and trigone (BL-T) tissues and intravesically instilled an H_2S donor prolonged intercontraction intervals in rats [11]. We also detected that at least the CAT/MPST pathway-mediated endogenous H_2S production was working in the rat BL-D and BL-T tissues [11]. These results indicate a possibility that H_2S can function as an endogenous relaxation factor in the rat bladder.

Recently, bladder ischemia induced by a decrease in bladder blood flow (BBF) has been recognized as an etiologic factor of lower urinary tract symptoms (LUTS) including overactive bladder (OAB) and detrusor overactivity (DO) in human and animal models [12–16]. Hypertension can decrease pelvic arterial blood flow, resulting in dysfunction of the bladder [17–19]. The spontaneously hypertensive rat (SHR) develops DO [20, 21] and is a valuable tool for exploring the pathogenesis of DO [22]. Our and other groups reported that BBF was lower in SHRs compared with normotensive rats [18, 23, 24], and chronic treatment with an α_1 adrenoceptor antagonist silodosin or an ATP-sensitive potassium (K_{ATP}) channel opener nicorandil prevented hypertension-related DO in SHRs via improving the decreased BBF [23, 24]. These lines of evidence indicate that hypertension is an important etiologic factor of LUTS via a decrease in BBF.

In SHRs, downregulation of the endogenous H_2S system is reported and systemically administered H_2S donors decreased blood pressure [25–27]. However, hypertension-related changes of the H_2S system in the bladder have not clarified yet. In this study, therefore, we compared effects of H_2S donors on the micturition reflex and on the bladder contractility, and the endogenous H_2S system in the bladder between SHRs and normotensive Wistar rats.

Materials and methods

Ethics approval

Animal experimental procedures were approved by the Kochi University Institutional Animal Care and Use Committee (IACUC) (#J-34/K-48) and were conducted in accordance with NIH guidelines.

Animals

Eighteen-week old male Wistar rats ($N=43$, Japan SLC, Hamamatsu, Japan) and male SHRs [$N=44$, SHR/Izm, provided from the Disease Model Cooperative Research Association (Kyoto, Japan)] were used and for in vitro studies, tissues (BL-D, BL-T, liver, and cerebellum) were isolated after sacrifice by sodium pentobarbital (80 mg/kg, ip). At the day before tissue isolation or in vivo experiments, blood pressure and heart rate were measured with the tail cuff method, which included warming the whole animal body in the absence of anesthesia (BP-98A-L, Softron, Tokyo, Japan) [28]. Some parts of these tissues were quickly frozen in liquid nitrogen and stored at − 80 °C until use for Western blot and quantification of H_2S content.

Cystometry

Cystometry was performed under urethane anesthesia (0.8 g/kg, ip) according to previous reports [11, 23]. Saline was intravesically instilled (2.4 mL/h) in all animals until 4–5 voidings were detected, and then either GYY4137 (a slow-releasing H_2S donor [29]) (10^{-8}, 10^{-7} and 10^{-6} M in saline) or vehicle (3.3×10^{-5} % *N*,*N*-dimethylformamide/saline) was instilled (2.4 mL/h). In the GYY4137-treated group, after detecting four–five voidings, the dose was increased in turn, and a total of 12–15 voidings were recorded after starting GYY4137 instillation. In the vehicle-treated group, vehicle instillation was continued until 15 voidings were recorded. Intercontraction intervals (ICI) and maximal voiding pressure (MVP) were evaluated from four to five voidings at each dose, and the relative values of these parameters were calculated as the ratio of averaged ICI and MVP measured at each dose to those measured during the first saline instillation. After cystometry, animals were euthanized by cervical dislocation under anesthesia. During the surgery and continuous cystometry, we monitored sufficient levels of anesthesia by confirming negative reflex responses to toe pinch every 30 min. If the level was insufficient, additional doses of urethane (0.05 g/kg/injection, ip) were administered.

In vitro organ bath experiments

Functional studies were performed according to previous reports [11, 30]. Strips of BL-D and BL-T were equilibrated and unstretched for 30 min in Krebs–Henseleit solution (10 mL) bubbled with 5% CO_2/95% O_2 (37 °C). A 1.0 g load was applied to each strip, and the load was readjusted to this level 30 min later. Following a 30-min period of equilibration, these strips were exposed to 100 mM KCl. After a 30 min-washout period, the relaxation response to NaHS (a

rapid-releasing H_2S donor [29]) (1×10^{-8}–3×10^{-4} M) was measured cumulatively in bladder strips pre-contracted by carbachol (1×10^{-5} M). NaHS was administered after stable contraction was reached. NaHS solutions were quickly prepared before use.

Quantification of H_2S

The content of H_2S in bladder tissue samples was measured using the methylene blue method [11, 31]. Tissues were homogenized in 210 µL of ice-cold 50 mM Tris–HCl (pH 7.4). The homogenate (75 µL) was mixed with 425 µL of distilled water, 250 µL of 1% zinc acetate, 133 µL of 20 mM *N*,*N*-dimethyl *p*-phenylenediamine sulfate in 7.2 M HCl and 133 µL of 30 mM $FeCl_3$ in 1.2 M HCl, then incubated for 30 min at room temperature. Next, 250 µL of 10% trichloroacetic acid was added, and the mixture was centrifuged (10,000 × *g* for 5 min). The absorbance of the supernatants (200 µL) was measured at 670 nm. All samples were assayed in duplicate, and the concentration of H_2S was calculated based on a standard curve of NaHS (0–400 µM). The amount of H_2S was normalized by protein content, determined using a commercially available kit (Protein Assay Rapid Kit, Wako, Osaka, Japan).

Western blot

Protein samples were prepared and Western blots were performed according to previous reports [11, 32]. Tissue samples (approximately 30 mg) were homogenized in 210 µL of ice-cold RIPA buffer (Wako) with a protease inhibitor cocktail (Roche Diagnosis, Basel, Switzerland). Protein (50 µg) was separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. After blocking with 5% skim milk, these membranes were incubated with primary antibodies as follows: rabbit polyclonal anti-CBS antibody (1:400 dilution, Proteintech, Rosemont, IL, USA), rabbit polyclonal anti-MPST antibody (1:400 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-CAT antibody (1:500 dilution, Abcam, Cambridge, UK), rabbit polyclonal anti-CSE antibody (1:200 dilution, Abcam), rabbit polyclonal anti-β-actin antibody (1:200 dilution, ANASPEC, Fremont, CA, USA) and mouse monoclonal anti-GAPDH antibody (1:3000 dilution, Acris Antibodies GmbH, Herford, Germany). The secondary antibodies were peroxidase-conjugated anti-rabbit IgG (1:3000) or anti-mouse IgG (1:2000) (GE Healthcare, Buckinghamshire, UK). The membranes were developed with Immobilon™ Western Chemiluminescent horseradish peroxidase substrate (Millipore, Billerica, MA, USA). Images were captured in a LAS4000mini (Fujifilm, Tokyo, Japan). Densitometric analysis was performed using MultiGauge software (Fujifilm). Target protein bands were normalized to β-actin or GAPDH.

Statistics

All values are expressed as mean ± SEM. Statistical differences were determined using an unpaired Student's *t* test or Welch's test. *P* values less than 0.05 were taken to indicate statistical significance.

Drugs and chemicals

GYY4137 [(*p*-methoxyphenyl)morpholino-phosphinodithioic acid] and NaHS were purchased from Cayman Chemical, Ann Arbor, MI, USA. All other chemicals (reagent grade) were commercially available.

Results

General features of the animals

Body weight, the bladder weight and the bladder body weight ratio (BBR) in SHRs were significantly lower than those in Wistar rats (Table 1). Systolic, mean and diastolic blood pressures in SHRs were significantly higher than those in Wistar rats (Table 1). There is no significant difference in heart rate between Wistar rats and SHRs (Table 1).

Effects of intravesically instilled GYY4137 on the micturition reflex

Results of continuous cystometry are shown in Figs. 1 and 2. The baseline values of ICI (sec) and MVP (cmH_2O) just before intravesical instillation of vehicle or GYY4137 were 2152 ± 153 and 32.6 ± 0.8 in Wistar rats ($N=17$) and 1362 ± 140 and 30.4 ± 0.5 in SHRs ($N=14$). The

Table 1 The general features

Factors	Groups	
	Wistar ($N=43$)	SHR ($N=44$)
Body weight (g)	461.8 ± 4.3	385.4 ± 3.0*
Bladder weight (mg)	172.3 ± 6.5	108 ± 2.5*
BBR ($\times10^{-3}$)	0.37 ± 0.01	0.28 ± 0.01*
Systolic blood pressure (mmHg)	120.4 ± 2.3	185.7 ± 2.7*
Mean blood pressure (mmHg)	101.1 ± 2.3	155.9 ± 2.3*
Diastolic blood pressure (mmHg)	91.7 ± 2.3	140.9 ± 2.5*
Heart rate (bpm)	404.9 ± 12.0	384.6 ± 9.0

Values are mean ± SEM

BBR bladder body weight ratio (bladder weight/body weight), *SHR* spontaneously hypertensive rat

*$P<0.05$, significantly different from the Wistar group (Welch's test for body weight, bladder weight and BBR; unpaired Student's *t* test for systolic, mean and diastolic blood pressure)

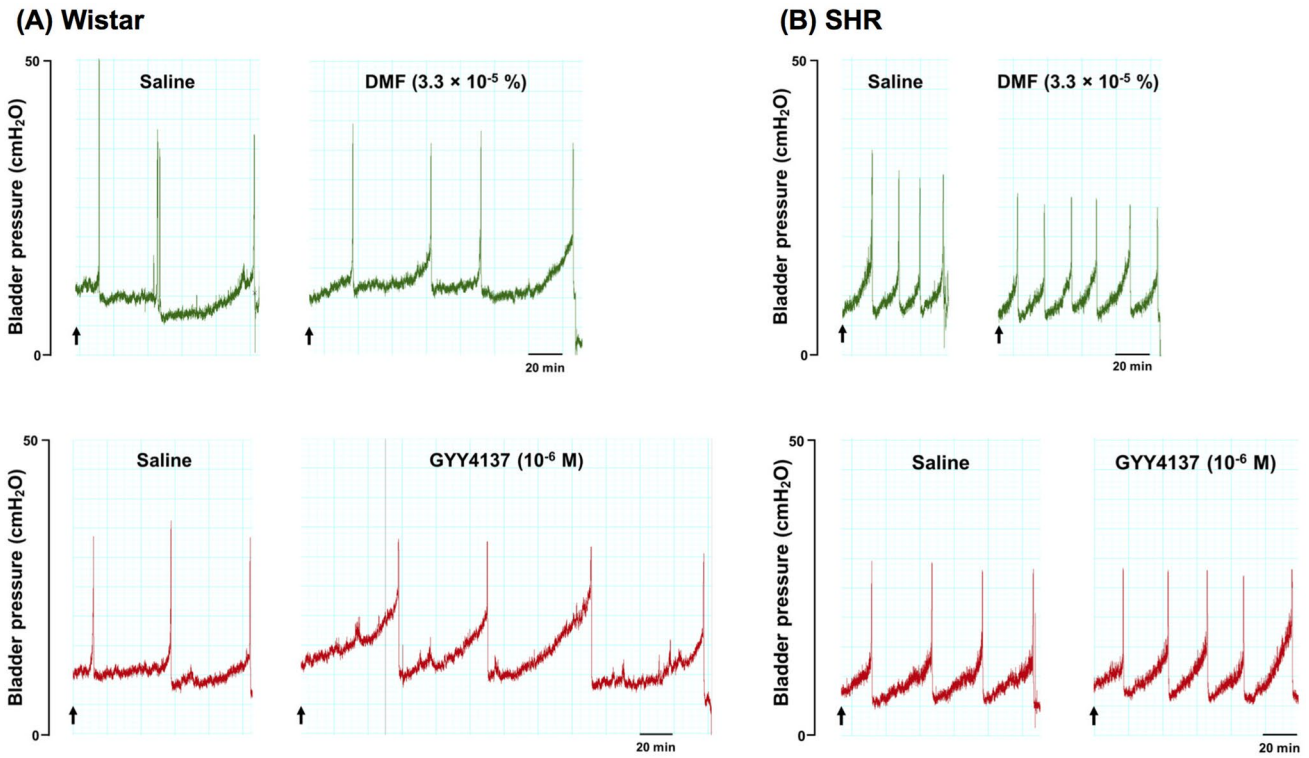

Fig. 1 Representative in vivo continuous cystometry traces in a Wistar rat (**a**) and a SHR (**b**). Vehicle (3.3×10^{-5} % DMF, upper traces) or GYY4137 (a slow-releasing H_2S donor, 10^{-6} M, lower traces) was intravesically instilled (2.4 ml/h) following saline instillation. Arrows indicate the timing of each instillation. *DMF* *N*,*N*-dimethylformamide, *SHR* spontaneously hypertensive rat

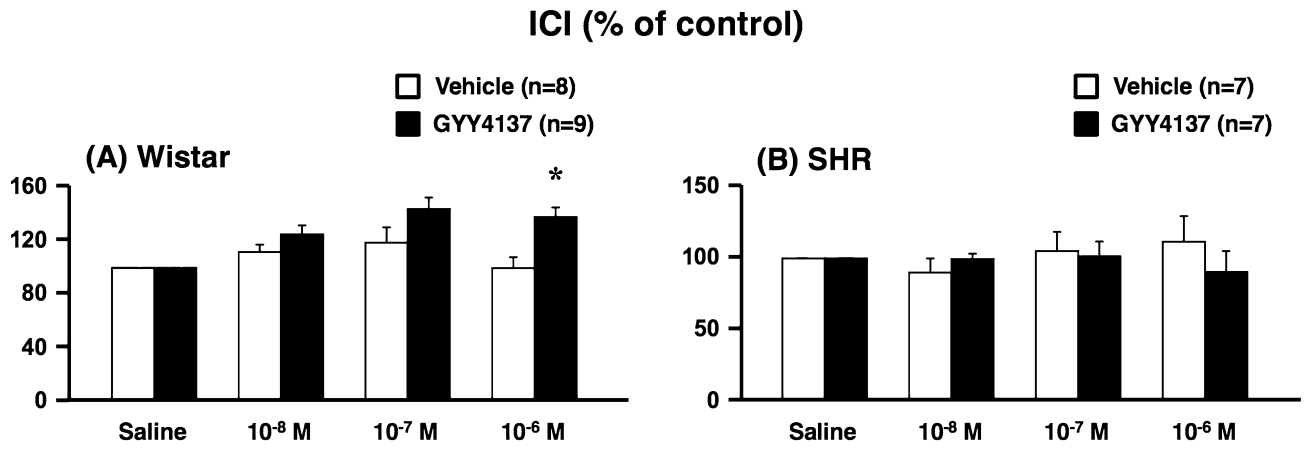

Fig. 2 Effect of intravesically instilled GYY4137 (a slow-releasing H_2S donor) on intercontraction intervals (ICI) in Wistar rats (**a**) and SHRs (**b**). Continuous cystometry (infusion rate, 2.4 ml/h) was performed under urethane anesthesia (0.8 g/kg, ip) in these rats. GYY4137 (10^{-8}–10^{-6} M) or vehicle was intravesically instilled following saline instillation. Values are mean ± SEM. $^*P<0.05$, significantly different from the vehicle-treated group (unpaired Student's *t* test). The number of samples per group is indicated in parentheses. *SHR*: spontaneously hypertensive rat

baseline values of ICI values of SHRs were significantly shorter than those of Wistar rats ($P<0.05$). In Wistar rats, GYY4137 significantly prolonged ICI at 10^{-6} M compared to the vehicle-treated group, while the GYY4137-induced prolongation was not detected in SHRs (Fig. 2). There was no significant difference in baseline values of MVP between Wistar rats and SHRs. GYY4137 showed no significant effect on MVP in Wistar rats or SHRs (data not shown).

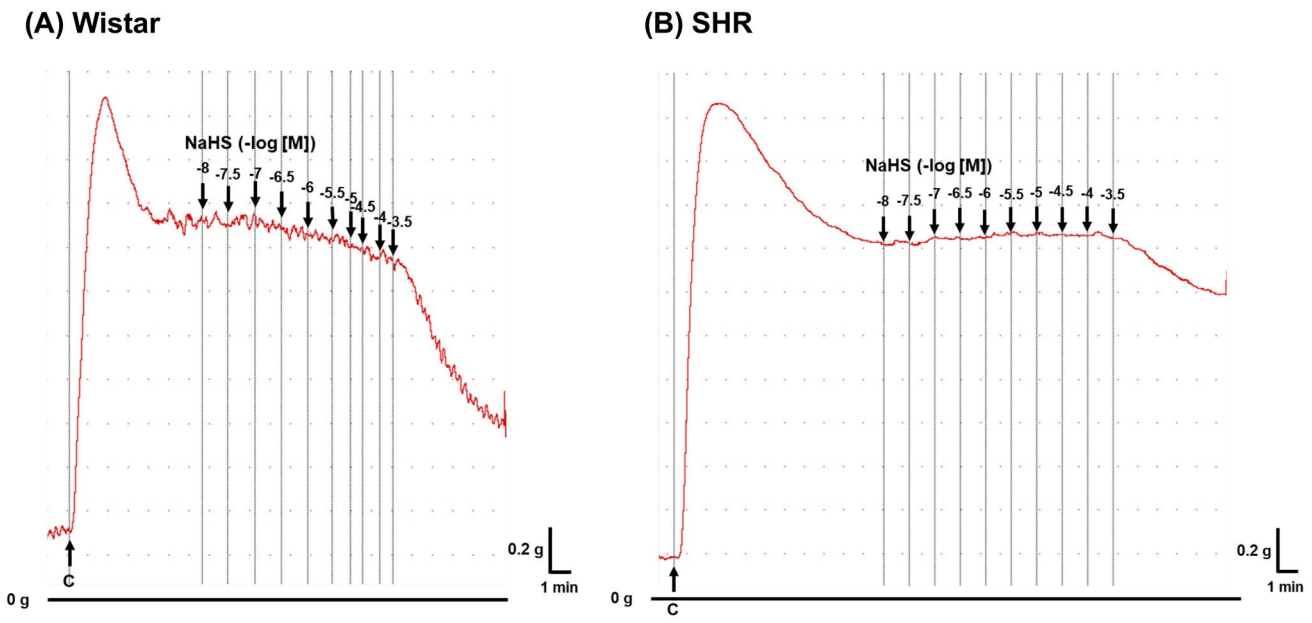

Fig. 3 Representative isometric force recordings in BL-D strips isolated from a Wistar rat (**a**) or a SHR (**b**). These strips were pre-contracted by carbachol (1×10^{-5} M) and after stable contraction was reached, NaHS (a rapid-releasing H_2S donor, 1×10^{-8}–3×10^{-4} M) was administered. *BL-D* bladder dome, *C* carbachol, *SHR* spontaneously hypertensive rat

Table 2 Data from functional studies in the rat bladder tissues

Group	Maximum relaxation (%)	EC_{50} (M)
BL-D		
Wistar	66.5 ± 3.2	$3.5\pm1.1\ (\times10^{-2})$
SHR	47.5 ± 5.9*	$2.6\pm1.0\ (\times10^{-2})$
BL-T		
Wistar	68.2 ± 5.2	$0.06\pm0.002\ (\times10^{-2})$
SHR	69.6 ± 8.1	$4.0\pm1.2\ (\times10^{-2})$*

Values are mean ± SEM ($N=12$). A rapid-releasing H_2S donor NaHS (1×10^{-8} to 3×10^{-4} M) was administered on the rat BL-D and BL-T strips pre-contracted by carbachol (10^{-5} M)

BL-D bladder dome, *BL-T* bladder trigone, *SHR* spontaneously hypertensive rat

*$P<0.05$, significantly different from the Wistar group (Welch's test for BL-D; unpaired Student's *t* test for BL-T)

In vitro organ bath experiments using pre-contracted rat bladder strips

Results of in vitro organ bath experiments are shown in Fig. 3 and Table 2. NaHS (1×10^{-8}–3×10^{-4} M) dose-dependently induced the relaxation of BL-D and BL-T strips pre-contracted by carbachol in both Wistar rats and SHRs. In the BL-D, maximum relaxation in SHRs was significantly lower than that in Wistar rats, while there was no significant difference in the EC_{50} values for NaHS-induced relaxation between Wistar rats and SHRs (Table 2). In the BL-T, the EC_{50} values for NaHS-induced relaxation in SHRs were significantly higher than those in Wistar rats, while there was no significant difference in maximum relaxation between Wistar rats and SHRs (Table 2). We verified that NaHS or Krebs–Henseleit solution alone showed no contraction or relaxation on each tissue strip (data not shown).

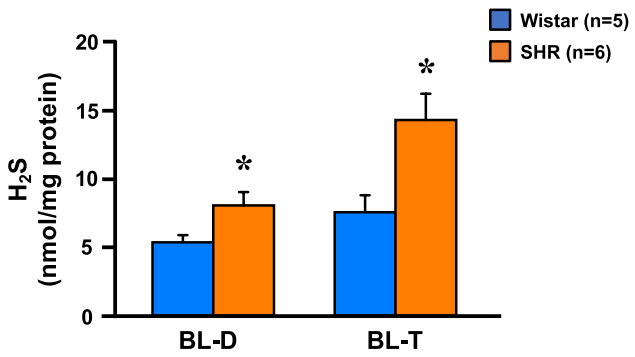

Fig. 4 H_2S content in the bladder of Wistar rats and SHRs. Values are mean ± SEM. *$P<0.05$, significantly different from the Wistar group (Welch's test for BL-D; unpaired Student's *t* test for BL-T). The number of samples per group is indicated in parentheses. *BL-D* bladder dome, *BL-T* bladder trigone, *SHR* spontaneously hypertensive rat

H_2S content in the rat bladder

H_2S was detectable in the bladder of Wistar rats and SHRs (Fig. 4). The level of H_2S was significantly higher in SHRs than that in Wistar rats both in the BL-D and BL-T (Fig. 4).

Expression levels of enzymes related to H_2S biosynthesis in the rat bladder

Protein bands of CBS, MPST and CAT were detected in the BL-D and BL-T of both Wistar rats and SHRs (Fig. 5a–c). The expression level of MPST in the SHR BL-D was significantly higher than that in the Wistar rat BL-D, and that in the SHR BL-T showed a non-significant tendency ($P=0.09$) to be higher than that in the Wistar rat BL-T (Fig. 5b). In contrast, there were no significant differences in the expression levels of CBS or CAT between Wistar rat and SHR bladder tissues (Fig. 5a, c). No protein bands for CSE were detected in the bladder of both Wistar rats and SHRs (data not shown).

Discussion

In the present study, GYY4137 prolonged ICI in normotensive Wistar rats, but not in SHRs. NaHS-induced relaxation of pre-contracted bladder strips was attenuated in SHRs compared with Wistar rats. The H_2S content in the bladder of SHRs was higher than that of Wistar rats. CBS, MPST, and CAT were detected in the bladder of both Wistar rats and SHRs, and the expression levels of MPST in the SHR bladder were significantly higher than those in the Wistar rat bladder.

It is reported that even at 6 weeks of age, SHRs show higher systolic blood pressure (180–200 mmHg) compared with normotensive controls (140–150 mmHg), and that the blood pressure of SHRs elevated with age [33]. On the other hand, hypertension-induced DO was detected at 17 and 21 weeks of age, but not at 12 weeks of age [20]. Based on the evidence and our previous reports [23, 24], in this study, we used SHRs and normotensive Wistar rats at 18 weeks of age. The SHRs showed obvious hypertension, and lower body weight, the bladder weight and the BBR compared with the Wistar rats. In addition, basal values of ICI in the SHRs were shorter than those in the Wistar rats, indicating that at 18 weeks of age, SHRs showed frequent urination due to DO. These differences between Wistar rats and SHRs were in line with previous reports [20, 23, 24]. At 18 weeks of age, intravesically instilled GYY4137, a slow-releasing H_2S donor [29], prolonged ICI in Wistar rats and NaHS, a rapid-releasing H_2S donor [29], relaxed pre-contracted bladder strips of Wistar rats, in line with our previous report [11]. These results suggest that intravesically administered GYY4137-induced inhibition of the micturition reflex in Wistar rats might be mediated at least by relaxation of the bladder smooth muscle. On the other hand, in SHRs, the GYY4137 showed no significant effect on ICI even at the same doses and SHR bladder strips showed NaHS-induced hypo-relaxation (BL-D) or lower potency in the

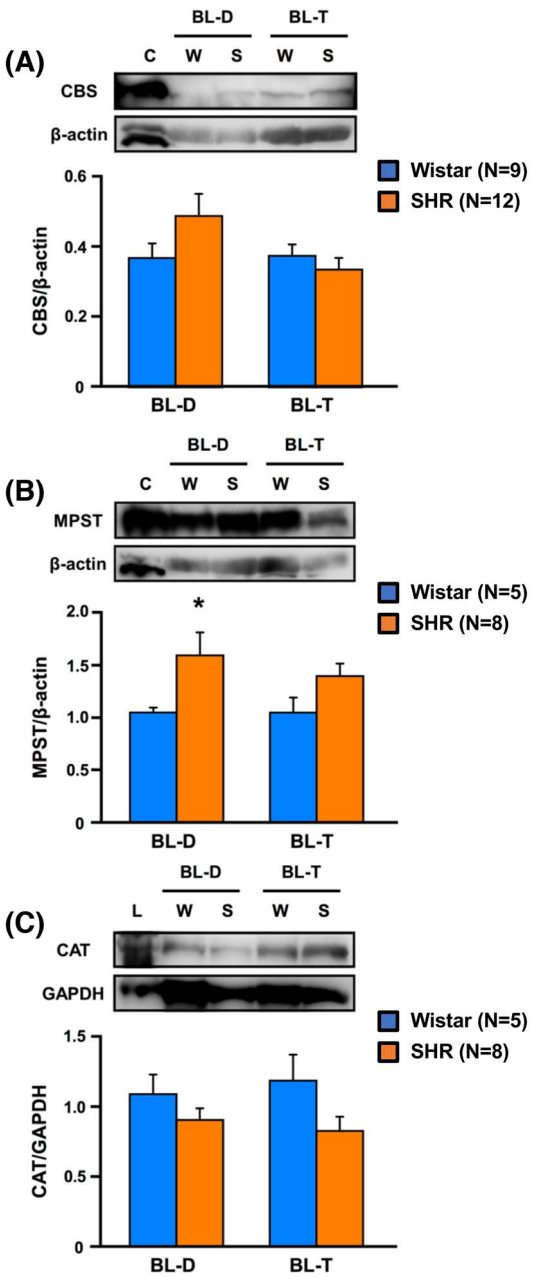

Fig. 5 Expression levels of enzymes related to H_2S biosynthesis in the bladder of Wistar rats and SHRs. The amount of protein for CBS (**a**), MPST (**b**) or CAT (**c**) was normalized to the amount of protein for β-actin/GAPDH. Values are mean ± SEM. $^*P<0.05$, significantly different from the Wistar group (Welch's test). The number of samples per group is indicated in parentheses. *C* and *L* indicate positive controls (cerebellum for CBS and MPST and liver for CAT). *W* and *S* indicate the Wistar and SHR groups. *CAT* cysteine aminotransferase, *CBS* cystathionine β-synthase, *MPST* 3-mercaptopyruvate sulfurtransferase, *BL-D* bladder dome, *BL-T* bladder trigone, *SHR* spontaneously hypertensive rat

NaHS-induced relaxation (BL-T) compared with the Wistar rat strips. These results suggest that intravesical H_2S-induced suppression of the micturition reflex might be impaired in SHRs at least by attenuating H_2S-induced relaxation of the

bladder smooth muscle. In the SHR thoracic aorta, however, enhanced maximal vasorelaxation was observed in response to Na_2S, another rapid-releasing H_2S donor [29], compared with normotensive controls [34]. Therefore, hypertension-mediated changes in responsiveness to H_2S might be different between the bladder and the vascular system.

Next, we compared endogenous H_2S system between SHR and Wistar rat bladder tissues. Higher H_2S content and an increase in MPST protein expression were detected in the SHR bladder compared with the Wistar rat bladder. These results indicate that endogenous H_2S system might be upregulated to compensate the attenuated relaxation response to H_2S in the SHR bladder. Enhancement of the bladder afferent activity, a cause of DO [35], is reported in SHRs [36]. Since it is reported that H_2S promotes the release of sensory neuropeptides from the bladder afferents to mediate inhibitory neurotransmission to the pig bladder neck [9], in SHRs, H_2S system might be upregulated to augment the inhibitory signal, thereby counteracting the enhanced bladder afferent activity. On the other hand, several groups reported that in SHRs, plasma H_2S levels were reduced and CSE mRNA expression and activity of CSE were decreased in the thoracic aorta compared with normotensive controls [26, 37, 38]. Since in the SHR thoracic aorta, Na_2S-induced vasorelaxation was enhanced [34], unlike the bladder, to compensate the decreased H_2S production in the vascular system, H_2S-induced vasorelaxation might be enhanced in SHRs compared with the control. In addition, responses to H_2S in the thoracic aorta are different between young pre-hypertensive and adult SHRs. At 4 weeks of age, higher potency of the vasorelaxant response to Na_2S was detected in the SHR thoracic aorta, while Na_2S-induced hyper-relaxation was observed in SHRs at 16 weeks of age compared with each control [34]. Since hypertension-induced DO is detected after hypertension is developed (at least after 12 weeks of age) [20], responses to H_2S and levels of the endogenous H_2S system in the bladder might be different among prehypertensive and pre-detrusor overactive SHRs, hypertensive and pre-detrusor overactive SHRs, and hypertensive and detrusor overactive SHRs. Further studies are needed to investigate "time-dependency" of hypertension-mediated changes of responses to H_2S and the endogenous H_2S system in the SHR bladder.

Although mechanisms for H_2S-induced bladder smooth muscle relaxation remain controversial, one of the targets for H_2S is K_{ATP} channels, because glibenclamide, a K_{ATP} channel blocker, reduced GYY4137-induced relaxation of the pre-contracted pig bladder strips [9]. In this study, H_2S-induced bladder relaxation was attenuated in SHRs, indicating a possibility that K_{ATP} channels-mediated relaxation might be impaired in the SHR bladder. However, it is reported that mRNA levels of K_{ATP} channel subunits are increased in the SHR bladder compared with normotensive controls [24], indicating that K_{ATP} channels-mediated responses can be enhanced. These findings suggest the involvement of other mechanisms rather than K_{ATP} channels in the impairment of H_2S-induced bladder relaxation in SHRs. Another possible mechanism for the impairment might be related to nitric oxide (NO), which is reported to show synergistic action with H_2S on vasorelaxation [39]. In general, NO plays an important role in the relaxation of the urethra sphincter smooth muscle, while the detrusor is less sensitive to the effects of NO [40, 41]. However, NO is reported to depress sensitized bladder afferent nerve activity in rats with cyclophosphamide-induced cystitis [42, 43], indicating that NO might modulate bladder hyperactivity induced by pathological conditions and H_2S has a possibility to enhance the NO-induced modulation synergistically. In SHRs, mRNA expression levels of neuronal NO synthase were lower in the bladder compared with the normotensive rat bladder [18]. Therefore, impairment of the synergistic action of NO and H_2S might be a cause of the attenuation of H_2S-induced bladder relaxation in SHRs. As it is unclear whether H_2S and NO modulate bladder smooth muscle relaxation and bladder afferent nerve activity synergistically, further studies are necessary to clarify the synergistic action and the attenuation mechanisms in the SHR bladder focusing on the interaction between H_2S and NO.

Conclusion

H_2S-induced bladder relaxation in SHRs is impaired, which might be a cause of hypertension-mediated induction of LUTD. To compensate the less responsiveness, endogenous H_2S levels might be increased in the SHR bladder.

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Compliance with ethical standards

Conflict of interest None of the contributing authors have any conflict of interest.

References

1. Huang CW, Moore PK (2015) H_2S synthesizing enzymes: biochemistry and molecular aspects. Handb Exp Pharmacol 230:3–25
2. Kimura H (2015) Signaling molecules: hydrogen sulfide and polysulfide. Antioxid Redox Signal 22:362–376
3. Gemici B, Elsheikh W, Feitosa KB et al (2015) H_2S-releasing drugs: anti-inflammatory, cytoprotective and chemopreventative potential. Nitric Oxide 46:25–31
4. Kimura H (2014) The physiological role of hydrogen sulfide and beyond. Nitric Oxide 41:4–10

5. Yang G, Wu L, Jiang B et al (2008) H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ-lyase. Science 322:587–590
6. Ozatik FY, Teksen Y, Kadioglu E et al (2019) Effects of hydrogen sulfide on acetaminophen-induced acute renal toxicity in rats. Int Urol Nephrol 51:745–754
7. Fernandes VS, Xin W, Petkov GV (2015) Novel mechanism of hydrogen sulfide-induced guinea pig urinary bladder smooth muscle contraction: role of BK channels and cholinergic neurotransmission. Am J Physiol Cell Physiol 309:C107–C116
8. Patacchini R, Santicioli P, Giuliani S et al (2005) Pharmacological investigation of hydrogen sulfide (H_2S) contractile activity in rat detrusor muscle. Eur J Pharmacol 509:171–177
9. Fernandes VS, Ribeiro AS, Martínez MP et al (2013) Endogenous hydrogen sulfide has a powerful role in inhibitory neurotransmission to the pig bladder neck. J Urol 189:1567–1573
10. Gai JW, Wahafu W, Guo H et al (2013) Further evidence of endogenous hydrogen sulphide as a mediator of relaxation in human and rat bladder. Asian J Androl 15:692–696
11. Zou S, Shimizu T, Shimizu S et al (2018) Possible role of hydrogen sulfide as an endogenous relaxation factor in the rat bladder and prostate. Neurourol Urodyn 37:2519–2526
12. Azadzoi KM, Tarcan T, Kozlowski R et al (1999) Overactivity and structural changes in the chronically ischemic bladder. J Urol 162:1768–1778
13. Nomiya M, Sagawa K, Yazaki J et al (2012) Increased bladder activity is associated with elevated oxidative stress markers and proinflammatory cytokines in a rat model of atherosclerosis-induced chronic bladder ischemia. Neurourol Urodyn 31:185–189
14. Pinggera GM, Mitterberger M, Steiner E et al (2008) Association of lower urinary tract symptoms and chronic ischaemia of the lower urinary tract in elderly women and men: assessment using colour Doppler ultrasonography. BJU Int 102:470–474
15. Pinggera GM, Mitterberger M, Pallwein L et al (2008) α-Blockers improve chronic ischaemia of the lower urinary tract in patients with lower urinary tract symptoms. BJU Int 101:319–324
16. Shimizu S, Tsounapi P, Shimizu T et al (2014) Lower urinary tract symptoms, benign prostatic hyperplasia/benign prostatic enlargement and erectile dysfunction: are these conditions related to vascular dysfunction? Int J Urol 21:856–864
17. Tarcan T, Azadzoi KM, Siroky MB et al (1998) Age-related erectile and voiding dysfunction: the role of arterial insufficiency. Br J Urol 82(Suppl 1):26–33
18. Yono M, Yamamoto Y, Yoshida M et al (2007) Effects of doxazosin on blood flow and mRNA expression of nitric oxide synthase in the spontaneously hypertensive rat genitourinary tract. Life Sci 81:218–222
19. Yono M, Tanaka T, Tsuji S et al (2011) Effects of age and hypertension on α1-adrenoceptors in the major source arteries of the rat bladder and penis. Eur J Pharmacol 670:260–265
20. Jin JH, Lee HJ, Shin HY et al (2011) Development and changes with age of detrusor overactivity in spontaneous hypertensive rats as observed by simultaneous registrations of intravesical and intraabdominal pressures. Int Neurourol J 15:192–198
21. Persson K, Pandita RK, Spitsbergen JM et al (1998) Spinal and peripheral mechanisms contributing to hyperactive voiding in spontaneously hypertensive rats. Am J Physiol 275:R1366–R1373
22. Steers WD, Clemow DB, Persson K et al (1999) The spontaneously hypertensive rat: insight into the pathogenesis of irritative symptoms in benign prostatic hyperplasia and young anxious males. Exp Physiol 84:137–147
23. Inoue S, Saito M, Tsounapi P et al (2012) Effect of silodosin on detrusor overactivity in the male spontaneously hypertensive rat. BJU Int 110:E118–E124
24. Saito M, Ohmasa F, Tsounapi P et al (2012) Nicorandil ameliorates hypertension-related bladder dysfunction in the rat. Neurouro Urodyn 31:695–701
25. Swan KW, Song BM, Chen AL et al (2017) Analysis of decreases in systemic arterial pressure and heart rate in response to the hydrogen sulfide donor sodium sulfide. Am J Phsiol Heart Circ Physiol 313:H732–H743
26. Yan H, Du J, Tang C (2004) The possible role of hydrogen sulfide on the pathogenesis of spontaneous hypertension in rats. Biochem Biophys Res Commun 313:22–27
27. Yu W, Liao Y, Huang Y et al (2017) Endogenous hydrogen sulfide enhances carotid sinus baroreceptor sensitivity by activating the transient receptor potential cation channel subfamily V member 1 (TRPV1) channel. J Am Heart Assoc 6:e004971
28. Saito M, Tsounapi P, Oikawa R et al (2014) Prostatic ischemia induces ventral prostatic hyperplasia in the SHR; possible mechanism of development of BPH. Sci Rep 4:3822
29. Szabo C, Papapetropoulos A (2017) International union of basic and clinical pharmacology. CII: pharmacological modulation of H_2S levels: H_2S donors and H_2S biosynthesis inhibitors. Pharmacol Rev 69:497–564
30. Saito M, Shimizu S, Kinoshita Y et al (2010) Bladder dysfunction after acute urinary retention in the rats: a novel over active bladder model. Mol Cell Biochem 333:109–114
31. Mok YY, Atan MS, Yoke Ping C et al (2004) Role of hydrogen sulphide in haemorrhagic shock in the rat: protective effect of inhibitors of hydrogen sulphide biosynthesis. Br J Pharmacol 143:881–889
32. Saito M, Tominaga L, Nanba E et al (2005) Expression of HSP 70 and its mRNAS during ischemia-reperfusion in the rat bladder. Life Sci 75:1879–1886
33. Fukuda S, Tsuchikura S, Iida H (2004) Age-related changes in blood pressure, hematological values, concentrations of serum biochemical constituents and weights of organs in the SHR/Izm, SHRSP/Izm and WKY/Izm. Exp Anim 53:67–72
34. Berenyiova A, Drobna M, Cebova M et al (2018) Changes in the vasoactive effects of nitric oxide, hydrogen sulfide and the structure of the rat thoracic aorta: the role of age and essential hypertension. J Physiol Pharmacol 69:1–12
35. de Groat WC (1997) Neurologic basis for the overactive bladder. Urology 50:36–56
36. Leon LA, Hoffman BE, Gardner SD et al (2008) Effects of the β3-adrenergic receptor agonist disodium 5-[(2*R*)-2-[[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL-316243) on bladder micturition reflex in spontaneously hypertensive rats. J Pharmacol Exp Ther 326:178–185
37. Sun Y, Huang Y, Zhang R et al (2015) Hydrogen sulfide upregulates K_{ATP} channel expression in vascular smooth muscle cells of spontaneously hypertensive rats. J Mol Med 93:439–455
38. Zhao X, Zhang LK, Zhang CY et al (2008) Regulatory effect of hydrogen sulfide on vascular collagen content in spontaneously hypertensive rats. Hypertens Res 31:1619–1630
39. Szabo C (2017) Hydrogen sulfide, an enhancer of vascular nitric oxide signaling: mechanisms and implications. Am J Physiol Cell Physiol 312:C3–C15
40. Fujiwara M, Andersson K, Persson K (2000) Nitric oxide-induced cGMP accumulation in the mouse bladder is not related to smooth muscle relaxation. Eur J Pharmacol 401:241–250
41. Mamas MA, Reynard JM, Brading AF (2003) Nitric oxide and the lower urinary tract: current concepts, future prospects. Urology 61:1079–1085

42. Ozawa H, Chancellor MB, Jung SY et al (1999) Effect of intravesical nitric oxide therapy on cyclophosphamide-induced cystitis. J Urol 162:2211–2216
43. Yu Y, de Groat WC (2013) Nitric oxide modulates bladder afferent nerve activity in the in vitro urinary bladder-pelvic nerve preparation from rats with cyclophosphamide induced cystitis. Brain Res 1490:83–94