Pharmacological Reports 71 (2019) 968–975

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

$GABA_A$ receptor, K_{ATP} channel and L-type Ca^{2+} channel is associated with facilitation effect of H_2S on the baroreceptor reflex in spontaneous hypertensive rats

Xu Teng[a,d,1], Hui Li[a,1], Hongmei Xue[a], Sheng Jin[a], Lin Xiao[a,d], Qi Guo[a], Yuming Wu[a,b,c,*]

[a] Department of Physiology, Institute of Basic Medicine, Hebei Medical University, Shijiazhuang, China
[b] Key Laboratory of Vascular Medicine of Hebei Province, Shijiazhuang, China
[c] Hebei Collaborative Innovation Center for Cardio–Cerebrovascular Disease, Shijiazhuang, China
[d] Hebei Key Lab of Laboratory Animal Science, Hebei Medical University, Shijiazhuang, China

ARTICLE INFO

Article history:
Received 26 October 2018
Received in revised form 8 April 2019
Accepted 14 May 2019
Available online 15 May 2019

Keywords:
Hydrogen sulfide
Baroreceptor
Hypertension

ABSTRACT

Background: We aimed to investigate whether the facilitating effect of H_2S on the baroreceptor reflex is associated with the $GABA_A$ receptor, K_{ATP} channel and L-type Ca^{2+} channel pathway.
Methods: Spontaneously hypertensive rats (SHRs) and Wistar Kyoto (WKY) rats were used to investigate the facilitating effect of H_2S on the baroreceptor reflex by perfusing the isolated carotid sinus. The mechanism by which H_2S facilitated the baroreceptor reflex was determined by using Bay K8644 (an agonist of calcium channels), glibenclamide (Gli, a K_{ATP} channel blocker), and picrotoxin (PIC, a blocker of γ-aminobutyric acid $[GABA]_A$ receptor).
Results: As compared with WKY rats, SHRs showed impaired baroreceptor reflex sensitivity, as demonstrated by a right and upward shift of the functional curve for the intrasinus pressure–arterial blood pressure relation. H_2S perfusion (25, 50, or 100 μmol/L) dose-dependently ameliorated the impaired sensitivity of the baroreceptor reflex. Bay K8644 (500 nmol/L), Gli (20 μmol/L) and PIC (50 μmol/L) all prevented H_2S ameliorating the impaired baroreceptor reflex.
Conclusions: H_2S facilitating the baroreceptor reflex might be associated with activating the $GABA_A$ receptor, opening the K_{ATP} channel, and closing the L-type Ca^{2+} channel. These areas should provide new targets for preventing and treating hypertension.

Introduction

Hypertension is a worldwide chronic disease and a crucial risk factor for multiple organ injuries, including myocardial, cerebral and renal impairment, mainly responsible for morbidity and mortality. Carotid sinus baroreflex is one of the most considerable neuroregulation mechanisms maintaining homeostasis of arterial blood pressure by negative feedback [1]. In patients with hypertension, baroreflex sensitivity is significantly impaired, linked to increased thickness of the vascular wall of the carotid sinus [2]. Hypertension, hypotension and orthostatic tachycardia may occur in patients with bilateral carotid sinus resection due to trauma or local tumor surgery [3]. Syncope caused by hypotension may occur in patients with hypersensitivity of the carotid sinus [4]. Thus, the carotid sinus plays crucial roles in regulating arterial blood pressure under both physiological and pathophysiological conditions [5,6].

The carotid sinus baroreceptor is a crucial component of the baroreflex and is predominant among baroreceptors that play important roles in regulating arterial blood pressure [7,8]. The increased mechanical stretch of vessel walls in the carotid sinus caused by elevated arterial blood pressure can directly stimulate the baroreceptors, whose sensitivity is controlled by many endogenous substances in telecrine and para/autocrine ways [9,10]. A thorough investigation of these endogenous factors may contribute new targets for ameliorating impaired baroreceptor sensitivity and thus treating hypertension [11–13].

Hydrogen sulfide (H_2S) was revealed as the third gaseous signal molecule after nitric oxide and carbon monoxide [14]. H_2S is produced from the cysteine metabolic pathway catalyzed by cystathionine-β-synthase (CBS), cystathionine clyase (CSE), and

* Corresponding author.
E-mail address: wuyum@yahoo.com (Y. Wu).
[1] The first two authors contributed equally.

http://dx.doi.org/10.1016/j.pharep.2019.05.009
1734-1140/

3-mercaptopyruvate sulfurtransferase [15] and has widespread biological effects, including maintaining homeostasis of vascular function [16]. Accumulating evidence has shown that H_2S plays an important role in short-term and long-term regulation of arterial blood pressure, under both physiological and pathological conditions [17–19]. We previously reported that exogenous H_2S injection in the rostral ventrolateral medulla inhibited sympathetic vasomotor tone to reduce arterial blood pressure [20]. The endogenous H_2S system is downregulated in spontaneously hypertensive rats (SHRs) [21]. Our and other studies confirmed the ameliorative effect of H_2S supplementation on hypertension in rats [22,23]. However, the mechanism by which H_2S rescues hypertension has not been fully investigated.

Several articles have confirmed the facilitating effect of H_2S on baroreceptor sensitivity. Our previous studies reported that exogenous H_2S treatment by perfusing the isolated carotid sinus facilitated baroreceptor sensitivity and inhibited sympathetic outflow [24,25]. Recently, the CBS/H_2S pathway was found downregulated in the carotid sinus of SHRs. Chronic systemic treatment of H_2S by intraperitoneal injection could ameliorate the impaired sensitivity of the baroreceptor reflex and reduce arterial blood pressure in SHRs, whereas inhibition of CBS by hydroxylamine in control rats decreased carotid sinus baroreceptor sensitivity and increased arterial blood pressure [26]. However, the mechanism by which H_2S facilitates baroreceptor sensitivity has not been fully investigated.

In this study, we perfused isolated carotid sinus from SHRs to investigate the mechanism by which H_2S facilitates carotid sinus baroreceptor sensitivity.

Materials and methods

Animals

Male Wistar-Kyoto (WKY) rats and SHRs (300-320 g) were from the Vital River Laboratory Animal Technology Co. (Beijing) and were housed under standard conditions (room temperature 25 °C, humidity 60 ± 10%, lights on from 6:00 to 18:00) with free access to standard rodent chow and water. All animal procedures complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (document no. 55, 2001) and the US National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the Animal Care Committee of Hebei Medical University.

Evaluation of hemodynamic parameters in rats

Rats were anesthetized with 25% urethane (1 g/kg) by intraperitoneal injection. After an incision, the right femoral artery was separated. One end of a polyethylene catheter was inserted into the right femoral artery, and the other end was connected to a pressure transducer (PT-100) and biological experimental system (RM-6240; both Chengdu Instrument Factory, Chengdu, China) for recording mean arterial pressure (MAP). Body temperature was maintained at 37–38 °C throughout the experiment.

Perfusion of left isolated carotid sinus

The perfusion of isolated carotid sinus was as described [24,25]. Briefly, the carotid sinus areas were revealed, and the superior laryngeal nerves, bilateral aortic nerves, right carotid sinus nerve, cervical sympathetic nerves and recurrent laryngeal nerves were all cut. The common, external and internal carotid arteries and smaller arteries branching from these vessels were ligated, carefully leaving the left carotid sinus nerve undisturbed. To exclude chemoreceptor activation, the occipital artery at its origin from the external carotid artery was ligated. One catheter inserted into the left common carotid artery was an inlet tube, and another catheter inserted retrogradely into the external carotid artery was an outlet tube. Warm (37 °C) oxygenated Krebs-Henseleit (K-H) solution was used to perfuse the carotid sinus. The intrasinus pressure (ISP) was controlled by using a peristaltic pump. The ISP and arterial blood pressure were simultaneously recorded on a polygraph (RM-6240; Chengdu Instrument Factory, Chengdu, China). Perfusion of the left carotid sinus with elevated ISP produced a functional curve of the ISP–MAP relation. After perfusion of the left carotid sinus for 15 min, the ISP was kept at 100 mmHg for 20 min and then lowered to 0 mmHg rapidly. From this point, the ISP was elevated to 250 mmHg *via* a pulsatile ramp by regulating the speed of the peristaltic pump, which was automatically controlled by a program designed by our laboratory. It took 0.5 min for the ISP to be increased from 0 to 250 mmHg. This process was repeated at an interval of 5 min to check the stability of the baroreflex. The reproducibility of the experimental set-up was confirmed by the repeated arterial blood pressure decrease in response to the increase in ISP.

By perfusing the left carotid sinus with K-H solution and elevating the ISP, a functional curve for the ISP–MAP relation was constructed, and the functional parameters of baroreflex, such as threshold pressure (TP), saturation pressure (SP), equilibrium pressure (EP), peak slope (PS), reflex decrease of arterial blood pressure (RD), and operating range (OR) were determined. TP was the ISP at which arterial blood pressure began to decrease in response to the increasing ISP. SP was the ISP at which arterial blood pressure showed no further reflex decreases with an increase in ISP. OR was calculated as SP minus TP.

Experimental protocols

Before administration of drugs, K-H solution was used as a control. Four experimental treatments were used. (1) To test the effect of NaHS on the carotid baroreflex of rats, the ISP was fixed at 100 mmHg for 20 min with K-H solution as a control, and baroreflex parameters were measured. Then K-H solution containing NaHS (25, 50, or 100 μmol/L, Sigma) was used to perfuse the isolated carotid sinus for 50 min, then parameters were measured again. Finally, the carotid sinus was perfused with K-H solution to wash out the NaHS. (2) To test the effect of Bay K8644 (an agonist of calcium channels) on NaHS activity in SHRs, baroreflex parameters were examined after perfusion of NaHS (50 μmol/L) before and after treatment with Bay K8644 (500 nmol/L, Sigma) for 30 min. The effect of Bay K8644 alone on baroreflex in SHR was also detected. (3) To test the effect of glibenclamide (Gli; a K_{ATP} channel blocker) on NaHS activity in SHRs, baroreflex parameters were examined after perfusion of NaHS (50 μmol/L) before and after treatment with Gli (20 μmol/L, Sigma) for 30 min. The effect of Gli alone on baroreflex in SHR was also detected. (4) To test the effect of picrotoxin (PIC, a blocker of γ-aminobutyric acid $[GABA]_A$ receptors) on NaHS activity in SHRs, baroreflex parameters were examined after perfusion of NaHS (50 μmol/L) before and after treatment with PIC (50 μmol/L, Sigma) for 30 min. The effect of PIC alone on baroreflex in SHR was also detected.

Statistical analysis

Statistical analysis involved use of GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). The differences between groups of means were assessed by one-way ANOVA and further analyzed by the Student-Newman-Keuls test.

Data are expressed as mean ± SD. A value of $p < 0.05$ was considered statistically significant.

Results

Sensitivity of baroreceptor in carotid sinus was impaired in SHRs

SBP was significantly higher in SHRs than WKY rats (Fig. 1a). To detect baroreceptor sensitivity, we used perfusion of the isolated carotid sinus. Compared with WKY rats, SHRs showed impaired carotid sinus baroreceptor sensitivity, as demonstrated by the right and upward shift of the functional curve for the ISP–MAP relation (Fig. 1b).

H_2S facilitated baroreceptor sensitivity in carotid sinus in SHRs

In the perfused isolated carotid sinus model, NaHS (25, 50, 100 μmol/L) was added to detect the effect of H_2S on baroreceptor sensitivity in SHRs and WKY rats. NaHS perfusion ameliorated the impaired carotid sinus baroreceptor sensitivity in SHRs. The functional curve for the ISP–mean arterial pressure (MAP) relation dose-dependently moved to the left and downward, and the curve for the ISP-peak slope moved upward (Fig. 2). TP, EP and SP were decreased, and PS and RD were elevated (Table 1).

Bay K8644 blocked the effect of NaHS on carotid sinus baroreceptor sensitivity in SHRs

To test whether the Ca^{2+} channel was involved in H_2S facilitating the carotid sinus baroreflex, we added the Ca^{2+} channel agonist Bay K8644 into the perfusate and incubated with low-speed perfusion for 30 min before perfusion with NaHS. Stimulating Ca^{2+} channel activity prevented NaHS facilitating the carotid sinus baroreceptor sensitivity in SHRs, represented by a right and upward shift of the functional curve for the ISP–MAP relation, a downward shift of the curve for the ISP–peak slope relation (Fig. 3), elevated TP, EP, and SP, and reduced RD and PS (Table 2) as compared with NaHS alone. Bay K8644 alone had no effect on baroreceptor sensitivity in SHRs.

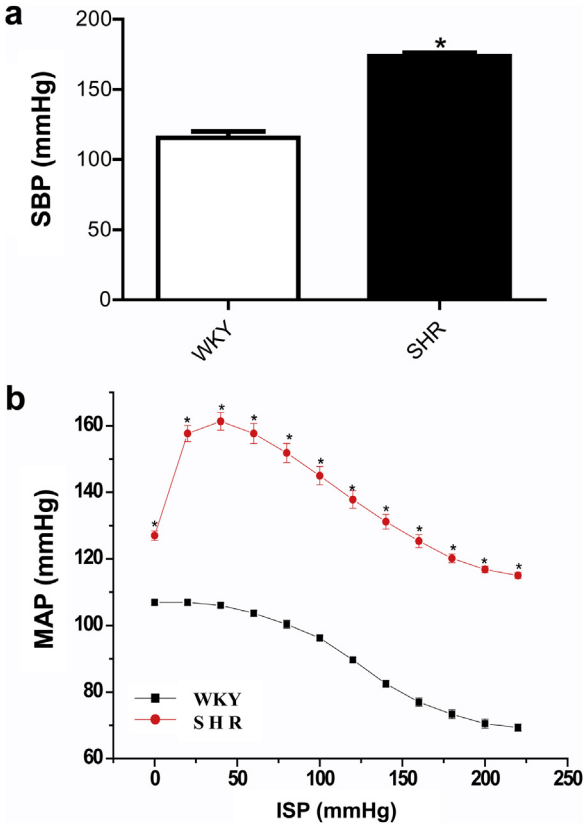

Fig. 1. Systolic blood pressure (SBP) in spontaneously hypertensive rats (SHRs) and Wistar Kyoto (WKY) rats (a), and the functional curve for the intrasinus pressure–mean arterial pressure (ISP–MAP) relation (b). Data are mean ± SD; * $p < 0.05$ *vs.* WKY, n = 10 in each group.

Glibenclamide blocked the effect of NaHS on carotid sinus baroreceptor sensitivity in SHRs

To test whether the K_{ATP} channel was involved in H_2S facilitating the carotid sinus baroreflex, we added the K_{ATP} antagonist Gli into the perfusate and incubated with low-speed perfusion for 30 min before perfusion with K-H solution containing NaHS. Inhibiting K_{ATP} activity prevented NaHS facilitating the carotid sinus baroreceptor sensitivity in SHRs, represented by a right and upward shift of the functional curve for the ISP–MAP relation, downward shift of the curve for the ISP–peak slope relation (Fig. 4), elevated TP, EP, and SP, and reduced RD and PS (Table 3) as compared with NaHS alone. Gli alone had no effect on baroreceptor sensitivity in SHRs.

PIC blocked the effect of NaHS on carotid sinus baroreceptor sensitivity in SHRs

To test whether $GABA_A$ receptors were involved in H_2S facilitating the carotid sinus baroreflex, we added the $GABA_A$ receptor blocker PIC into the perfusate and incubated with low-speed perfusion for 30 min before perfusion with NaHS. Blocking $GABA_A$ receptors prevented NaHS facilitating the carotid sinus baroreceptor sensitivity in SHRs, represented by a right and upward shift of the functional curve for the ISP–MAP relation, downward shift of the curve for the ISP–peak slope relation (Fig. 5), elevated TP, EP, and SP, and reduced RD and PS (Table 4) as compared with NaHS alone. PIC alone had no effect on baroreceptor sensitivity in SHRs.

Discussion

By perfusion of the isolated carotid sinus in rats, we found that as compared with WKY rats, in SHRs, the functional curve of the ISP–MAP relation moved right and upward; the curve of ISP–peak slope relation moved downward; TP, EP and SP were increased; and RD and PS was decreased, for impaired baroreceptor reflex. All these features could be ameliorated by NaHS treatment. The effect of NaHS on carotid sinus baroreceptor reflex was blocked by the K_{ATP} antagonist Gli, Ca^{2+} channel agonist Bay K8644 and $GABA_A$ receptor blocker PIC. These findings should provide new targets for preventing and treating hypertension.

Our and other previous articles have confirmed that H_2S facilitates baroreceptor sensitivity in physiological conditions [24] and also rescues the impaired sensitivity of the baroreceptor in hypertension [26]. Endogenous H_2S levels and expression of CSE, a crucial enzyme producing H_2S in the cardiovascular system, were significantly lower in SHRs than WKY rats, accompanied by decreased sensitivity of the baroreceptor. Both daily chronic supplement of a general H_2S donor, NaHS, or acute NaHS treatment in perfusate rescued the impaired sensitivity of the baroreceptor in SHRs [26]. These results suggest the key regulatory role of H_2S in baroreceptor sensitivity. Consistent with previous studies, here we also demonstrate that H_2S rescued the impaired ISP–MAP and ISP-peak slope relations, increased TP, EP and SP, and deceased RD and PS in SHRs. These results confirm the ameliorating effect of H_2S on impaired baroreceptor sensitivity in hypertension.

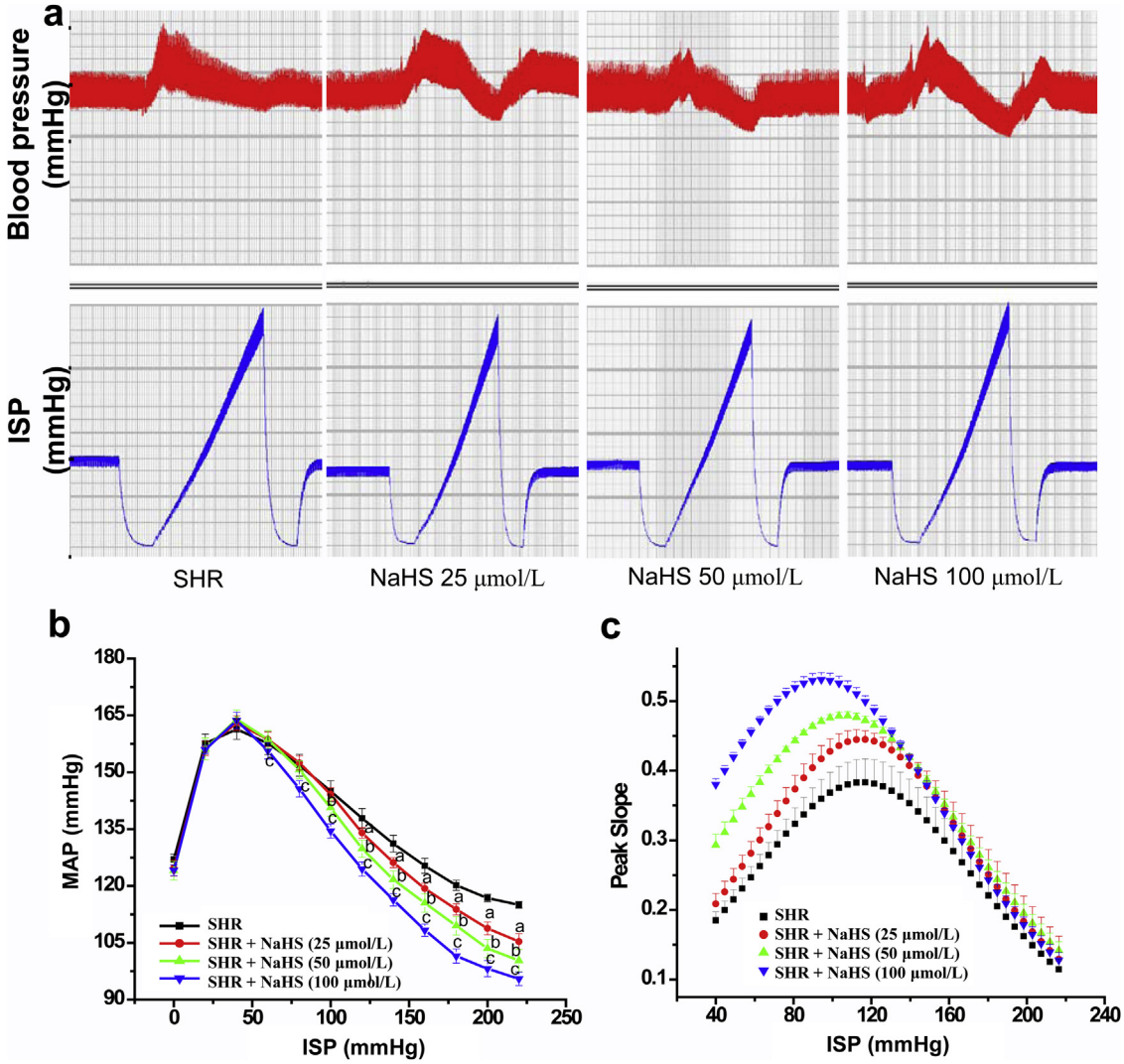

Fig. 2. NaHS treatment dose-dependently facilitated carotid sinus baroreceptor sensitivity in SHRs. (a) Representative images of arterial blood pressure and ISP. (b) Functional curve for ISP-MAP relation. (c) Curve for ISP-peak slope relation. Data are mean ± SD; a, $p < 0.05$ *vs.* SHR. b, $p < 0.05$ *vs.* NaHS 25 μmol/L. c, $p < 0.05$ *vs.* NaHS 50 μmol/L, n = 10 in each group.

Table 1
Effects of NaHS on the parameters of carotid sinus baroreceptor in SHR.

	TP (mmHg)	EP (mmHg)	SP (mmHg)	PS	OR (mmHg)	RD (mmHg)
SHR	63.22 ± 0.74	133.77 ± 1.48	204.83 ± 2.90	0.37 ± 0.01	141.61 ± 3.12	12.37 ± 0.90
NaHS (μmol/L)						
25	60.20 ± 1.10[a]	130.30 ± 0.89[a]	200.10 ± 1.51[a]	0.45 ± 0.01[a]	139.90 ± 1.78	18.93 ± 0.48[a]
50	55.53 ± 0.68[a,b]	127.59 ± 1.56[a,b]	197.09 ± 1.48[a,b]	0.48 ± 0.01[a,b]	141.56 ± 1.69	23.28 ± 1.09[a,b]
100	52.36 ± 0.47[a,b,c]	122.91 ± 1.10[a,b,c]	191.81 ± 1.56[a,b,c]	0.53 ± 0.01[a,b,c]	139.15 ± 1.76	28.88 ± 0.85[a,b,c]

TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; PS, peak slope; OR, operating range; RD, reflex decrease of arterial blood pressure.
[a] $p < 0.05$ *vs.* SHR.
[b] $p < 0.05$ *vs.* NaHS 25 μmol/L.
[c] $p < 0.05$ *vs.* NaHS 50 μmol/L. n = 10 in each group.

L-type Ca^{2+} channels play an important role in vascular contraction and are abundantly expressed in arterial smooth muscle cells. Detected by pressure myography, inhibiting the L-type Ca^{2+} channels reduces arterial tone when the vessel is depolarized [27]. L-type Ca^{2+} channel inhibition also mediates the diastolic effect of NaHS on arteries [28]. Bay K8644 is well known as a specific agonist of L-type Ca^{2+} channels. Our previous studies found that Bay K8644 could block the enhancing effect of H_2S on the baroreflex in Sprague-Dawley rats [20,22]. Here, we used Bay K8644 to evaluate whether L-type Ca^{2+} channels mediate the effect of H_2S on the baroreceptor reflex in SHRs. The facilitating effects of H_2S on the baroreceptor reflex in SHRs was significantly blocked by Bay K8644, so closing L-type Ca^{2+} channels mediated the effect of H_2S on smooth muscle cells and sinus wall dilation and then facilitated the baroreceptor reflex.

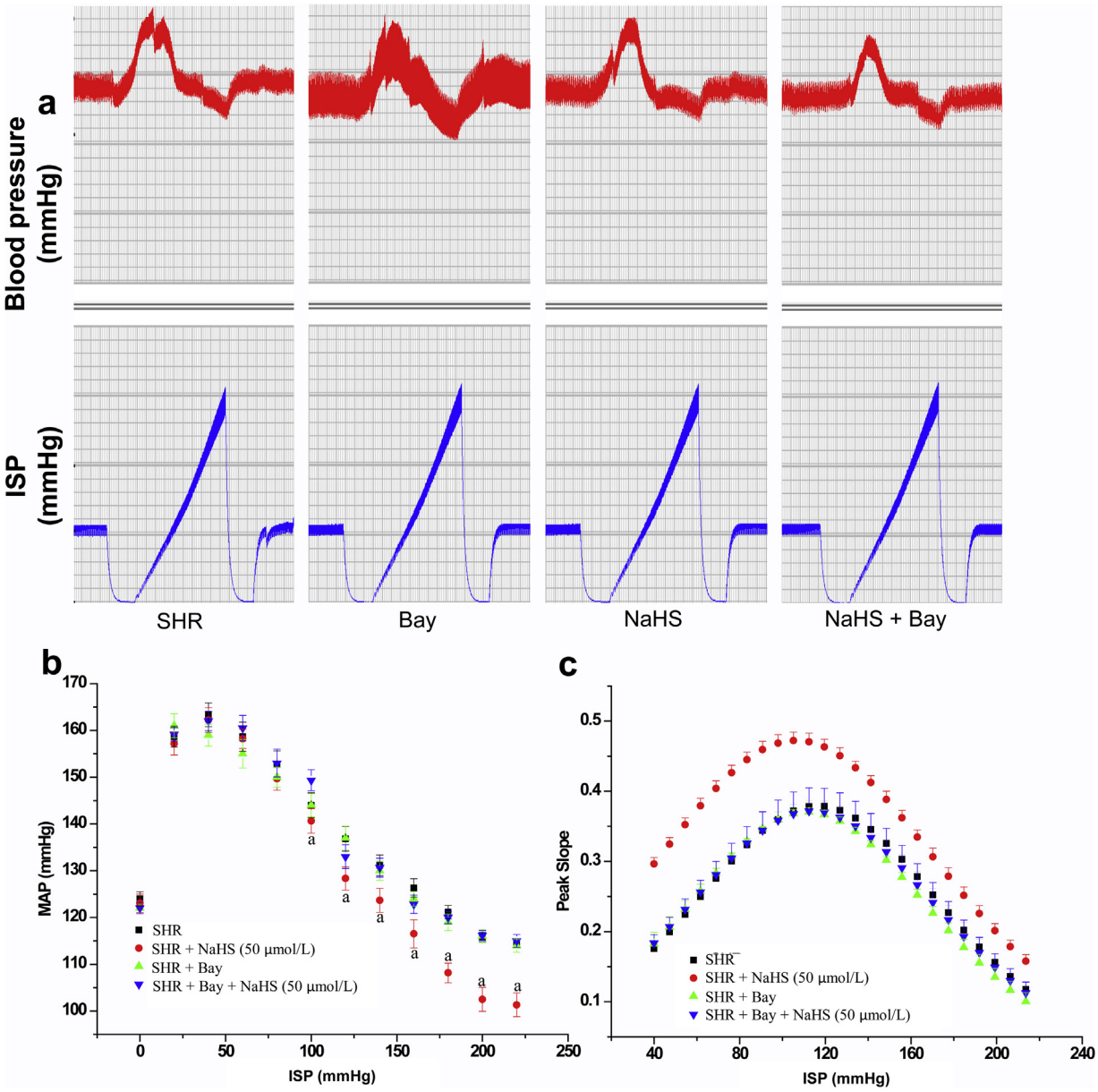

Fig. 3. Bay K8644 treatment blocked the effect of NaHS on carotid sinus baroreceptor sensitivity in SHRs. (a) Representative images of arterial blood pressure and ISP. (b) Functional curve for ISP–MAP relation. (c) Curve for ISP–peak slope relation. Data are mean ± SD; a, $p < 0.05$ *vs.* SHR, n = 10 in each group.

Table 2
Bay K8644 blocked the facilitated effect of NaHS on baroreceptor sensitivity in SHR.

	TP (mmHg)	EP (mmHg)	SP (mmHg)	PS	OR (mmHg)	RD (mmHg)
SHR(A)	62.91 ± 0.74	133.47 ± 1.36	203.02 ± 2.26	0.37 ± 0.01	140.11 ± 2.29	12.01 ± 1.03
NaHS(A)	54.77 ± 0.93[a]	128.19 ± 1.37[a]	198.03 ± 1.11[a]	0.49 ± 0.01[a]	142.80 ± 1.95	22.71 ± 1.32[a]
Bay	62.98 ± 1.10[b]	133.92 ± 1.06[b]	204.77 ± 1.56[b]	0.37 ± 0.01[b]	141.86 ± 1.56	12.05 ± 1.07[b]
Bay + NaHS	63.37 ± 1.06[b]	133.32 ± 1.85[b]	204.32 ± 1.09[b]	0.36 ± 0.01[b]	141.40 ± 1.48	11.88 ± 1.33[b]

TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; PS, peak slope; OR, operating range; RD, reflex decrease of arterial blood pressure.
[a] $p < 0.05$ *vs.* SHR.
[b] $p < 0.05$ *vs.* NaHS. n = 10 in each group.

Ca^{2+} influx *via* L-type Ca^{2+} channels can be prevented by K_{ATP} channels [29,30]. Several studies have demonstrated that K_{ATP} channels mediate the vasodilatation of H_2S in mesenteric arteries and aorta [31,32]. The vasorelaxation caused by H_2S is attenuated by a high concentration of K^+ in the aortic ring, and Gli or 5-hydroxydecanoate (5-HD), K_{ATP} channel antagonists, could effectively block the H_2S-induced vasodilation [33–35]. Also, in phenylephrine-precontracted human internal mammary arteries, the relaxant response to H_2S was blocked by Gli [36,37]. Therefore, we also assessed whether K_{ATP} channels were associated with the ameliorative effect of H_2S on the impaired baroreceptor reflex in SHRs. We found that Gli attenuated the effect of H_2S. H_2S might

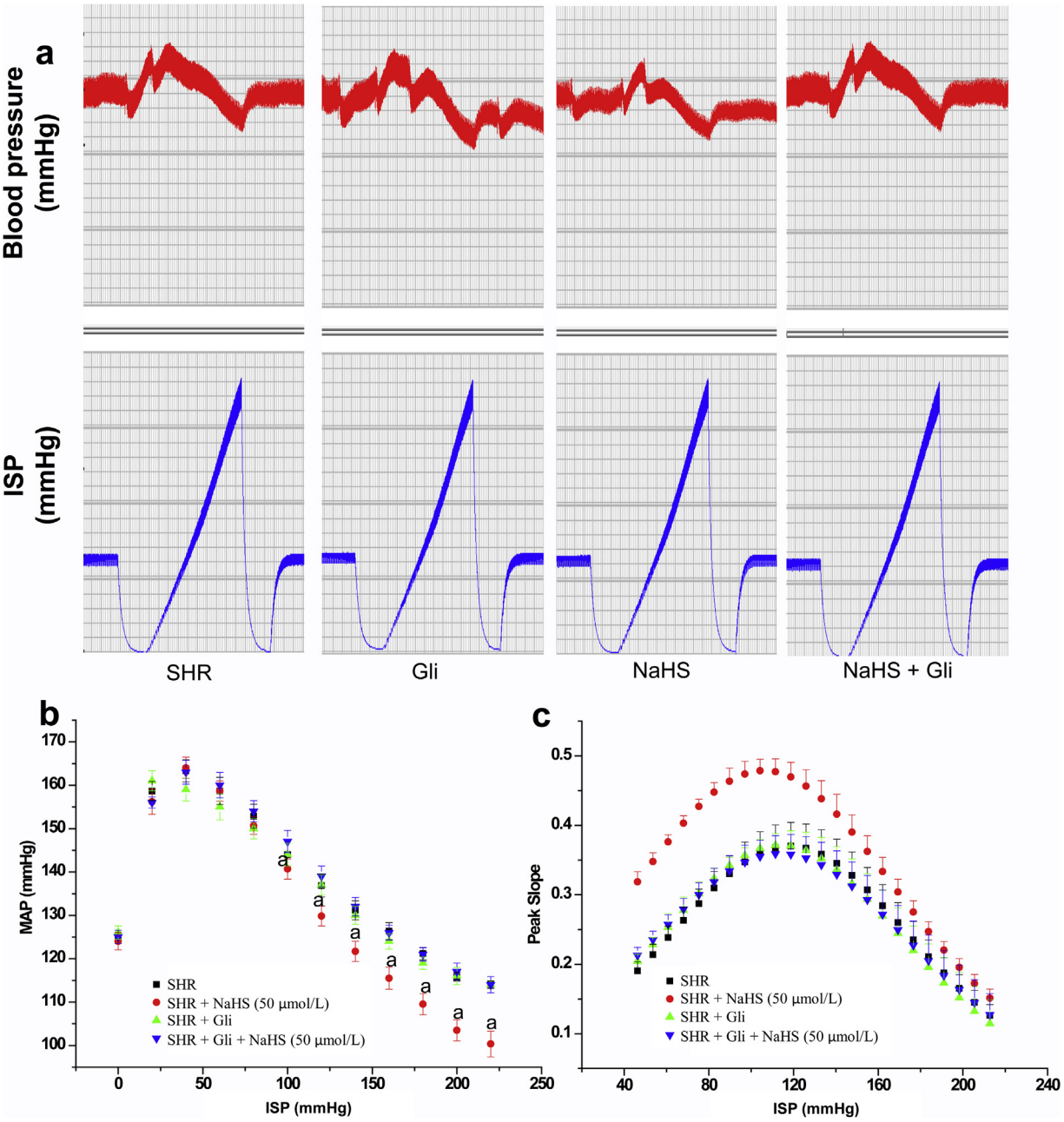

Fig. 4. Glibenclamid treatment blocked the effect of NaHS on carotid sinus baroreceptor sensitivity in SHRs. (a) Representative images of arterial blood pressure and ISP. (b) Functional curve for ISP–MAP relation. (c) Curve for ISP–peak slope relation. Data are mean ± SD; a, $p < 0.05$ *vs.* SHR, n = 10 in each group.

Table 3
Glibenclamide blocked the facilitated effect of NaHS on baroreceptor sensitivity in SHR.

	TP (mmHg)	EP (mmHg)	SP (mmHg)	PS	OR (mmHg)	RD (mmHg)
SHR(A)	63.52 ± 0.57	133.92 ± 1.33	204.77 ± 2.58	0.37 ± 0.01	141.25 ± 2.65	12.36 ± 0.91
NaHS(A)	55.83 ± 0.76[a]	128.04 ± 0.93[a]	196.43 ± 0.89[a]	0.48 ± 0.01[a]	140.60 ± 1.58	23.47 ± 1.00[a]
Gil	63.37 ± 0.89[b]	132.32 ± 1.33[b]	203.47 ± 3.36[b]	0.36 ± 0.01[b]	140.10 ± 3.34	11.91 ± 0.90[b]
Gil + NaHS	62.52 ± 0.99 [b]	133.32 ± 0.68[b]	204.02 ± 2.78[b]	0.36 ± 0.01[b]	141.50 ± 3.01	12.25 ± 0.95[b]

TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; PS, peak slope; OR, operating range; RD, reflex decrease of arterial blood pressure.
[a] $p < 0.05$ *vs.* SHR.
[b] $p < 0.05$ *vs.* NaHS. n = 10 in each group.

activate the K_{ATP} channels and stimulate K^+ outflow, followed by hyperpolarisation, which subsequently blocks Ca^{2+} influx *via* inhibiting L-type voltage-gated calcium channels and prevents excess Ca^{2+} influx in vascular smooth muscle cells.

$GABA_A$ receptor is located on vascular smooth muscle cells and is an Cl^- channel. When the $GABA_A$ receptor is activated, an increase in intracellular Cl^- leads to vascular relaxation [38,39]. $GABA_A$ receptors and K_{ATP} channels can interact, and activation of

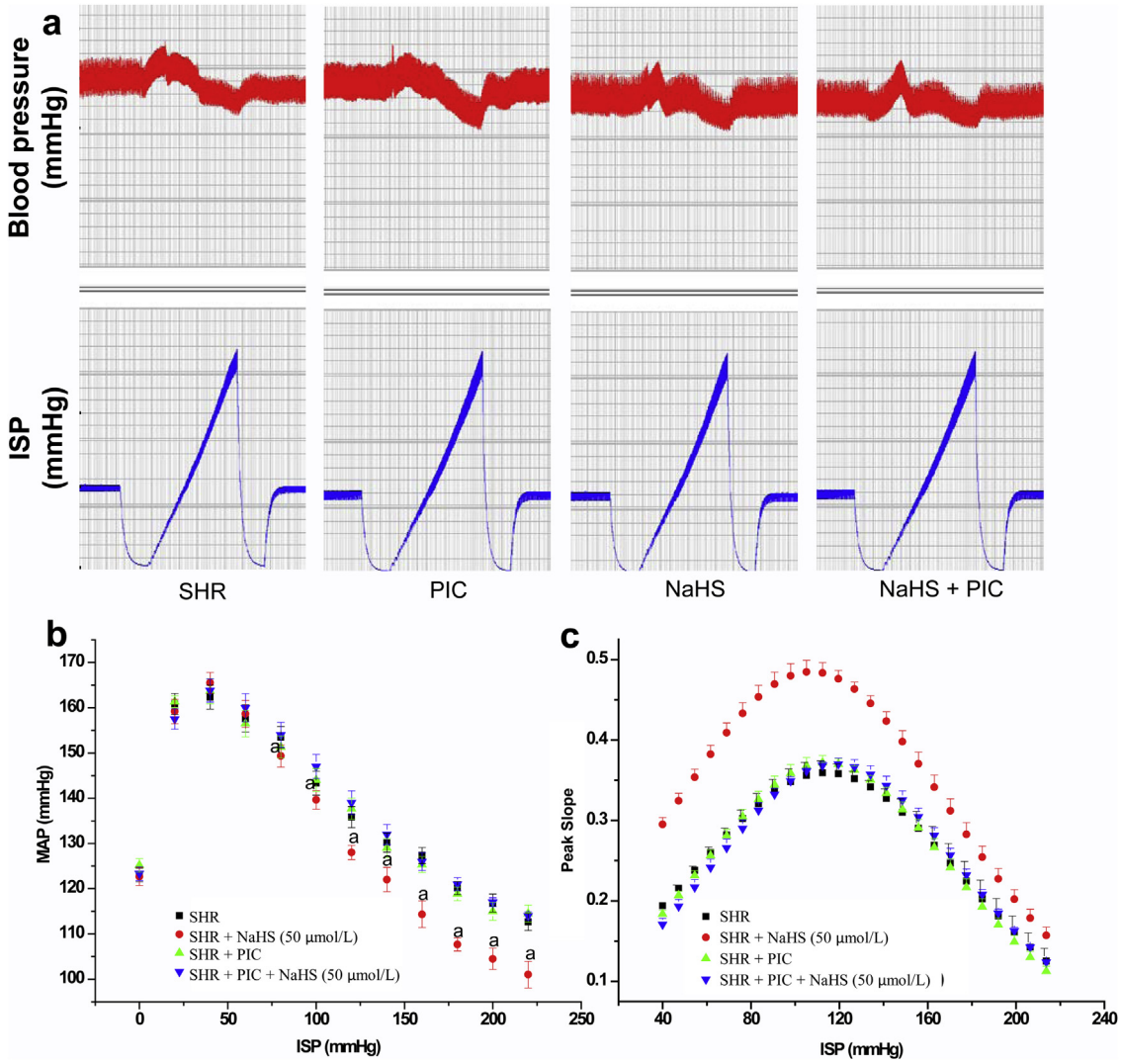

Fig. 5. PIC treatment blocked the effect of NaHS on carotid sinus baroreceptor sensitivity in SHRs. (a) Representative images of arterial blood pressure and ISP. (b) Functional curve for ISP–MAP relation. (c) Curve for ISP–peak slope relation. Data are mean ± SD; a, $p < 0.05$ *vs.* SHR, n = 10 in each group.

Table 4
PIC blocked the facilitated effect of NaHS on baroreceptor sensitivity in SHR.

	TP (mmHg)	EP (mmHg)	SP (mmHg)	PS	OR (mmHg)	RD (mmHg)
SHR(A)	62.91 ± 0.93	132.92 ± 1.20	203.29 ± 2.11	0.37 ± 0.01	140.38 ± 2.23	12.30 ± 0.92
NaHS(A)	56.13 ± 1.06[a]	128.19 ± 1.25[a]	196.73 ± 0.89[a]	0.49 ± 0.01[a]	140.60 ± 1.36	22.37 ± 1.04[a]
PIC	63.97 ± 0.77[b]	133.47 ± 1.48[b]	205.23 ± 2.60[b]	0.37 ± 0.01[b]	140.26 ± 2.52	12.37 ± 0.93[b]
PIC + NaHS	63.35 ± 1.06[b]	133.77 ± 1.48[b]	204.47 ± 1.76[b]	0.36 ± 0.01[b]	141.12 ± 2.36	12.27 ± 0.93[b]

TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; PS, peak slope; OR, operating range; RD, reflex decrease of arterial blood pressure.
[a] $p < 0.05$ *vs.* SHR.
[b] $p < 0.05$ *vs.* NaHS. n = 10 in each group.

K_{ATP} channels is involved in the $GABA_A$ receptor effect [40]. Therefore, we determined whether $GABA_A$ receptors mediate the facilitating effect of H_2S on baroreceptor reflex. The $GABA_A$ receptor blocker PIC could attenuate the effect of H_2S in SHRs. Along with other results, our findings suggest that H_2S ameliorated the impaired baroreceptor reflex by stimulating $GABA_A$ receptors, followed by opening K_{ATP} channels and closing L-type Ca^{2+} channels.

Because we could not directly detect the state of the channels by patch clamp, we could not confirm the causal relation among H_2S, $GABA_A$ receptors, K_{ATP} channels and L-type Ca^{2+} channels, which is a major limitation of the study and should be investigated in the future. Although H_2S may ameliorate the impaired baroreceptor reflex in SHRs *via* a $GABA_A$ receptor–K_{ATP} channel–L-type Ca^{2+} channel pathway, the direct effect of H_2S on the channels could not be excluded. The thorough mechanism by which H_2S regulates the channels is worth exploring.

Conclusions

H2S could ameliorate the impaired baroreceptor reflex in SHRs, which might be associated with activating $GABA_A$ receptors,

opening K_{ATP} channels and closing L-type Ca^{2+} channels. These might suggest new targets for preventing and treating hypertension.

Conflicts of interest

The authors declare no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31671185, 81770499, 31871154, 91849120), the Natural Science Foundation of Hebei [CN] (No. H2016206264) and Research Foundation for Higher Education (ZD2018068).

References

[1] Lohmeier TE, Iliescu R. The baroreflex as a long-term controller of arterial pressure. Physiology (Bethesda) 2015;30:148–58.
[2] Labrova R, Honzıkova N, Maderova E, Vysocanova P, Novakova Z, Zavodna E, et al. Age-dependent relationship between the carotid intimamedia thickness, baroreflex sensitivity, and the inter-beat interval in normotensive and hypertensive subjects. Physiol Res 2005;54:593–600.
[3] Ketch T, Biaggioni I, Robertson RM, Robertson D. Four faces of baroreflex failure: hypertensive crisis, volatile hypertension, orthostatic tachycardia, and malignant vagotonia. Circulation 2002;105:2518–23.
[4] Freitas J. Carotid sinus syndrome. Management and approach. Rev Port Cardiol 2004;23:903–10.
[5] Grassi G, Mark A, Esler M. The sympathetic nervous system alterations in human hypertension. Circ Res 2015;116:976–90.
[6] Smith S, Rossignol P, Willis S, Zannad F, Mentz R, Pocock S, et al. Neural modulation for hypertension and heart failure. Int J Cardiol 2016;214:320–30.
[7] Tomiyama H, Matsumoto C, Kimura K, Odaira M, Shiina K, Yamashina A. Pathophysiological contribution of vascular function to baroreflex regulation in hypertension. Circ J 2014;78:1414–9.
[8] Lohmeier TE, Iliescu R, Tudorancea I, Cazan R, Cates AW, Georgakopoulos D, et al. Chronic interactions between carotid baroreceptors and chemoreceptors in obesity hypertension. Hypertension 2016;68:227–35.
[9] Chapleau MW, Li Z, Meyrelles SS, Ma X, Abboud FM. Mechanisms determining sensitivity of baroreceptor afferents in health and disease. Ann N Y Acad Sci 2001;940:1–19.
[10] Shimoura CG, Lincevicius GS, Nishi EE, Girardi AC, Simon KA, Bergamaschi CT, et al. Increased dietary salt changes baroreceptor sensitivity and intrarenal renin-angiotensin system in Goldblatt Hypertension. Am J Hypertens 2017;30:28–36.
[11] Prasad AM, Morgan DA, Nuno DW, Ketsawatsomkron P, Bair TB, Venema AN, et al. Calcium/calmodulin-dependent kinase II inhibition in smooth muscle reduces angiotensin II-induced hypertension by controlling aortic remodeling and baroreceptor function. J Am Heart Assoc 2015;4:e001949.
[12] Sata Y, Kawada T, Shimizu S, Kamiya A, Akiyama T, Sugimachi M. Predominant role of neural arc in sympathetic baroreflex resetting of spontaneously hypertensive rats. Circ J 2015;79:592–9.
[13] Yoruk A, Bisognano JD, Gassler JP. Baroreceptor stimulation for resistant hypertension. Am J Hypertens 2016;29:1319–24.
[14] Chen CQ, Xin H, Zhu YZ. Hydrogen sulfide: third gaseous transmitter, but with great pharmacological potential. Acta Pharmacol Sin 2007;28:1709–16.
[15] Kimura H. Hydrogen sulfide: its production, release and functions. Amino Acids 2010;41:113–21.
[16] Xie ZZ, Liu Y, Bian JS. Hydrogen sulfide and cellular redox homeostasis. Oxid Med Cell Longev 2016;2016:6043038.
[17] Sun Y, Huang Y, Zhang R, Chen Q, Chen J, Zong Y, et al. Hydrogen sulfide upregulates KATP channel expression in vascular smooth muscle cells of spontaneously hypertensive rats. J Mol Med 2015;93:439–55.
[18] Li L, Liu D, Bu D, Chen S, Wu J, Tang C, et al. Brg1-dependent epigenetic control of vascular smooth muscle cell proliferation by hydrogen sulfide. Biochim Biophys Acta 2013;1833:1347–55.
[19] Zhong G, Chen F, Cheng Y, Tang C, Du J. The role of hydrogen sulfide in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase. J Hypertens 2003;21:1879–85.
[20] Guo Q, Jin S, Wang XL, Wang R, Xiao L, He RR, et al. Hydrogen sulfide in the rostral ventrolateral medulla inhibits sympathetic vasomotor tone through ATP-sensitive K+ channels. J Pharmacol Exp Ther 2011;338:458–65.
[21] Yan H, Du JB, Tang CS. The possible role of hydrogen sulfide on the pathogenesis of spontaneous hypertension in rats. Biochem Biophys Res Commun 2004;313:22–7.
[22] Xiao L, Dong JH, Jin S, Xue HM, Guo Q, Teng X, et al. Hydrogen sulfide improves endothelial dysfunction via downregulating BMP4/COX-2 pathway in rats with hypertension. Oxid Med Cell Longev 2016;2016:8128957.
[23] Jin HF, Sun Y, Liang JM, Tang CS, Du JB. Hypotensive effects of hydrogen sulfide via attenuating vascular inflammation in spontaneously hypertensive rats. Chin J Cardiol. 2008;36:541–5.
[24] Xiao L, Wu YM, Zhang H, Liu YX, He RR. Hydrogen sulfide facilitates carotid sinus baroreflex in anesthetized rats. Acta Pharmacol Sin 2006;27:294–8.
[25] Guo Q, Wu Y, Xue H, Xiao L, Jin S, Wang R. Perfusion of isolated carotid sinus with hydrogen sulfide attenuated the renal sympathetic nerve activity in anesthetized male rats. Physiol Res 2016;65:413–23.
[26] Yu W, Liao Y, Huang Y, Chen SY, Sun Y, Sun C, et al. 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 2017;6: pii: e004971.
[27] Abd El-Rahman RR, Harraz OF, Brett SE, Anfinogenova Y, Mufti RE, Goldman D, et al. Identification of L- and T-type Ca^{2+} channels in rat cerebral arteries: role in myogenic tone development. Am J Physiol Heart Circ Physiol 2013;304:H58–71.
[28] Tian XY, Wong WT, Sayed N, Luo J, Tsang SY, Bian ZX, et al. NaHS relaxes rat cerebral artery in vitro via inhibition of l-type voltage-sensitive Ca2+ channel. Pharmacol Res 2012;65:239–46.
[29] Cifelli C, Boudreault L, Gong B, Bercier JP, Renaud JM. Contractile dysfunctions in ATP-dependent K+ channel-deficient mouse muscle during fatigue involve excessive depolarization and Ca2+ influx through L-type Ca2+ channels. Exp Physiol 2008;93:1126–38.
[30] Jovanovic S, Jovanovic A. Pinacidil prevents membrane depolarisation and intracellular Ca2+ loading in single cardiomyocytes exposed to severe metabolic stress. Int J Mol Med 2001;7:639–43.
[31] Cheng Y, Ndisang JF, Tang G, Cao K, Wang R. Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. Am J Physiol Heart Circ Physiol 2004;287:H2316–23.
[32] Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. EMBO J 2001;20:6008–16.
[33] Ali MY, Ping CY, Mok YY, Ling L, Whiteman M, Bhatia M, et al. Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide? Br J Pharmacol 2006;149:625–34.
[34] Johansen D, Ytrehus K, Baxter GF. Exogenous hydrogen sulfide (H2S) protects against regional myocardial ischemia-reperfusion injury–Evidence for a role of K ATP channels. Basic Res Cardiol 2006;101:53–60.
[35] Zhu YZ, Wang ZJ, Ho P, Loke YY, Zhu YC, Huang SH, et al. Hydrogen sulfide and its possible roles in myocardial ischemia in experimental rats. J Appl Physiol 1985;2007(102):261–8.
[36] Webb GD, Lim LH, Oh VM, Yeo SB, Cheong YP, Ali MY, et al. Contractile and vasorelaxant effects of hydrogen sulfide and its biosynthesis in the human internal mammary artery. J Pharmacol Exp Ther 2008;324:876–82.
[37] Tang G, Wu L, Liang W, Wang R. Direct stimulation of K(ATP) channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. Mol Pharmacol 2005;68:1757–64.
[38] Kamran M, Bahrami A, Soltani N, Keshavarz M, Farsi L. GABA-induced vasorelaxation mediated by nitric oxide and GABAA receptor in non diabetic and streptozotocin-induced diabetic rat vessels. Gen Physiol Biophys 2013;32:101–6.
[39] Bhatt S, Zalcman S, Hassanain M, Siegel A. Cytokine modulation of defensive rage behavior in the cat: role of GABAA and interleukin-2 receptors in the medial hypothalamus. Neuroscience 2005;133:17–28.
[40] Sheikhi M, Shirzadian A, Dehdashtian A, Amiri S, Ostadhadi S, Ghasemi M, et al. Involvement of ATP-sensitive potassium channels and the opioid system in the anticonvulsive effect of zolpidem in mice. Epilepsy Behav 2016;62:291–6.