Perfusion of Isolated Carotid Sinus With Hydrogen Sulfide Attenuated the Renal Sympathetic Nerve Activity in Anesthetized Male Rats

Qi GUO[1], Yuming WU[1], Hongmei XUE[1], Lin XIAO[1], Sheng JIN[1], Ru WANG[1]

[1]Department of Physiology, Institute of Basic Medicine, Hebei Medical University, Shijiazhuang, China

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Summary

The purpose of the present study was to define the indirect central effect of hydrogen sulfide (H_2S) on baroreflex control of sympathetic outflow. Perfusing the isolated carotid sinus with sodium hydrosulfide (NaHS), a H_2S donor, the effect of H_2S was measured by recording changes of renal sympathetic nerve activity (RSNA) in anesthetized male rats. Perfusion of isolated carotid sinus with NaHS (25, 50, 100 μmol/l) dose and time-dependently inhibited sympathetic outflow. Preconditioning of glibenclamide (20 μmol/l), a ATP-sensitive K^+ channels (K_{ATP}) blocker, the above effect of NaHS was removed. With 1, 4-dihydro-2, 6-dimethyl-5-nitro-4-(2-[trifluoromethyl] phenyl) pyridine-3-carboxylic acid methyl ester (Bay K8644, 500 nmol/l) pretreatment, which is an agonist of L-calcium channels, the effect of NaHS was eliminated. Perfusion of cystathionine γ-lyase (CSE) inhibitor, DL-propargylglycine (PPG, 200 μmol/l), increased sympathetic outflow. The results show that exogenous H_2S in the carotid sinus inhibits sympathetic outflow. The effect of H_2S is attributed to opening K_{ATP} channels and closing the L-calcium channels.

Key words

Hydrogen sulfide • Renal sympathetic nerve activity • Isolated carotid sinus • Baroreflex • ATP-sensitive K^+ channels

Corresponding author

Y. Wu, Department of Physiology, Institute of Basic Medicine, Hebei Medical University, 361 Zhongshan East Road, Shijiazhuang 050017, China. E-mail: wuyum@yahoo.com

Introduction

Hydrogen sulfide (H_2S) is considered as a toxic gas for the past decades. However, H_2S has recently been known as a new gaseous messenger molecule in many physiological and pathophysiologic processes, similar to the other gastransmitters, nitric oxide (NO) and carbon monoxide (CO) (Eto and Kimura 2002, Fiorucci *et al.* 2006, Kimura 2002). Endogenous H_2S is produced from cysteine by pyridoxal-5'-phosphate (PLP)-dependent enzymes, including cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Stipanuk 2004, Stipanuk and Beck 1982). 3-mercaptopyruvate sulfurtransferase (3-MST), a PLP-independent enzyme, is additional possible way to produce H_2S (Shibuya *et al.* 2009, Stipanuk and Beck 1982). The distribution of these enzymes is tissue specific, and CSE is the mainly enzyme which produced hydrogen sulfide in the cardiovascular system (Zhao *et al.* 2001).

A growing number of reports suggested H_2S is involved in many fundamental physiopathology processes including nociception, neuroprotection, cardiovascular functions, inflammation and apoptosis (Li and Moore 2008, Wang 2003, Zhao *et al.* 2001). H_2S can activate ATP-sensitive K^+ channels (K_{ATP}) in smooth muscle to induce vasodilation in the blood vessel (Zhao *et al.* 2001). Kubo *et al.* (2007) also have shown sodium hydrosulfide (NaHS), a H_2S donor, causes relaxation of aorta in rat and mouse while the relaxation effect of NaHS is partially mediated by K_{ATP} channels (Kubo *et al.* 2007). Moreover, H_2S can regulate Ca^{2+} homeostasis of human vascular endothelial, suggesting that H_2S may

Fax +420 241 062 164, e-mail: physres@biomed.cas.cz, www.biomed.cas.cz/physiolres

decrease blood pressure *via* the above effect (Bauer *et al.* 2010). There is a result suggests that H_2S play a negative chronotropic action on pacemaker cells in sinoatrial nodes of rabbits. These effects are likely due to opening K_{ATP} channels and increasing in potassium efflux (Xu *et al.* 2008). H_2S also activates transient receptor potential ankyrin 1 (TRPA1) channels in sensory nerves and then causes vasodilatation in isolated small pressurized mesenteric arteries from rats (White *et al.* 2013).

Arterial baroreflex has been considered to be the major negative-feedback system that steadies systemic arterial pressure (AP) against pressure disturbance. The baroreflex consists of two subsystems: neural and peripheral arc (Ikeda *et al.* 1996). The neural arc characterizes the input-output relation of baroreceptor pressure and sympathetic nerve activity (SNA), while the peripheral arc represents the relationship between SNA and AP (Ikeda *et al.* 1996, Kawada *et al.* 2005). In our laboratory, we have demonstrated that H_2S may dose-dependently facilitate the carotid sinus baroreflex and baroreceptor activity (Xiao *et al.* 2006, 2007). We also have demonstrated H_2S inhibits sympathetic vasomotor tone by opening a K_{ATP} channels in the rostral ventrolateral medulla (RVLM) (Guo *et al.* 2011). Nevertheless, the indirect central effects of H_2S on baroreflex control of sympathetic outflow have not been reported.

In the present study, we want to reveal the indirect central effect of perfusion of isolated carotid sinus with NaHS on sympathetic outflow by recording renal sympathetic nerve activity (RSNA) and to define the possible mechanisms.

Materials and Methods

Animal

Male Sprague-Dawley rats, weighting 290-310 g Grade II, were obtained from the Experimental Animal Center of Hebei Province, China. Rats were housed in 12 h light/dark cycle. Food and water were freely available. The animals were adapted to the environment for about a week before the experiment. All protocols and procedures used in this study were reviewed and approved by the Institutional Animal Ethics Committee of Hebei Medical University and in accordance with the Guide for the Care and Use of Laboratory Animals (1985, NIH).

Recording of RSNA

General operation was performed as described before (Guo *et al.* 2011). Anesthetized was induced by urethane (1.0 g/kg i.p.). The trachea was cannulated for breathing. Body temperature was maintained at 37-38 °C by using a thermostatic bed. A left flank incision was made and then the left kidney was visualized by retroperitoneal. One branch of the renal sympathetic nerves was hooked up with a bipolar platinum electrode for recording efferent potential. The distal end of the nerve was clamped to eliminate the afferent activity and then immersed in warm (37 °C) liquid paraffin oil to keep moist. The recording electrode was connected with a set of biological polygraph (RM6240BD, Chengdu Technology) to record RSNA simultaneously. Integrate of RSNA was automatically by the computer and the integrated time was 0.16 s. At the end of the experiment, the head end of the nerve was clamped to get the noise level of RSNA.

Perfusion of left isolated carotid sinus

Isolated carotid sinus was perfused with a method as we previously reported (Xiao *et al.* 2006). Turn the trachea and esophagus to head in order to fully expose the areas of carotid sinus. The left carotid sinus nerve was carefully retention. The rest buffer nerves and other nerves around the carotid sinus were all cut. The vascular of the left carotid sinus was isolated from systemic circulation by ligating the external and internal carotid arteries and its branches originating from the carotid sinus regions. In order to exclude the effect of chemoreceptors, occipital artery was ligated at its initial part that preventing activation of chemoreceptor secondary to decrease carotid sinus pressure. Plastic catheters were placed into the left carotid artery and the external carotid artery respectively served as inlet and outlet tubes. The carotid sinus was then perfused with warm (37 ºC) oxygenated modified Krebs-Henseleit (K-H) solution (mmol/l: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.6, pH 7.35-7.45) bubbled with 95 % O_2 and 5 % CO_2.

The inlet tube connected with a pressure transducer (YPJ01, Chengdu Technology) was recorded intrasinus pressure (ISP). ISP was controlled by a peristaltic pump and a program designed by our laboratory (Xiao *et al.* 2006). After finished the above operation, ISP was kept at 100 mm Hg for 20 min. When checked the baroreflex, ISP was lowered to 0 mm Hg rapidly and then increased to 250 mm Hg in the form of

pulsatile ramp. It took 0.5 min for ISP to be increased from 0 to 250 mm Hg. ISP and RSNA were simultaneously recorded on a polygraph (RM6240BD, Chengdu Technology). Every 5 min, repeat the above process to check the stability of the baroreflex. Reproducibility drops of RSNA in response to the increase in ISP were documented.

Protocols

The left carotid sinus was perfused with K-H solution. As the ISP change from 0 to 250 mm Hg, we observe the difference between the maximum and the minor integral value of RSNA which was recorded as a 100 %.

Each experimental group was performed on six rats. ISP was fixed at 100 mm Hg for 20 min with K-H solution. Baseline ISP-RSNA was measured when the isolated carotid sinus was perfused with K-H solution. After we got the baseline ISP-RSNA, then NaHS (25, 50 or 100 μmol/l) were added into K-H solution. The isolated carotid sinus was perfused with the solution for 50 min, and then ISP-RSNA was measured again. The concentrations of NaHS were perfused in random order. One dose was performed on one rat.

We tested the effect of glibenclamide, a K_{ATP} channels-antagonist (20 μmol/l), on NaHS-induce effect of ISP-RSNA. We first perfused the isolated carotid sinus with NaHS (50 μmol/l) to observe the effect on ISP-RSNA. After the ISP-RSNA returned to baseline, glibenclamide (20 μmol/l) was perfused for 15 min before another dose of NaHS (50 μmol/l). To determine whether Ca^{2+} was involved in the effect of NaHS on ISP-RSNA, Bay K8644 (500 nmol/l), an agonist of Ca^{2+} channels, was perfused into the isolated carotid sinus to open the Ca^{2+} channels. To further determine the effect of endogenous H_2S on ISP-RSNA, we compared ISP-RSNA recorded during the administration of cystathionine γ-lyase (CSE) inhibitor, DL-propargylglycine (PPG) (200 μmol/l).

Western blot analysis

We used Western blot to determine the expression of CSE in carotid sinus. After experiment, the bilateral carotid sinus were rapidly removed and put in liquid nitrogen and then stored at –80 °C for further analysis. The tissue were homogenized in 100 μl lysing buffer and then centrifuged at 15,000 *g* for 20 min at 4 °C. We collected supernatant for protein assay. Bradford assay was used to determine the concentration of protein in tissue. The protein was denatured at 99 °C for 10 min. Then protein of 50 μg was loaded in each lane of SDS-PAGE gels. After electrophoresis, the protein was separated then transferred onto polyvinylidene fluoride (PVDF) membranes. The transferred PVDF membranes were blocked with 5 % skim milk in TBST (1.37 mmol/l NaCl, 200 mmol/l Tris, 0.05 % Tween-20, pH 7.5) for 1 h. The PVDF membranes were incubated with primary antibody (anti-mouse CSE polyclonal antibody, 1:500, Proteintech Biotechnology) overnight followed by appropriate secondary horseradish peroxidase-conjugated antibody (1:2000, Proteintech Biotechnology). Western blotting reagents (Millipore Corporation, Billerica, MA01821, USA) were used to detect the signal. The chemiluminescent signals obtained were recorded. We have used Photoshop to modulate the lightness of the photographies (Fig. 6).

Drugs

NaHS (purity 99 %, Sigma, St Louis, MO, USA) was dissolved in saline. Bay K8644 (1, 4-dihydro-2, 6-dimethyl-5-nitro-4-(2-[trifluoromethyl]phenyl) pyridine -3-carboxylic acid methyl ester, $C_{16}H_{15}F_3N_2O_4$) was purchased from Sigma and dissolved in 99 % ethyl alcohol. Glibenclamide (5-chloro-N-[4-(cyclo hexylureidosulfonyl) phenethyl]-2-methoxybenzamide, $C_{23}H_{28}ClN_3O_5S$) was obtained from Alfa Aesar (Ward Hill, MA, USA) and dissolved in dimethyl sulphoxide (DMSO, $(CH_3)_2SO$). The final concentration of dimethyl sulphoxide or ethyl alcohol in the K-H solution was lower than 0.05 %. PPG (DL-propargylglycine, $C_5H_7NO_2$) was obtained from Sigma and dissolved in K-H solution.

Statistics

All data were reported as means ± SD. ANOVA was applied to compare differences between groups and Student-Newman-Keuls test and Dunnett's t-test were used to further analyze. The lever of significance was set at $P<0.05$.

Results

Effect of NaHS on ISP-RSNA

Perfused the left carotid sinus with NaHS (25, 50, 100 μmol/l) reflexly decreased RSNA in a concentration-dependent manner (Fig. 1). After NaHS (25, 50, 100 μmol/l) were perfused, RSNA was decreased to 84.95±3.58 % ($P<0.01$), 63.89±2.53 % ($P<0.01$) and

48.70±4.16 % (P<0.01) respectively, compared with control. The response of ISP-RSNA to NaHS appeared obvious changes approximately 30 min after perfusing the isolated carotid sinus with NaHS, reached maximum response at about 40 min. Recoveries were of no effect on ISP-RSNA at 30-50 min after washout. When we perfused 100 μmol/l NaHS, the response appeared earlier but it was needed long time to recover completely about 50 min.

NaHS 50 μmol/l produced reproducible effect on ISP-RSNA. It took about 40 min to recover. Therefore, NaHS 50 μmol/l was selected to test mechanistic evaluation and also present time-dependent changes in Figure 2.

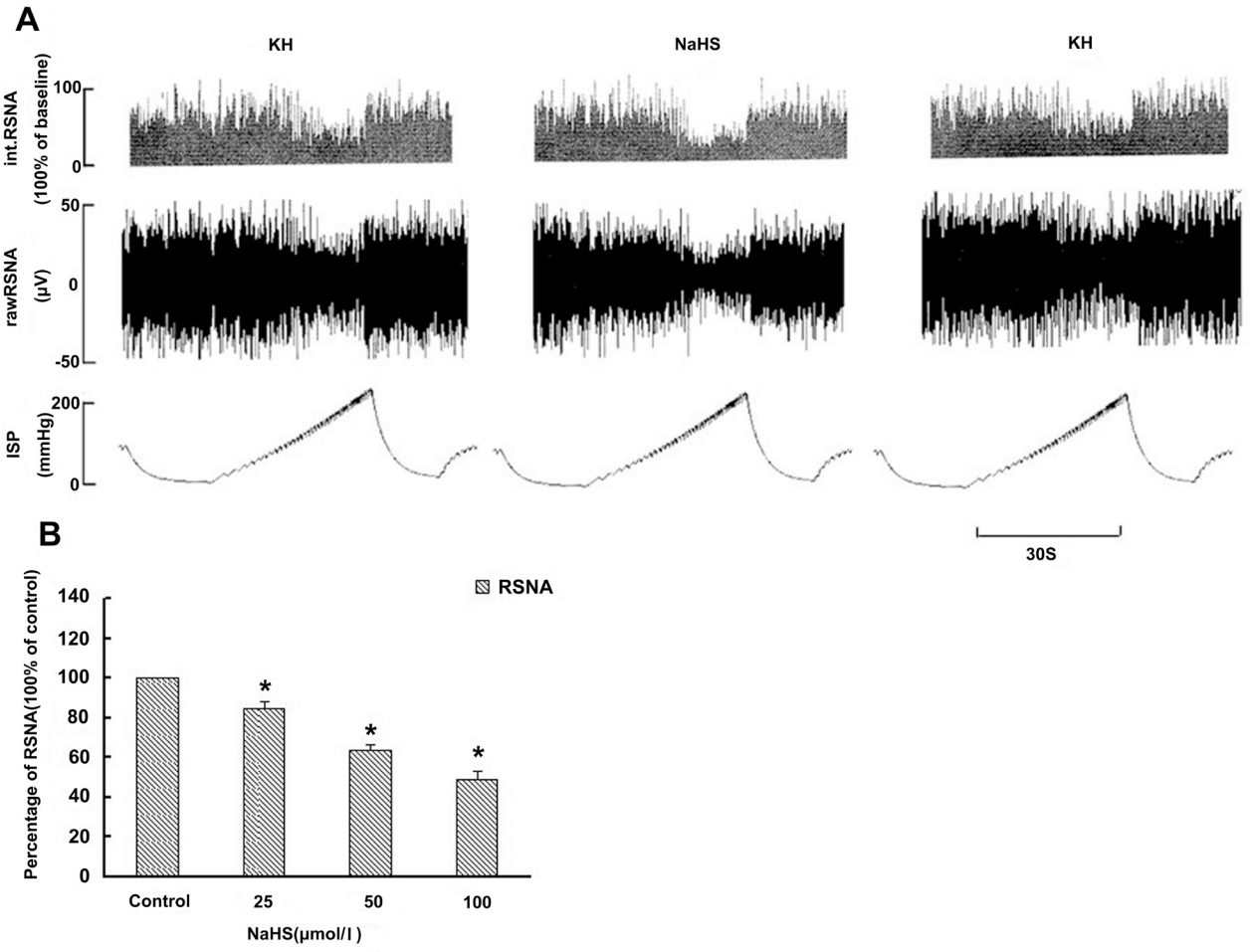

Fig. 1. Effect of isolated carotid sinus perfused with NaHS. (**A**) Original tracing recordings showing the effect of NaHS (50 μmol/l) perfused into the isolated carotid sinus on RSNA. "int.RSNA" means integral of RSNA, the unit is changes of baseline (%); "rawRSNA" means original RSNA, the unit is μV. (**B**) Summary data showing the effects of isolated carotid sinus perfused with NaHS (25, 50, 100 μmol/l) on RSNA in anesthetized male rats (n=6). Data are means ± SD. * P<0.05 compared with control value.

Effect of glibenclamide on NaHS-induced response

To testify the role of K_{ATP} in the action of NaHS (50 μmol/l) in this study, glibenclamide (20 μmol/l) was perfused the isolated carotid sinus followed by initial NaHS (50 μmol/l). ISP-RSNA was compared between NaHS group and group given NaHS plus glibenclamide. The effect of NaHS on ISP-RSNA was blocked by glibenclamide (Fig. 3).

The vehicle of glibenclamide (0.01 % dimethyl sulphoxide in K-H solution) showed no effect on the above parameters.

Effect of Bay K8644 on the response of ISP-RSNA to NaHS

We used Bay K8644 to prove whether Ca^{2+} is involved in the action of NaHS to ISP-RSNA. In six rats, after the initial effect of NaHS (100±0 % to 65.56±6.87 %) perfusion into the isolated carotid sinus on ISP-RSNA, Bay K8644 (500 nmol/l) was perfused into the isolated carotid sinus. ISP-RSNA did not respond to

Bay K8644. Following the subsequent NaHS (50 μmol/l) perfusion, ISP-RSNA decreased from 100±0 % to 95.15±8.2 % (P<0.01). There is significance difference compared with the initial effect of NaHS perfusion into the isolated carotid sinus (P<0.05) (Fig. 4). The vehicle of Bay K8644 (0.05 % ethyl alcohol in K-H solution) was perfused again, and showed no statistical effect on the above parameters.

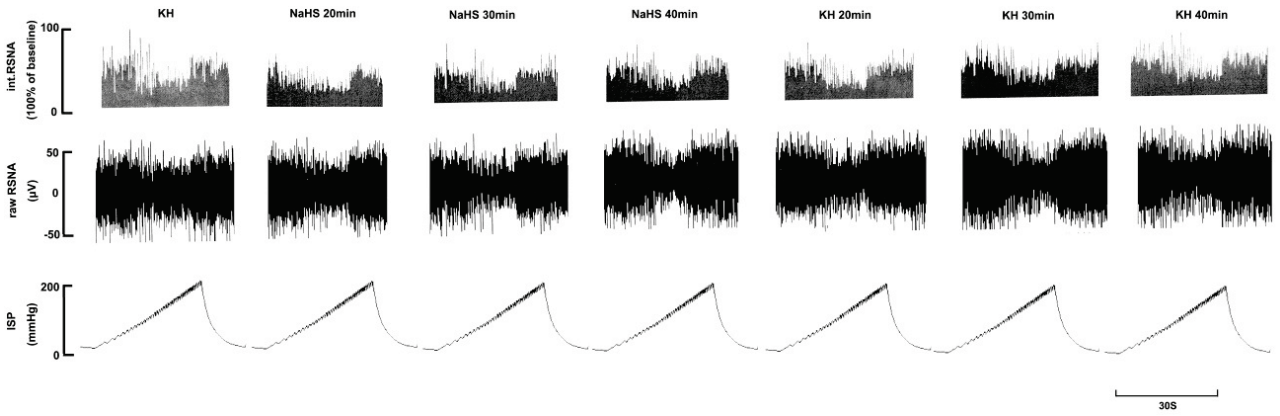

Fig. 2. A time dependent changes of the ISP-RSNAS before and after isolated carotid sinus perfused with NaHS. Original tracing recordings showing the effect of NaHS (50 μmol/l) perfused into the isolated carotid sinus on RSNA. "int.RSNA" means integral of RSNA, the unit is changes of baseline (%); "rawRSNA" means original RSNA, the unit is μV.

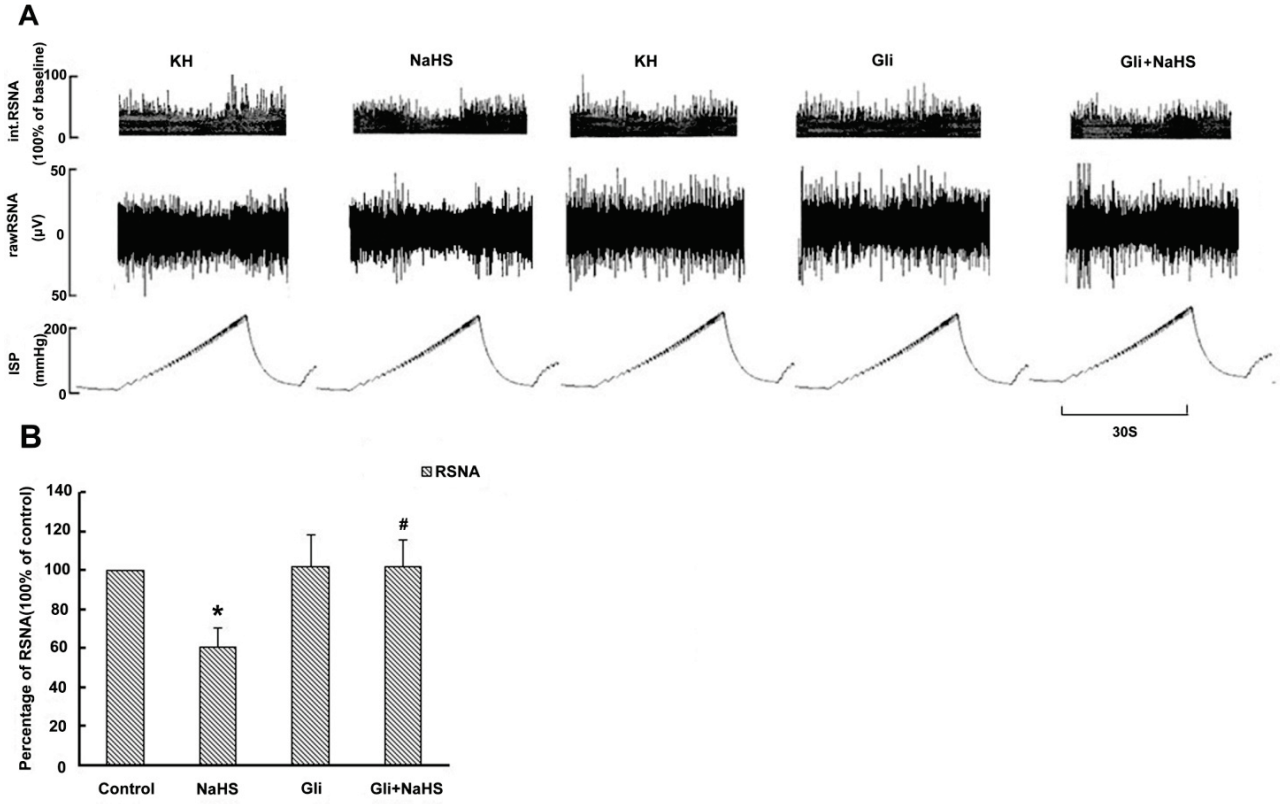

Fig. 3. Effect of glibenclamide (Gli, 20 μmol/l) on the response of RSNA to 50 μmol/l NaHS. (**A**) Original tracing recordings showing the effect of NaHS (50 μmol/l), glibenclamide, and NaHS plus glibenclamide perfused into the isolated carotid sinus on RSNA. "int.RSNA" means integral of RSNA, the unit is changes of baseline (%); "rawRSNA" means original RSNA, the unit is μV. (**B**) Summary data showing the effect of NaHS, glibenclamide, and NaHS plus glibenclamide perfusion into the isolated carotid sinus on RSNA (n=6). Data are means ± SD. * P<0.05 compared with control value. # P<0.05, compared with NaHS (50 μmol/l).

Effect of PPG on ISP-RSNA

To determine the effect of endogenous H_2S on ISP-RSNA, we perfused PPG, to inhibit synthesis of H_2S. After perfusion of PPG (200 μmol/l), RSNA increased significantly from 100±0 % to 118.43±8.04 % ($P<0.01$). PPG inhibits the CSE in male rats and suppresses the response of RSNA to the increased ISP (Fig. 5).

Expression of CSE in isolated carotid sinus

Western blot was performed to detect the protein expression of CSE in carotid sinus of control, PPG and Bay K8644. As the result shown in Figure 6, grey bands represented the CSE positive signals.

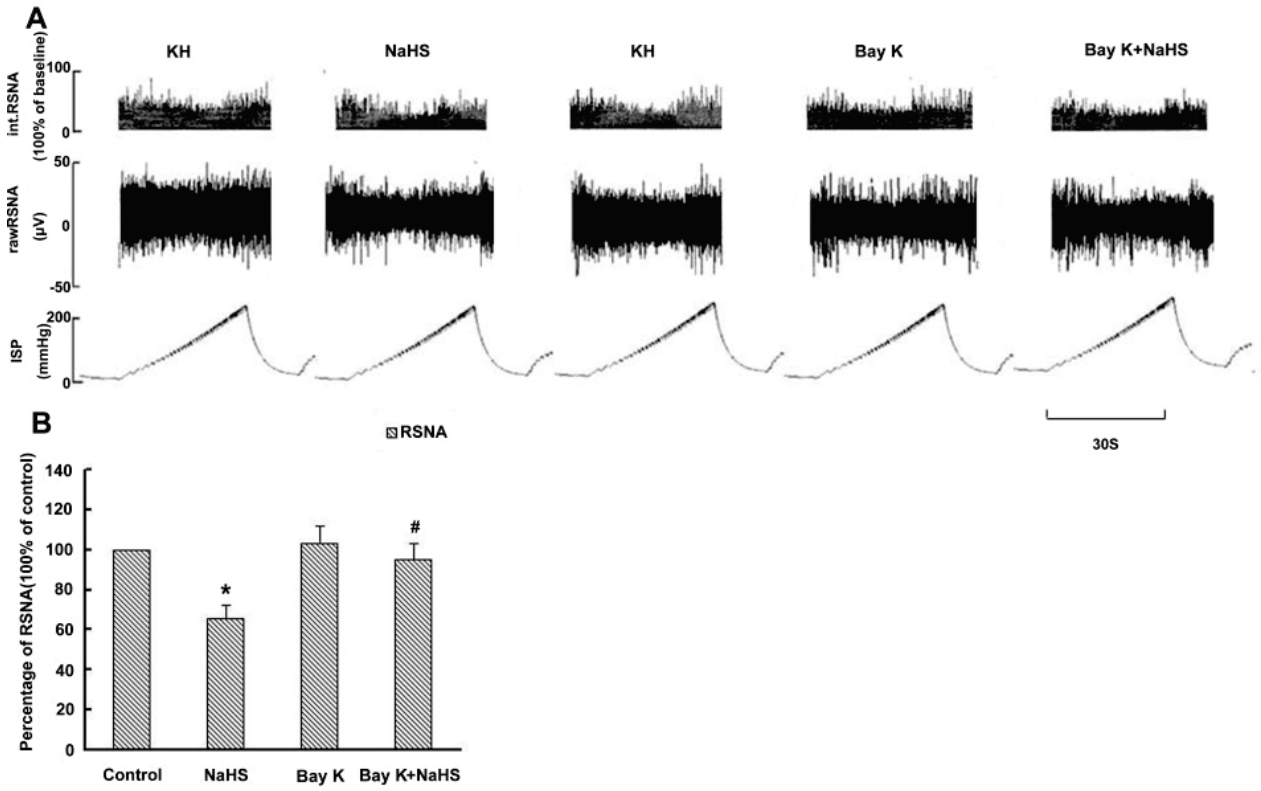

Fig. 4. Effect of Bay K8644 (500 nmol/l) on the response of RSNA to 50 μmol/l NaHS. (**A**) Original tracing recordings showing the effect of NaHS (50 μmol/l), Bay K8644, and NaHS plus Bay K8644 perfused into the isolated carotid sinus on RSNA. "int.RSNA" means integral of RSNA, the unit is changes of baseline (%); "rawRSNA" means original RSNA, the unit is μV. (**B**) Summary data showing the effect of NaHS, Bay K8644, and NaHS plus Bay K8644 perfusion into the isolated carotid sinus on RSNA (n=6). Data are means ± SD. * $P<0.05$ compared with control value. # $P<0.05$, compared with NaHS (50 μmol/l).

Discussion

The present study was designed to demonstrate the indirect central effect of H_2S on baroreflex control of the sympathetic outflow. The finding showed that perfusion isolated carotid sinus with NaHS dose and time-dependently inhibited sympathetic outflow that is in the form of increasing response of RSNA to ISP. When we perfused largest dose, the inhibit effect appeared earlier and needed long time to wash out. Compared with our previous studies, we further revealed the effect of hydrogen sulfide on baroreflex control of sympathetic outflow. As we all known, activation of the sympathetic system is the main cause of hypertension. The results imply H_2S modulate SNA by baroreflex and sensitization of the baroreflex control of RSNA in order to stability the blood pressure.

Resistance to the treatment of hypertension may be due in part to inadequate inhibition of the sympathetic nervous system (Egan *et al.* 2010). Renal sympathetic nerve activity is an important direct indicator for the evaluation of sympathetic central activity. Increased RSNA can contribute to the genesis of hypertension both directly by increasing reabsorption of tubular water and sodium and indirectly by increasing the secretion of renin, which activates the renin-angiotensin system resulting in increased vascular resistance and reduced GFR (DiBona 2000). The preclinical researches target

carotid sinuses (Baroreflex Activation Therapy) invasive therapy for the treatment of drug-resistant hypertension. Stimulation of carotid baroreceptors by suppressing sympathetic tone can induce sustained decrease in arterial pressure and heart rate (Briasoulis and Bakris 2012, 2014). Thereafter, H_2S may have possible therapeutic potential in some cardiovascular disease especially in patients with resistant hypertension.

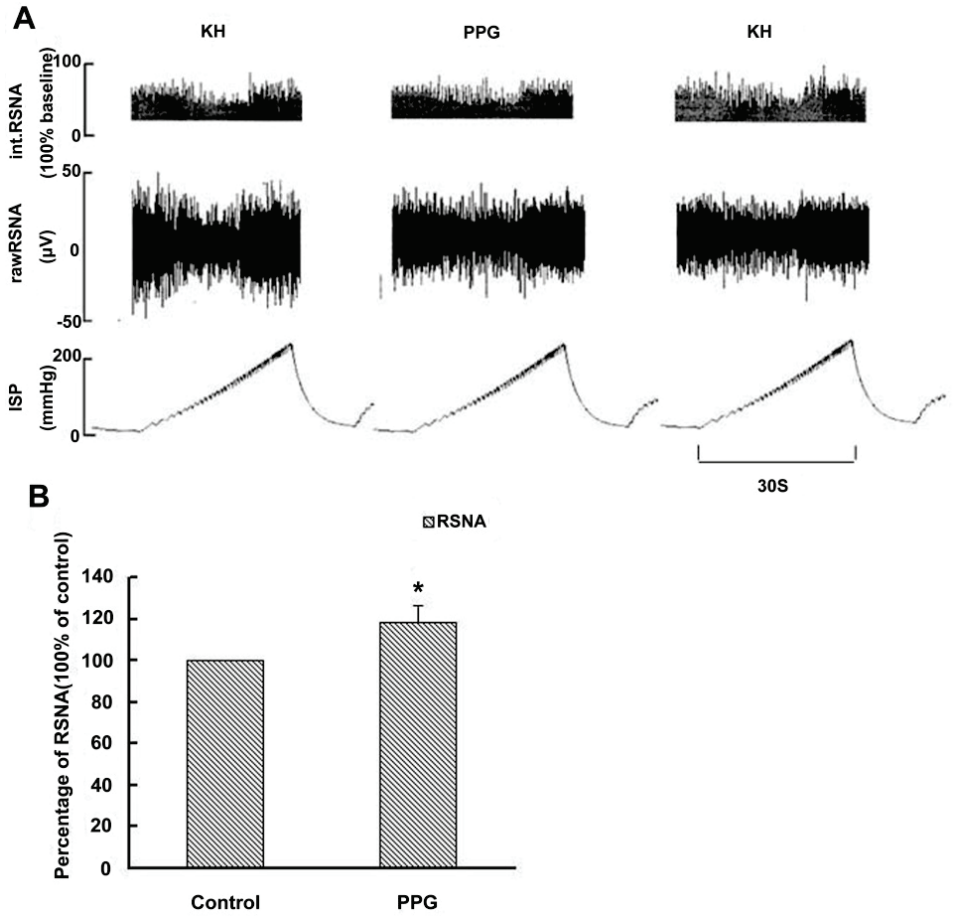

Fig. 5. Effect of PPG (200 μmol/l) on the response of RSNA. (**A**) Original tracing recordings showing the effect of PPG (200 μmol/l) perfusion into the isolated carotid sinus on RSNA. "int.RSNA" means integral of RSNA, the unit is changes of baseline (%); "rawRSNA" means original RSNA, the unit is μV. (**B**) Summary data showing the effect of PPG (200 μmol/l) perfusion into the isolated carotid sinus on RSNA. Data are means ± SD. * $P<0.05$ compared with control value.

NaHS is commonly used as an H_2S donor since it dissociates to Na^+ and HS^-, the latter then partially binds H^+ to form undissociated H_2S (Lowicka and Beltowski 2007). H_2S readily dissolves in water, and dissociates to H^+, HS^-, and S^{2-}. Under physiological conditions, approximately 20 % exist as H_2S and the remaining 80 % as HS^-, with only trace amounts of S^{2-}. The term "hydrogen sulfide" has been used to refer to H_2S, HS^-, and S^{2-} (Abe and Kimura 1996). Once generated, H_2S can be oxidized to generate reductant-labile sulfane sulfur pools, which include hydrodisulfides/persulfides. When we perfused carotid sinus with NaHS, it is possible to restore oxygen to produce superoxide anion. However, superoxide may contribute to the pathogenesis of many diseases and damage the function of baroreflex (Zhang *et al.* 2014). It is opposite to our results. Maybe superoxide play minimum role in our results. Polysulfide is a bound sulfur species derived from endogenous H_2S (Koike *et al.* 2013). Polysulfide contains sulfane sulfur and also exerts much more cytoprotective effects. Oxidized sulfide, such as persulfide thiosulfate ($S_2O_3^{2-}$) and sulfate (SO_4^{2-}) which a downstream product of H_2S, also plays cytoprotective effects (Sakaguchi *et al.* 2014, Schreurs and Cipolla 2013). Meanwhile, H_2S can also be released from bound sulfane sulfur pools (Kimura 2014). There is a dynamic balance between them (Bailey *et al.* 2014, Vitvitsky *et al.* 2012). In our present experiment, it is difficult to make a distinction effect between them. Even if the other products exert cytoprotective effects and that is still to be attributed to H_2S.

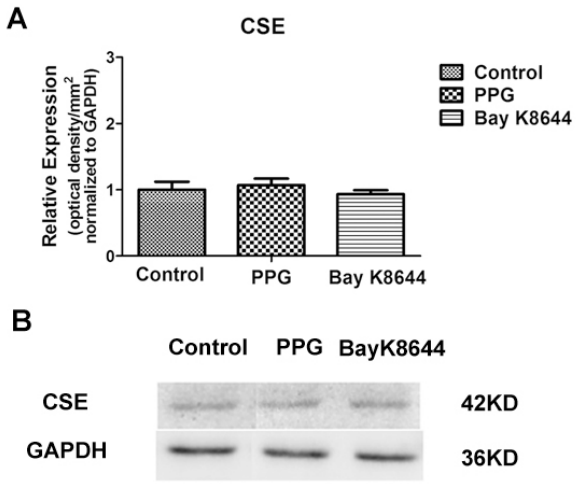

Fig. 6. Expression of CSE in isolated carotid sinus. (**A**) Protein expression of CSE in carotid sinus from control, PPG and Bay K8644 group (n=6). GAPDH was used to normalize. Data are means ± SD. (**B**) Representative original illustrating of Western blot in control, PPG and Bay K8644 group.

Evidences have been present to show that endogenous H_2S in mammalian tissues play a significant role in the cardiovascular system (Elrod *et al.* 2007, Geng *et al.* 2004, Zhao *et al.* 2001). There are some studies have reported that H_2S can relax isolated aorta and the mesenteric artery in rat, and that the vasodilatation induced by H_2S is attributable to activation of K_{ATP} channels (Cheng *et al.* 2004, Zhao and Wang 2002, Zhao *et al.* 2001). High concentration of K^+ ions attenuated vasorelaxation induced by H_2S in aortic ring, and H_2S-mediated vasodilatation was effectively blocked by glibenclamide or 5-hydroxydecanoate (5-HD), K_{ATP} channels antagonists (Ali *et al.* 2006, Johansen *et al.* 2006, Zhu *et al.* 2007). A similar study also has been reported by Webb *et al.* (2008) and by Tang *et al.* (2005) glibenclamide can block the relaxant response to H_2S in phenylephrine precontracted human internal mammary artery. However, glibenclamide was also reported to inhibit chloride intracellular channels (Kominkova *et al.* 2013). If glibenclamide inhibit chloride channels, it will cause hyperpolarization and then relaxation of sinus wall which will increase the effect of H_2S. But in our study, the effect of H_2S was blocked by glibenclamide. As a result, glibenclamide may be possible mainly play a role as a K_{ATP} channels blocker in our experiment. It is conceivable that the effect of H_2S is mediated by opening a K_{ATP} channels in smooth muscle cell and then dilation of sinus wall. The relaxation of sinus wall will enhance stretch of baroreceptors. On the other side, mechanosensitive channels on baroreceptors will be activated and result in reducing RSNA. However, we cannot rule out the directly effect of H_2S on mechanosensitive channels on baroreceptor.

Bay K8644, a specific agonist of L-type Ca^{2+} channels, is used to further detect whether H_2S acts on L-type Ca^{2+} channels. The result of the experiment that the inhibitory effect of H_2S on sympathetic outflow was significantly blocked by Bay K8644, powerfully imply that H_2S-mediate-inhibitory effect might be attributed to the close of L-type Ca^{2+} channels. The present data is similar to that of our previous study, which shows that H_2S can act on L-type Ca^{2+} channels and significantly inhibit Ca^{2+} influx (Xiao *et al.* 2006, Xu *et al.* 2008). As we all known that K_{ATP} channels can inhibit the Ca^{2+} influx through L-type Ca^{2+} channels (Cifelli *et al.* 2008, Jovanovic and Jovanovic 2001). Therefore, it may be proposed that H_2S might first open the K_{ATP} channels and cause K^+ outflow then result in hyperpolarisation, which subsequently inhibit Ca^{2+} influx *via* L-type voltage-gated calcium channels and prevent excessive Ca^{2+} in smooth muscle cell. However, Bay K8644 can open calcium channels and the protein of CSE can be affected by calcium in the cell. We tested the expression of CSE after perfusion Bay K8644, there was no significance difference compared with control. This result implied Bay K8644 has not affected the product of endogenous H_2S and only inhibited the effect of H_2S.

Previously data have shown expression of CSE in vascular tissues and that the production of H_2S was inhibited by PPG (Yan *et al.* 2004, Zhao *et al.* 2001). PPG, a potent nonreversible inhibitor of CSE, was used to inhibit the production of H_2S (Thompson *et al.* 1982). In our current experiments, perfusing the left carotid sinus with PPG induced an increase in RSNA compared with control application of K-H solution. In addition, our present study has shown CSE positive signal is distributed in carotid sinus even though there is no significant difference between PPG and control group. The effect of PPG is due to the down production of endogenous H_2S by inhibiting CSE in carotid sinus wall. Moreover, the inhibition did not affect the quantity of CSE protein and only inhibit the function of CSE in our experiment. Meanwhile, the expression of CSE in carotid sinus is an extra evidence that endogenous H_2S produced by CSE tonically suppresses the sympathetic vasomotor by activation of the carotid sinus baroreflex (CSB). And that distinguish from the exogenous H_2S which depended on dose and time.

In summary, the present study has demonstrated

that perfusion of isolated carotid sinus with NaHS inhibited sympathetic outflow in the form of increased the response of ISP-RSNA. Endogenous H_2S maintain the blood pressure in a basal level through modulating the carotid sinus baroreflex. Sympathetic outflow play an important role in some cardiovascular disease. Therefore, our data imply that H_2S may be a novel intervention that can be used in clinical.

Conflict of Interest

There is no conflict of interest.

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