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Hydrogen sulfide facilitates carotid sinus baroreflex in anesthetized rats

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Key words

Abstract

Aim: To study effects of hydrogen sulfide (H_2S) on the carotid sinus baroreflex (CSB). **Methods:** The functional curve of the carotid sinus baroreflex was measured by recording changes in arterial pressure in anesthetized male rats with perfused carotid sinus. **Results:** H_2S (derived from sodium hydrosulfide) at concentrations of 25, 50, and 100 μmol/L facilitated the CSB, shifting the functional curve of the baroreflex downward and to the left. There was a marked increase in peak slope (PS) and reflex decrease in blood pressure (RD). Effects were concentration-dependent. Pretreatment with glibenclamide (20 μmol/L), a K_{ATP} channel blocker, abolished the above effects of H_2S on CSB. Pretreatment with Bay K8644 (an agonist of calcium channels; 500 nmol/L) eliminated the effect of H_2S on CSB. An inhibitor of cystathionine γ-lyase (CSE), *DL*-propargylglycine (PPG; 200 μmol/L), inhibited CSB in male rats and shifted the functional curve of the baroreflex upward and to the right. **Conclusion:** These data suggest that exogenous H_2S exerts a facilitatory role on isolated CSB through opening K_{ATP} channels and further closing the calcium channels in vascular smooth muscle. Endogenous H_2S may activate the activity of the CSB *in vivo*.

Introduction

Hydrogen sulfide (H_2S) is a toxic gas that is found in contaminated environments. Many studies have shown that H_2S affected the nervous system and vascular and intestinal smooth muscle of mammals[1–6]. Although toxic at high doses, there was increasing evidence to suggest that H_2S has important physiological functions in the mammalian cardiovascular system[4]. The production of H_2S from *L*-cysteine can be catalyzed by two pyridoxal-5'-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine α-lyase (CSE), in mammalian tissue[7–9]. The expression of these two enzymes is tissue-type specific[4]. H_2S is directly produced in arterial and venous tissue by CSE[10,11]. *DL*-propargylglycine (PPG) is a specific inhibitor of CSE, which can suppress endogenous H_2S production[12].

H_2S has been proposed to be an endogenous gaseous transmitter similar to 2 other vasoactive gases, NO (nitric oxide) and CO (carbon monoxide). The vascular effect of H_2S is exerted by direct activation of K_{ATP} channels and hyperpolarization of the membrane potential of vascular smooth

muscle cells (VSMCs)[10]. It has been found that the vascular effect of H_2S was partially mediated by a functional endothelium, and its action was dependent on extracellular calcium entry, but is independent of activation of the cGMP pathway, unlike NO and CO[13]. Recently, H_2S has been found to play a negative inotropic role in the heart[14].

The baroreflex is the major method of blood pressure modulation. Whether H_2S affects the carotid sinus baroreflex (CSB) remains to be clarified. The aim of our study was to observe the action of exogenous H_2S, derived from sodium hydrosulfide (NaHS), and endogenous H_2S on the isolated CSB and to elucidate the mechanism involved.

Materials and methods

Drugs NaHS, Bay K8644 and PPG were purchased from Sigma. NaHS was used as a donor of H_2S. Glibenclamide (a K_{ATP} channel blocker) was purchased from the Tianjin Institute of Medical and Pharmaceutical Industry. Glibenclamide was initially dissolved in dimethylsulfoxide (100 μmol/L). The final concentration of dimethylsulfoxide in the K-H solution

was 0.01% (*v/v*). No change was observed in the CSB during perfusion with the final concentration of dimethylsul-foxide. Bay K8644 (an L-type calcium channel agonist) was dissolved in 99% ethyl alcohol. No changes in the CSB were observed during perfusion with ethyl alcohol (1:2000). PPG (an inhibitor of CSE) was dissolved in distilled water.

General surgical procedure Sprague-Dawley rats (male, 320±20 g), which were obtained from the Experimental Animal Center of Hebei Province, were anesthetized with 25% urethane (1.0 g/kg, ip). In each rat, the trachea was cannulated for ventilation, and the right femoral artery was cannulated for recording blood pressure (BP) with a transducer (MPU-0.5A; Nihon Kohden). Body temperature was maintained at 37–38 °C throughout the experiment.

Perfusion of left isolated carotid sinus The perfusion of isolated carotid sinus area was carried out using a previously reported method with modifications[15]. Carotid sinus areas were fully exposed by turning the trachea and esophagus in the rostral direction. The sternohyoideus muscles and superior laryngeal nerves were cut, then the bilateral aortic nerves, right carotid sinus nerve, cervical sympathetic nerves and recurrent laryngeal nerves were all sectioned. The common, external and internal carotid arteries and smaller arteries originating from these vessels were exposed and ligated, while carefully leaving the left carotid sinus nerve undisturbed. Ligation of the occipital artery at its origin from the external carotid artery excluded chemoreceptors from the isolated carotid sinus, thereby preventing chemoreceptor activation secondary to decrease in carotid sinus pressure. Plastic catheters were inserted anterograde into the left common carotid artery (inlet tube) and retrograde into the external carotid artery (outlet tube). The carotid sinus was then perfused with warm (37 °C) oxygenated modified Krebs-Henseleit (K-H) solution (in 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 intrasinus pressure (ISP) was monitored by using a pressure transducer (MPU-0.5A; Nihon Kohden) connected to the inlet tube. The ISP was controlled by using a peristaltic pump.

After perfusion of the left carotid sinus, the ISP was kept at 100 mmHg for 20 min and was 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[16]. It took 0.5 min for the ISP to be increased from 0 to 250 mmHg. The ISP and BP were simultaneously recorded on a polygraph (RM-6240; Chengdu Instrument Factory). 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 recurrent drop of BP in response to the increase in ISP.

Experimental protocols By perfusing the left carotid sinus with K-H solution and elevating the ISP, a functional curve for the ISP-BP relationship 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 BP (RD), and operating range (OR) were determined. TP was the ISP at which BP began to decrease in response to the increase of the ISP. SP was the ISP at which BP just showed no further reflex decreases with an increase in the ISP. OR was calculated as SP minus TP.

Before administration of the drugs, the K-H solution was used as a control. Four experimental treatments were used. (1) To test the effect of NaHS on carotid baroreflex (*n*=18), the ISP was fixed at 100 mmHg for 20 min with K-H solution as a control, and the baroreflex parameters were measured. Then K-H solution containing NaHS (25, 50, or 100 μmol/L) was used to perfuse the isolated carotid sinus for 50 min, then the 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 glibenclamide (Gli; 20 μmol/L) on the actions of NaHS (*n*=6), baroreflex parameters were examined following the application of NaHS before and after pretreatment with Gli for 20 min. (3) To test the effect of Bay K8644 (500 nmol/L) on the actions of NaHS (*n*=6), baroreflex parameters were examined following the application of NaHS before and after pretreatment with Bay K8644 for 15 min. (4) To test the effect of PPG (200 μmol/L) on the carotid baroreflex (*n*=6), after the baroreflex parameters of the control were recorded, PPG was added into a K-H solution and used to perfuse the isolated carotid sinus, then washed out. Finally, the carotid sinus was perfused with K-H solution containing NaHS (50 μmol/L) to check the carotid baroreflex activity.

Data analysis All data are expressed as mean±SD. The differences between groups of means were assessed by one-way ANOVA and further analyzed using the Student-Newman-Keuls test. *P*<0.05 was considered statistically significant.

Results

Effects of NaHS on carotid sinus baroreflex By perfusing the left carotid sinus with K-H solution and elevating the ISP from 0 to 250 mmHg, BP was reflex decreased. NaHS induced obvious changes in baroreflex parameters, which appeared approximately 30 min after perfusing the isolated

carotid sinus with K-H solution containing NaHS, and disappeared 30–60 min after washout. Compared with the control group, NaHS increased RD and PS in a concentration-dependent manner, and decreased TP and EP, shifting the functional curve of the baroreflex downward and to the left (Table 1, Figure 1). The functional curve was fit using Origin 6.0 procedures. The effects described indicate that exogenous NaHS exerts a facilitatory effect on the carotid baroreflex (Figure 2).

Effects of Gli and Bay K8644 on the actions of NaHS
Neither Gli (20 µmol/L) nor Bay K8644 (500 nmol/L) induced

any changes in the functional parameters of the baroreflex, but both completely blocked the effects of NaHS (Table 2).

Effect of PPG on carotid sinus baroreflex PPG inhibited the CSB in male rats and shifted the functional curve of the baroreflex upward and to the right (Table 3).

Discussion

The present study showed that H_2S could facilitate the CSB in a dose-dependent manner. By perfusing the left isolated carotid sinuses of rats with exogenous H_2S derived from NaHS, the functional curve of the CSB was shifted downward and to the left, with increases in PS and RD and decreases in TP, indicating the facilitatory action of H_2S on the CSB.

It has been shown that H_2S has cardiovascular effects, and that H_2S can relax isolated aortic tissue *in vitro* in a K_{ATP} channel-dependent manner. Furthermore, in isolated VSMCs, H_2S can directly increase K_{ATP} channel currents and hyperpolarize membrane[10]. So H_2S is considered to be an important endogenous vasoactive factor and the first identified gaseous opener of K_{ATP} channels in vascular smooth muscle cells. It is known that K_{ATP} channels exist in vascular smooth muscle[17]. A previous study from our laboratory reported that the opener of K_{ATP} channels can facilitate the carotid baroreflex[18]. On the basis of these data, we added glibenclamide (20 µmol/L), a K_{ATP} channel blocker, to the perfusate and found that it completely eliminated the effect of H_2S on the carotid baroreflex. Such results suggest that the opening of the K_{ATP} channel is involved in the action of H_2S on the CSB.

Brayden recently reported that K_{ATP} channel opening could hyperpolarize smooth muscle, which led to closure of the voltage-dependent Ca^{2+} channels, thereby causing a reduction in intracellular Ca^{2+} and vasodilation[19]. It is also known that distention of the carotid sinus can activate mechanosensitive ion channels, which would enhance the
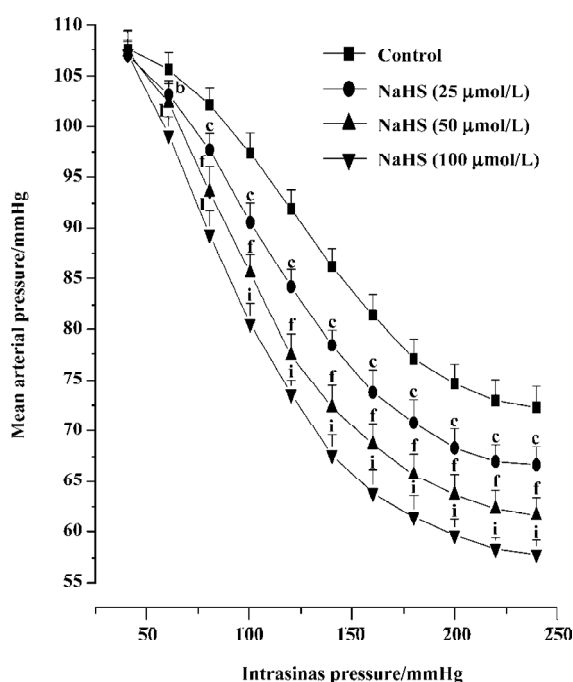

Figure 1. Changes in the functional curves of the baroreflex during intrasinus perfusion with different doses of NaHS in rats. $n=6$. $^bP<0.05$, $^cP<0.01$ *vs* control. $^eP<0.05$, $^fP<0.01$ *vs* 25 µmol/L NaHS. $^hP<0.05$, $^iP<0.01$ *vs* 50 µmol/L NaHS.

Table 1. Effects of exogenous H_2S on the functional parameters of carotid baroreflex in rats.

	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Control	77.17±1.47	97.33±1.37	194.50±2.07	117.33±2.88	0.29±0.01	35.50±0.55
NaHS (µmol/L)						
25(A)	63.83±1.47[c]	92.67±1.21[c]	185.67±2.42[c]	121.83±2.04[b]	0.34±0.01[c]	40.50±0.55[c]
50 (B)	56.00±1.10[cf]	89.00±1.55[cf]	176.17±1.60[cf]	120.17±1.72[ad]	0.42±0.01[cf]	45.83±0.75[cf]
100 (C)	53.17±2.32[cfi]	86.00±1.41[cfi]	174.67±4.03[cfg]	121.50±2.88[bdg]	0.48±0.02[cfi]	49.50±1.05[cfi]

$n=6$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ compared with Control. $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ compared with A. $^gP>0.05$, $^hP<0.05$, $^iP<0.01$ compared with B. TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; OR, operating range; PS, peak slope; RD, reflex decrease.
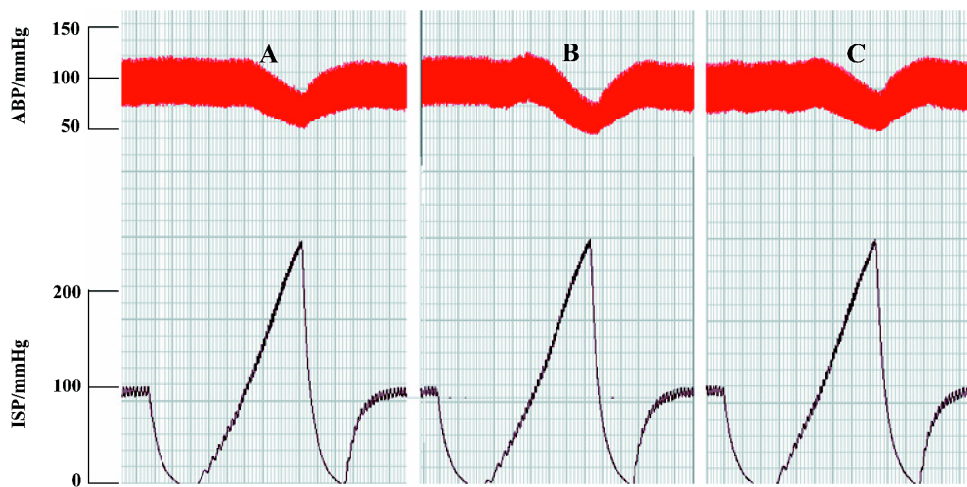
Figure 2. Original recording showing the reflex response of arterial blood pressure (ABP) to intrasinus perfusion with NaHS (50 µmol/L). (A) Control; (B) NaHS; (C) Washout.

Table 2. Effect of glibenclamide (Gli, 20 µmol/L) and Bay K8644 (500 nmol/L) on the reflex response induced by perfusing rat carotid sinus with NaHS (50 µmol/L). *n*=6. [b]*P*<0.05, [c]*P*<0.01 compared with control; [e]*P*<0.05, [f]*P*<0.01 compared with NaHS; [h]*P*<0.05, [i]*P*<0.01 compared with Gli; [k]*P*<0.05, [l]*P*<0.01 compared with Bay K8644.

	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Control	75.00±1.10	97.33±1.03	194.00±2.45	119.00±2.19	0.30±0.01	34.50±1.05
NaHS	54.17±1.47[c]	88.17±1.47[c]	176.67±2.73[c]	122.50±2.51[b]	0.41±0.01[c]	44.50±1.38[c]
Gli	76.50±1.64[f]	97.00±0.89[f]	193.17±2.48[f]	116.67±1.97[f]	0.29±0.01[f]	34.50±0.55[f]
Gli+NaHS	75.33±1.75[f]	96.17±1.83[f]	193.00±2.68[f]	117.67±2.66[f]	0.30±0.01[f]	34.83±0.75[f]
Control	73.67±2.50	97.00±1.79	192.50±2.66	118.83±1.60	0.29±0.01	35.50±1.05
NaHS	55.33±1.86[c]	89.17±1.17[c]	177.17±1.60[c]	121.83±1.33[b]	0.42±0.01[c]	46.00±1.41[c]
Bay K8644	75.17±1.72[f]	96.83±1.17[f]	194.67±1.86[f]	119.50±1.97[e]	0.30±0.01[f]	36.00±1.79[f]
Bay K8644+NaHS	73.83±1.72[f]	96.17±1.72[f]	193.33±1.63[f]	119.33±2.07[d]	0.29±0.01[f]	35.67±1.86[f]

Table 3. Reverse action of NaHS (50 µmol/L) on the reflex response induced by perfusing rat carotid sinus with cystathione-γ-lyase inhibitor PPG (200 µmol/L). *n*=6. [b]*P*<0.05, [c]*P*<0.01 compared with control; [e]*P*<0.05, [f]*P*<0.01 compared with PPG.

	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Control	74.67±1.97	96.50±1.38	198.33±1.21	123.67±2.34	0.32±0.01	35.50±1.05
PPG+NaHS	76.83±1.47	97.50±1.38	194.33±1.63[c]	117.50±1.37[c]	0.26±0.01[c]	31.17±0.98[c]
NaHS	65.50±1.87[cf]	93.17±1.33[cf]	184.83±1.83[c]	119.33±2.73[d]	0.32±0.01[af]	37.83±0.98[cf]

activity of the baroreceptors[20]. In the present study, pretreatment with L-type calcium channel agonist Bay K8644 completely abolished the facilitating effect of H$_2$S on CSB. Based on the above observations, it can be concluded that H$_2$S facilitates CSB through opening K$_{ATP}$ channels and further closing calcium channels in VSMCs.

The results so far discussed only relate to the actions of exogenous H$_2$S. In order to determine the function of endogenous H$_2$S, PPG (an inhibitor of CSE) was used in our experiment. Zhao reported that PPG might be a membrane-permeable drug, and that it had the potential to be used to study the physiological function of endogenously produced H$_2$S[12]. In the present study, PPG shifted the functional curve of CSB upward and to the right, with decreases in PS and RD, and increases in TP. These results indicate that endogenous H$_2$S may have an activating role with respect to CSB activity *in vivo*.

It is known that arterial baroreceptors play an important

role in the short-term modulation of CSB activity. Facilitating the CSB would tend to decrease arterial blood pressure, which could antagonize the hypertension caused by other stimulators. Some diseases, such as hypoxic pulmonary hypertension[21], result from a deficiency in endogenous H$_2$S production, so the modulation activity of the CSB is suppressed and the arterial blood pressure will increase. Deficits in the H$_2$S/CSE system are responsible for the development of spontaneous hypertension accompanying aorta remodeling[22,23]. Exogenous supply of an H$_2$S donor may exert a protective effect in the pathogenesis of hypertension.

In summary, H$_2$S facilitated the carotid baroreflex, which may result from the opening of K$_{ATP}$ channels and further closing of the calcium channels. It is possible that H$_2$S activates the carotid baroreflex in physiological conditions.

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