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Original Article

Activation of Inward Rectifier Potassium Channels in High Salt Impairment of Hydrogen Sulfide-Induced Aortic Relaxation in Rats

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Abstract

Introduction: Hydrogen sulfide (H_2S) plays a key role in the regulation of vascular tone and protection of blood vessels against endothelial dysfunction. Since the mechanism of salt impairing H_2S-induced vascular relaxation is not fully clear, therefore this study was designed to investigate the role of potassium (K^+) channels in the vasodilatory effects of exogenous H_2S in rat aortic rings.

Materials and Methods: Isolated thoracic aortic rings of adult male albino rats fed 8% NaCl diet for six weeks were used for isometric tension recording using PowerLab tissue bath system.

Results: The relaxation response to sodium disulfide (Na_2S, an H_2S donor) was reduced in aortic rings of rats that were either fed high salt (HS) or incubated in a medium containing 1,3 or 5mM/L of extra NaCl compared with control rings. Na_2S-induced relaxation was lower in rings precontracted by high K^+ than phenylephrine (PE, a selective α1adrenergic receptor agonist). In addition, incubation of aortic rings of HS loaded rats with inward-rectifier K^+ (K_{IR}) channels blocker individually or simultaneously with either ATP-dependent (K_{ATP}) or voltage-sensitive K^+ (K_V) channels blockers inhibited Na_2S-induced relaxation in PE-precontracted rings; however it had no effects on rings pretreated with K_{ATP} channels blocker. In contrast, incubation of aortic rings of HS loaded rats with Ca^{+2} activated K^+ (K_{Ca}) channels blocker individually or in combination with K_{IR} channels blocker significantly enhanced Na_2S-induced relaxation.

Conclusion: These results revealed that HS partially impairs aortic relaxation caused by H_2S, and that the mechanism of relaxation is mainly mediated by the stimulation of K_{IR} channels and inhibition of K_{Ca} channels.

Keywords:
Hydrogen sulfide;
K_{IR} channels;
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Introduction

Salt-induced hypertension is associated with endothelial dysfunction (Sofola *et al.*, 2003; Altaany *et al.*, 2014), structural changes in arterioles, reductions in microvessel density (Lombard *et al.*, 2003), enhanced vascular reactivity to vasoconstrictor stimuli (Adegunloye and Sofola, 1998; Giardina *et al.*, 2001; Sofola *et al.*, 2003), as well as impaired endothelium-dependent dilation (Lombard *et al.*, 2003; Zhu *et al.*, 2004) due to increased oxidative stress (Simon, 2003; Edwards and Farquhar, 2015) and reduced nitric oxide (NO) signaling (Callera *et al.*, 2004).

Gasotransmitters are endogenously generated gaseous signaling molecules (Li and Moore, 2007). Beside NO, carbon monoxide (CO) more recently interest has been directed towards the third naturally occurring group of gases, notably Hydrogen sulfide (H_2S) (Moore *et al.*, 2003). H_2S is generated endogenously from L-cysteine by pyridoxal-5′-phosphate-dependent enzymes, including cystathionine β-synthase (CBS) and/or cystathionine γ-lyase (CSE) in mammalian cells. So far, only the expression of CSE has been detected in vascular smooth muscle cells (VSMCs) (Yang *et al.*, 2004). H_2S plays key roles in the regulation of vessel diameter, protection of endothelium from redox stress, ischemia reperfusion injury and chronic inflammation (Pushpakumar *et al.*, 2014). It dilates different blood vessels via activation of ATP-sensitive K^+ (K_{ATP}) channels in VSMCs (Zhao *et al.*, 2001; Tang *et al.*, 2005), and Calcium (Ca^{+2})-activated K^+ (K_{Ca}) channels in endothelium (Cheng *et al.*, 2004).

VSMCs express at least four different functional types of K+ channels, including K_{ATP}, K_{Ca}, inward rectifier K^+ (K_{IR}), and voltage-dependent K^+ (K_V) channels (Jackson, 2000). Opening of K^+ channels and increase in K^+ efflux in VSMCs cause membrane potential hyperpolarization and decrease Ca^{+2} entry via closing of voltage activated Ca^{+2} channels (Nelson and Quayle, 1995). This leads to stimulation of electrogenic sodium (Na^+)-K^+ pump and/or activation of K_{IR} channels and vasodilation (Haddy *et al.*, 2006). Alteration in the function and expression of K^+ channels has been observed in different models of hypertension (Callera *et al.*, 2004). Expression and densities of K_V (Joseph *et al.*, 2013), K_{IR} (Sobey, 2001) and K_{ATP} channels (Sobey, 2001; Blanco-Rivero *et al.*, 2008) are impaired in several models of hypertension. Meanwhile, the density of membrane K_{Ca} is increased in VSMCs of arteries from genetic, renal and salt-induced hypertension (Rusch and Liu, 1997).

Several studies have suggested that acetylcholine (ACh)-induced relaxation has been impaired in both inherently hypertensive rats (Konishi and Su, 1983; Izzard and Heagerty, 1999; Kagota *et al.*, 2002) and salt-sensitive rats (Luscher *et al.*, 1987; Nishida *et al.*, 1998). The mechanism linking salt to relaxation impairment appears to be complex and involve either decrease in NO production (Vapaatalo *et al.*, 2000) or suppression of eNOS activity (Li *et al.*, 2009) via a rise in plasma Na^+ concentration (Li *et al.*, 2009). Moreover, NO synergizes with H_2S, regulated gene expression and enzymatic activity of CSE (Dombkowski *et al.*, 2004; Lowicka and Beltowski, 2007) and the vascular H_2S synthase/H_2S pathway was found to be dysfunctional in hypertensive rats (Zhong *et al.*, 2003). Furthermore, CSE knockout mice displayed pronounced hypertension and highly reduced endothelium dependent relaxation (Yang *et al.*, 2008; Zoccali *et al.*, 2009). While the impairment of endothelium-dependent vasodilation is a known fact, the mechanism of salt impairing H_2S-induced vascular relaxation and the role of different types of K^+ channels are not completely understood. Therefore, the aim of the current study is to investigate the contribution of different subtypes of K^+ channels to the mechanism of H_2S-induced vascular relaxation in HS- incubated rats, with focus on K_{IR} channels.

Materials and methods

Experimental Animals

The animal experimental procedure compatible with the “Guide for the Care and Use of Laboratory Animals” of National Institutes of Health in the United States and was approved by the Animal Research Committee of Salahaddin University-Erbil. Adult male Wistar rats (*Rattus norvegicus*) weighing about 200–300gm were fed a diet of normal chow with 0.4% sodium chloride (NaCl) or a HS with 8% NaCl (Cordaillat *et al.*, 2007). The HS was prepared by mixing 76g of NaCl with 924g of chow. The rats were supplemented with these diets for 6 weeks with water *ad libitum* before the study. The rats were kept in an air-conditioned room (22±2°C) under an artificial 12 hour light/dark cycle.

Tissue preparation

After anaesthetizing the rats with Ketamine (40 mg/Kg) and Xyalzine (10mg/Kg) intraperitonealy (Struck *et al.*, 2011), the chest cavity was opened. After removal of excess tissue and fat, thoracic aorta was isolated and transferred to beaker containing cold Krebs solution (composition in mM: NaCl-136.9, KCl-5.4, Glucose-5.5, $NaHCO_3$-23.8, $MgCl_2$-1, $CaCl_2$-1.5, and EDTA-0.003). It was brought to equiblirium with 95% O_2 and 5% CO_2.

We followed the (Al-Habib and Salihi, 2013) protocol to study the vascular reactivity in the isolated aorta with some modifications. Two stainless steel wires were carefully inserted into lumen of the aortic rings. One wire was anchored to the hook at the base of a tissue bath (Model 166051, Radnoti, Monrovia Ca, USA) and other wire was connected to force transducer (MLT0201/RAD 5 mg - 25 mg, AD instruments, Sydney, Australia) which was then coupled to the transbridge amplifier (ML 224, Quad Bridge Amp, AD instruments). Data was acquired with a PowerLab Data Acquisition System (ML 870, Power Lab, AD instruments) using the chart software (Version 7) for measurement of isometric tension. The extent of contraction and relaxation were indicated by the level of tension development in the recording system and expressed in gram.

Aortic Relaxation Studies

Rings were allowed to equilibrate for 60 minutes at a resting tension of 2 grams with changes of buffer every 15 minutes. When the isometric tension had stabilized, after a number of preliminary tests, inhibitory concentration-response curve of the sodium disulfide (Na_2S; 1-6 mM) was constructed against contractions induced by phenylephrine (PE; 1µM).

After stabilization period, the medium was replaced with 10mL of PBS in the presence of excess NaCl (1,3 and 5mM/L), then concentration-response curve of the Na_2S (1-6 mM) was constructed against contractions induced with PE (1µM). To examine whether the Na_2S-induced vasorelaxation were mediated by increased K^+ conductance or by activation of α1-adrenoceptor subtype, aortic rings were contracted with either potassium chloride (KCl; 60 mM) or PE (1µM) (Sun *et al.*, 2013). Then, to test the role of different K^+ channels in the process of relaxation induced by Na_2S, the aortic rings were pre-incubated for 20 minutes with the following K^+ channel inhibitors, tetraethylammonium (TEA; 1mM), glibenclamide (GLIB; 10 µM), barium chloride ($BaCl_2$;1mM) and 4-aminopyridine (4-AP; 1mM), for inhibiting of K_{Ca}, K_{ATP}, K_{IR} and K_V channels. The inhibitors were used individually or in combinations.

The concentration-response curves were fitted with a Hill equation, from which the half maximal inhibitory concentration (IC_{50}) values were obtained as geometric mean. Maximum contractile responses to Na_2S were calculated as a percentage of the contraction produced by PE and were expressed as the means ± standard error of the mean (SEM). The tension produced by PE was defined as 0% relaxation, and the baseline tension before addition of vasoconstrictors were defined as 100% relaxation.

Chemicals

Phenylephrine, TEA, GLIB and $BaCl_2$ were obtained from Fluka (Fluka Chemical, Germany). Na_2S was purchased from Nakarai Chemicals (Japan) and 4-AP from Himedia Laboratories (Mumbai, India).

All chemicals were diluted in physiological saline solution. GLIB was diluted in a solution of dimethylsulfoxide (DMSO) 10%. The final concentration of DMSO did not exceed 0.01% in the tissue bath.

Statistical Analysis

The statistical analysis was performed using two-way analysis of variance (ANOVA) supported by Sidak post *hoc* test when carrying out pair wise comparison between the same doses of different groups. P-value less than 0.05 ($P<0.05$) was considered as statistically significant. All the graph, calculation and statistical analyses were performed using GraphPad Prism software version 6.0 for Windows (GraphPad Software, San Diego, California, USA).

Results

The vasodilator effects of Na_2S in HS rats or Normal diet

Aortic rings from HS rats showed a significant ($P<0.01$) attenuated relaxation to PE when compared with those of normal rats. The maximum relaxation (E_{max}) developed by Na_2S to 1µM PE in aortic rings from rats fed normal diet were significantly ($P<0.01$) greater (50.27±4.11%) than rats on HS (33.24±2%). Meanwhile, the IC_{50} of Na_2S was significantly ($P<0.001$) lower (1.23±0.29 mM) in HS rats than rats fed normal diet (2.28±0.12 mM), (Fig 1A). Also, typical traces of the dose-dependent vasodilator effects of Na_2S on isolated aortic rings from normal rats and rats fed 8% NaCl diet for 6 weeks and precontracted with PE (1µM) are shown in Fig. 1B. This implies that HS diminished Na_2S-induced aortic relaxation.

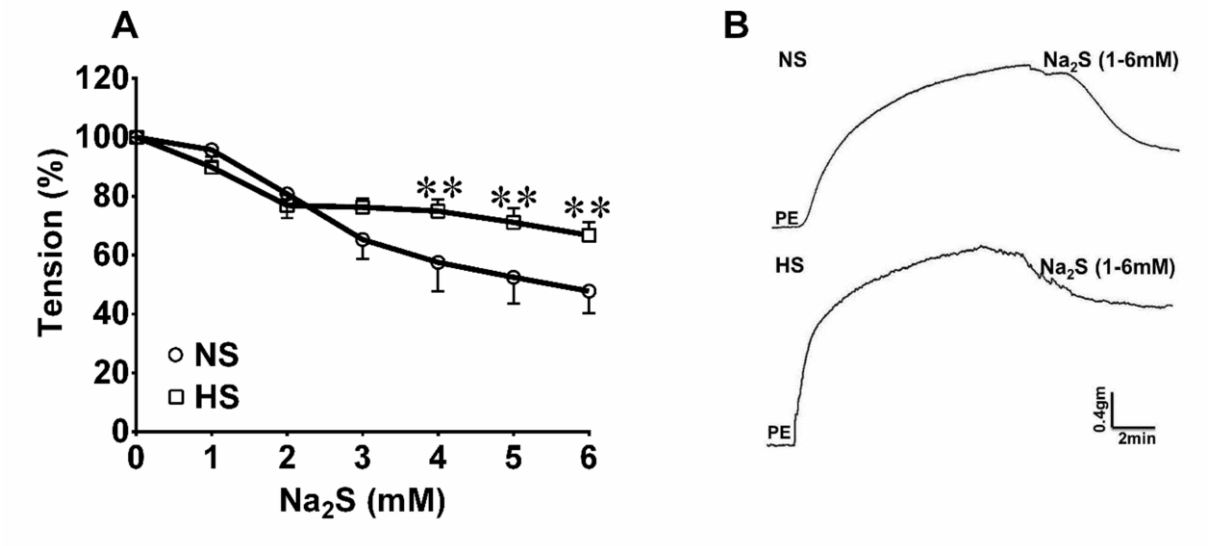

Fig.1. (**A**) Dose-response relationship curve to Na_2S induced relaxations in aorta of rats on normal salt diet (○; *n*=9) or 8% NaCl diet (□; *n*=14). Responses were significantly attenuated in HS rats (8% NaCl) diet. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE. Statistical differences were determined using two-way ANOVA supported by Sidak post hoc when carrying out pair wise comparison between the same doses of different groups. ** P<0.01 versus normal diet.(**B**) Typical traces showing the dose-dependent vasodilator effects of Na_2S (1-6mM) on isolated aortic rings from normal rats and rats fed 8% NaCl diet for 6 weeks and precontracted with PE (1 µM).

The effects of extra NaCl incubation on the vasodilator activity of Na_2S

Incubation of aortic rings in a medium of Krebs solution containing either 1mM, 3mM or 5mM of excess NaCl significantly (P<0.001) increased IC_{50} to (3.78±0.18, 4.19±, 0.27 and 3.6± 0.25mM) and shifted the dose-response relationship curve of Na_2S to the left, respectively. Meanwhile, excess NaCl did not change significantly E_{max} (58.07±5.06, 71.28±9.05 and 61.18±15.65) in comparison to control as shown in Fig. 2A, B and C. Typical traces of the dose-dependent vasodilator effects of Na_2S on isolated aortic rings from aortic rings incubated in a medium of Krebs solution containing either 1mM, 3mM or 5mM of extra NaCl precontracted with 1µM PE are shown in Fig. 2D, E and F.

Effect of Na_2S on aortic constriction evoked by PE or KCl

Aortic rings from HS rats had significantly (P<0.001) attenuated E_{max} in response to Na_2S in KCl-precontracted rings (-15.87±2.46%) when compared with those of PE- precontracted rings. While, the value of IC_{50} was significantly (P<0.001) higher in KCl (4.1±0.39 mM) than PE-precontracted aortic rings (Fig 3A). Furthermore, typical traces of the dose-dependent vasodilator effects of Na_2S on isolated aortic rings from rats fed 8% NaCl diet for 6 weeks, precontracted with either PE or KCl are shown in Fig. 3B. This result indicates that Na_2S relaxes aorta in HS rats via activation of K^+ channels.

The role of K^+ channels in the aortic effects of Na_2S

To identify the role of specific type of K^+ channels in the Na_2S-induced vasorelaxation, aortic rings were incubated with either $BaCl_2$, GLIB, TEA or 4-AP individually or in combination for 20 minutes prior to the application of Na_2S. Typical traces showing the role of K^+ channels in the dose-response vasodilator effects of Na_2S on aortic rings of HS rats incubated in buffer containing TEA, GLIB, $BaCl_2$ and 4-AP for 20 min and then contracted with PE are shown in Fig. 5A, B, C and D, respectively.

Fig 4C summarizes the dose-response curve for the inhibitory effect of $BaCl_2$ on Na_2S-induced relaxation in thoracic aortic rings precontracted with PE. The prior addition of $BaCl_2$ significantly (P<0.001) enhanced the IC_{50} (4.54±0.6 mM), whereas significantly reduced E_{max} to (9.12±2%), suggesting that K_{IR} channels are responsible for the Na_2S-induced relaxation in HS rats. Whereas, dose-response curve taken from the rings pretreated with GLIB showed that IC_{50} and E_{max} did not differ

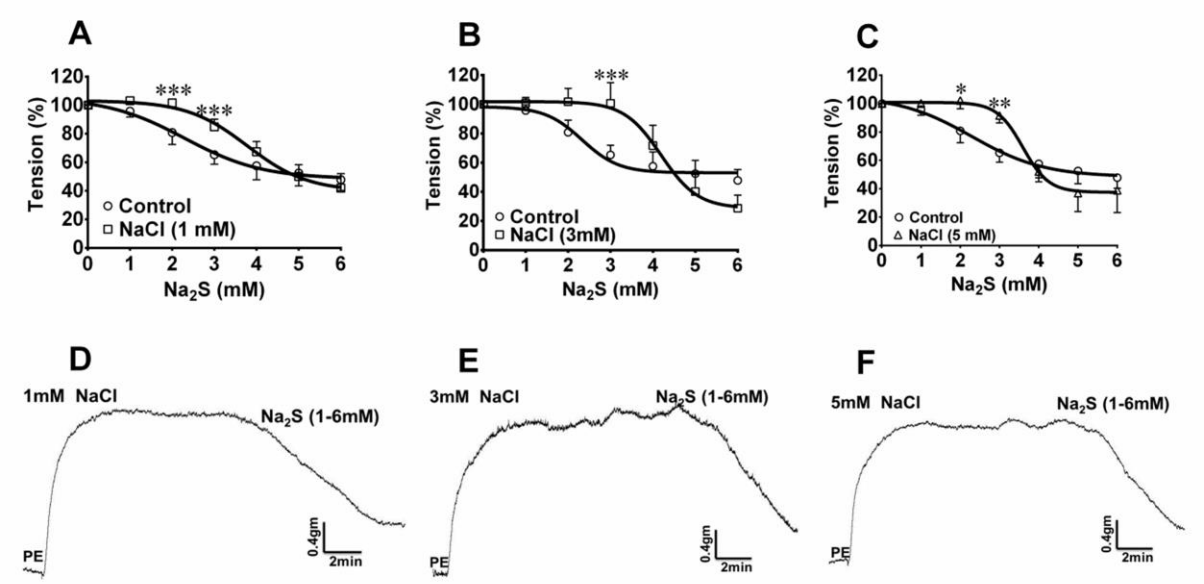

Fig.2. (**A, B and C**) Dose-response relationship curve to Na_2S induced aortic relaxations in a medium of Krebs solution containing either 1mM (□; *n*=6), 3mM (□; *n*=4) and 5mM (Δ; *n*=4) extra NaCl, precontracted with 1 µM PE. Although extra NaCl significantly increased IC_{50} and shifted the dose-response curve of Na_2S to the left, but the E_{max} did not differ significantly in comparison with control rings. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE Statistical differences were determined using two-way ANOVA supported by Sidak post hoc when carrying out pair wise comparison between the same doses of different groups. * P<0.05 versus control; ** P<0.01 versus normal diet; *** P<0.001 versus control. (**D, E and F**) Typical traces showing the dose-dependent vasodilator effects of Na_2S induced aortic relaxations in a medium of Krebs solution containing either 1mM, 3mM and 5mM extra NaCl, respectively.

significantly in comparison to HS control rings (Fig 4B).

Preincubation of aortic rings with TEA for 20 minute significantly (P<0.001) enhanced relaxation induced by Na_2S with an IC_{50}; 2.64±0.16mM, and E_{max} was increased to 73.43±3.37%, (Fig 4A). On the other hand, preincubation of aortic rings with 4-AP had negligible effect on Na_2S-induced relaxation with an IC_{50} (2.54±0.19mM) and E_{max} (44.46±2.66%), (Fig 4D).

To determine the possible role of Na_2S in the activation of more than one K^+ channels simultaneously, rings were incubated with a combination of $BaCl_2$ with either GLIB, 4-AP or TEA. Typical traces showing the role of K^+ channels in the dose-response vasodilator effects of Na_2S on aortic rings incubated in buffer containing $BaCl_2$ with either TEA; GLIB or 4-AP, are shown in Fig. 6D, E and F, respectively.

Combination of $BaCl_2$ with either GLIB or 4-AP significantly (P<0.001) shifted the curve to the left and reduced Na_2S-induced relaxation with IC_{50} 8±2.72 mM and 2.25±1.1mM, and reduced the E_{max} to -1.37±2.1% and -4±1.1% respectively, (Fig 6B and C). Meanwhile, Fig 6A indicates that combination of $BaCl_2$ with TEA significantly (P<0.001) enhanced Na_2S-induced relaxation with an IC_{50} 2.53±0.18 mM, and increased the E_{max} to 60±3.1%. These results further clarify the role of K_{IR} channels in Na_2S-induced relaxation in HS rats.

Discussion

Excess dietary salt is an important risk factor linked to hypertension (Elliot *et al.*, 1996), endothelial dysfunction (Zhu *et al.*, 2004), thickening and stiffening of conduit arteries and thickening and narrowing of resistance arteries (Wardener and MacGregor, 2002). These abnormalities lead to impairment of the vascular relaxation mediated by ACh (Lombard *et al.*, 2003) and NO (Kagota *et al.*, 2002). Although it was described earlier that H_2S relaxes different arteries in normal rats (Zhao *et al.*, 2001; Zhao and Wang, 2002) mutant mice lacking CSE display diminished vasorelaxation after muscarinic and cholinergic stimulation of vascular endothelial cells (Bernatova, 2014). But the vasoactivity of H_2S in rats who remained for

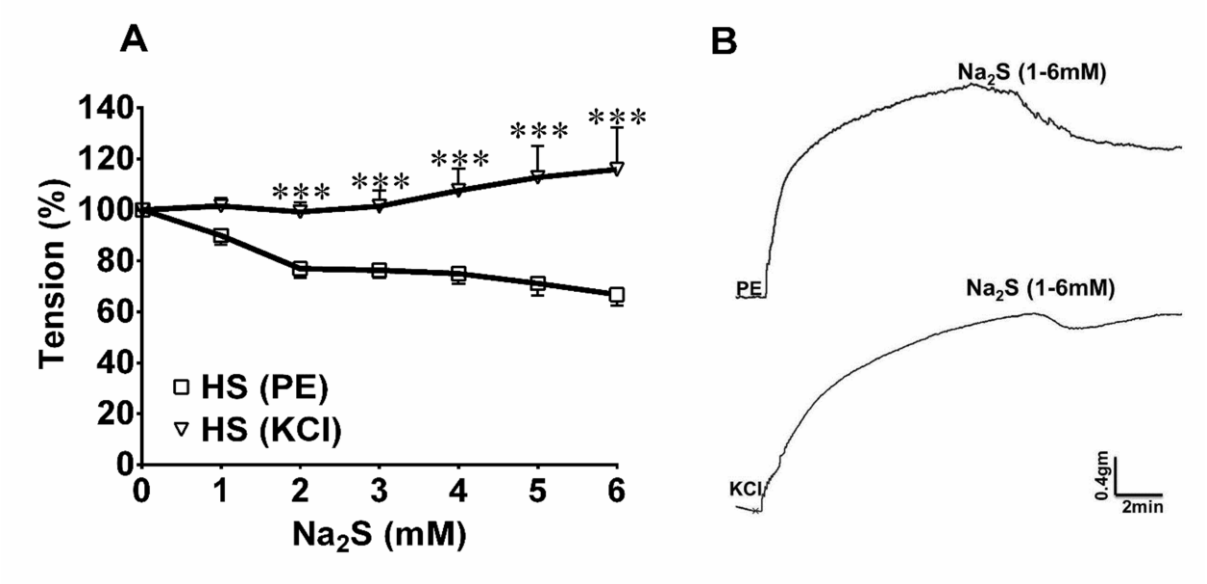

Fig.3. (**A**) Dose-response relationship curve to Na_2S induced relaxations in rat thoracic aortic rings precontracted with either 1 µM PE (□; n=14) or 60 mM K-Krebs buffer (Δ; *n*=9). Na_2S caused a dose-dependent relaxation after 1 µM PE precontraction, while relaxation completely blocked by 60 mM KCl. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE Statistical differences were determined using two-way ANOVA supported by Sidak post hoc when carrying out pair wise comparison between the same doses of different groups. *** P<0.001 versus control. (**B**) Typical traces showing the dose-dependent vasodilator effects of Na_2S (1-6mM) on isolated aortic rings from rats fed 8% NaCl diet for 6 weeks, precontracted with either PE (1 µM) or KCl (60mM).

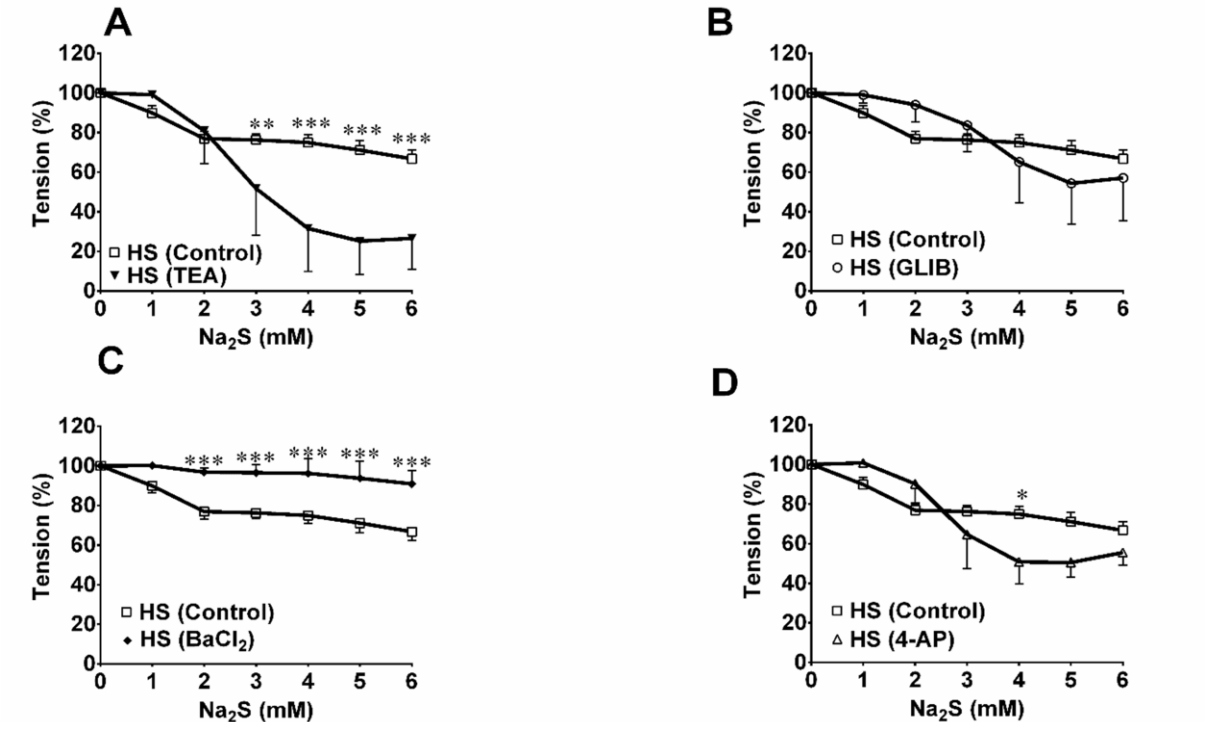

Fig.4. Role of K^+ channels in the vasodilator effects of Na_2S on PE-constricted aortic rings of HS rats. Aortic rings were first incubated in buffer containing (**A**) 1mM TEA (▼; n=8), (**B**) 10 mM GLIB (○; n=8), (**C**) 1mM $BaCl_2$ (♦; n=8) and (**D**)1mM 4-AP (Δ; n=4) for 20 min and then contracted with 1 µM PE. Dose-response relaxation induced by Na_2S significantly blocked by each of $BaCl_2$ and 4-AP; in contrast enhanced by TEA preincubation. While, GLIB did not change significantly dose-response relaxation induced by Na_2S. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE. Statistical differences were determined using two-way ANOVA supported by Sidak post hoc test when carrying out pair wise comparison between the same doses of different groups. * P<0.05 versus control; ** P<0.01 versus normal diet; *** P<0.001 versus control.

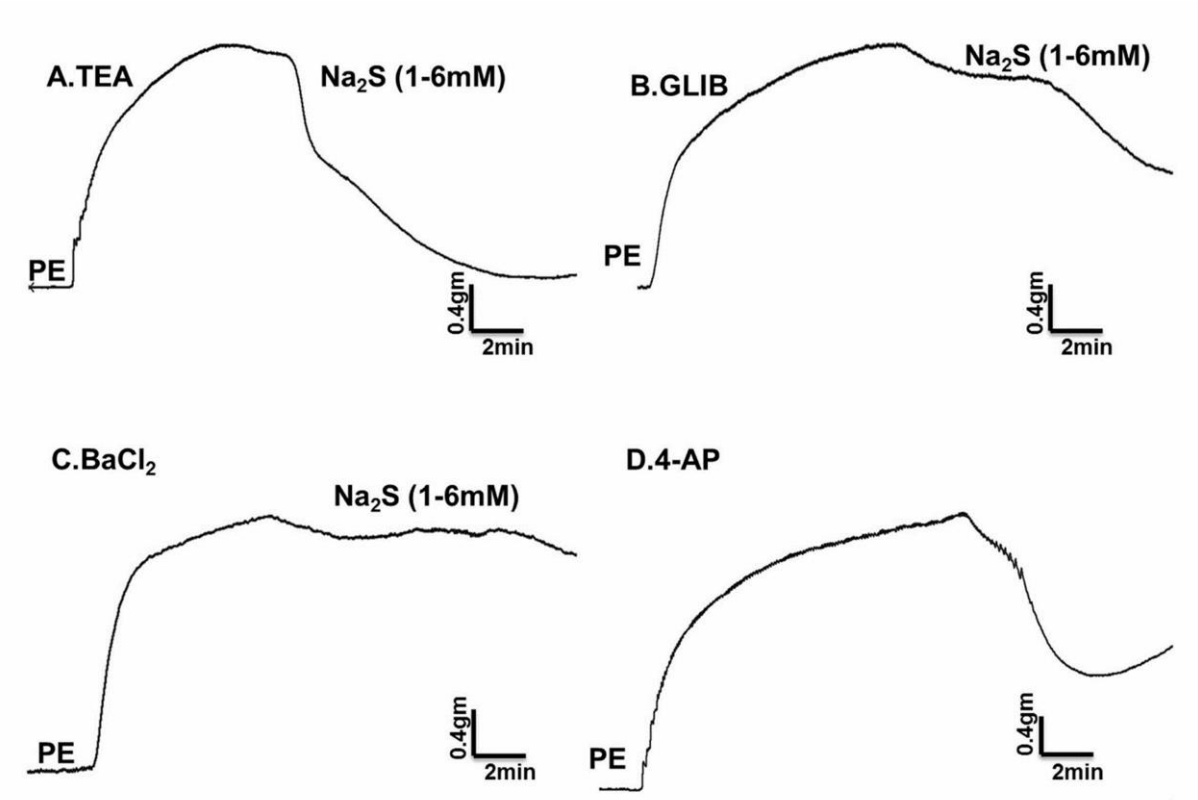

Fig.5. Typical traces showing the role of K^+ channels in the dose-response vasodilator effects of Na_2S (1-6mM) on aortic rings of HS rats incubated in buffer containing (**A**) 1mM TEA, (**B**) 10 mM GLIB, (**C**) 1mM $BaCl_2$ and (**D**) 1mM 4-AP for 20 min and then contracted with 1 mM PE.

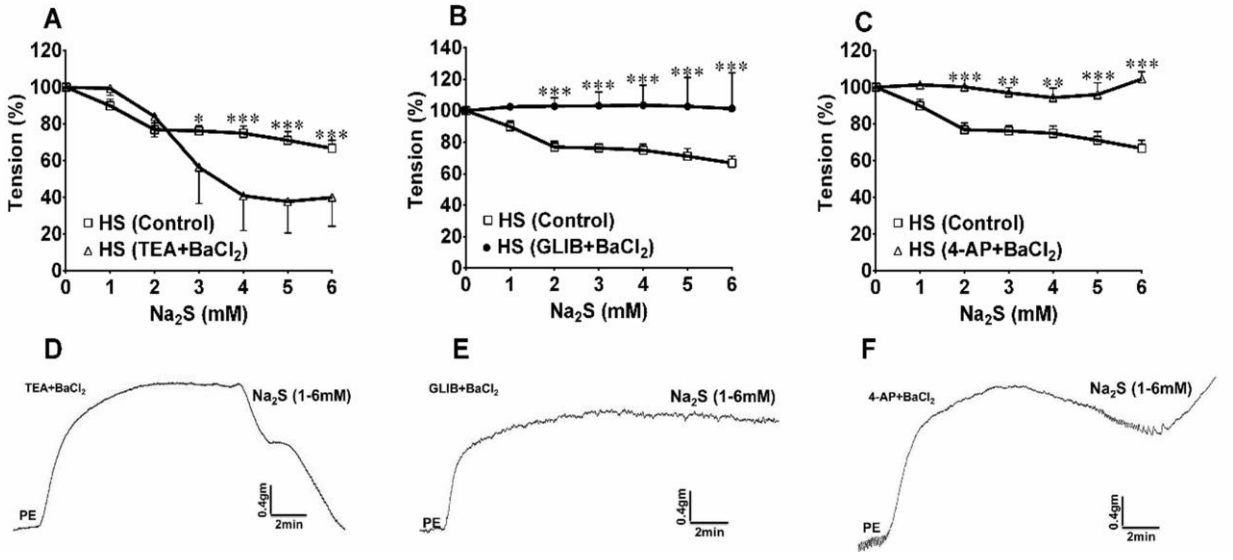

Fig.6. Role of K^+ channels combination in the vasodilator effects of Na_2S on PE-constricted aortic rings of rats fed 8% NaCl diet. Aortic rings were first incubated in buffer containing either (**A**) 1mM $BaCl_2$ and 1mM TEA (Δ; n=6); (**B**) 1mM $BaCl_2$ and 10 mM GLIB (●; n=7) or (**C**) 1 mM $BaCl_2$ and 1mM 4-AP (Δ; n=7) for 20 min and then contracted with 1mM PE. Combination of $BaCl_2$ with either GLIB or 4-AP significantly blocked Na_2S-induced aortic relaxation, while combination of $BaCl_2$ and TEA significantly enhanced dose-response relaxation induced by Na_2S. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE. Statistical differences were determined using two-way ANOVA supported by Sidak post hoc when carrying out pair wise comparison between the same doses of different groups. * P<0.05; ** P<0.01; *** P<0.001 versus control. (**D**, **E** and **F**) Typical traces showing the role of K^+ channels in the dose-response vasodilator effects of Na_2S (1-6mM) on aortic rings incubated in buffer containing 1mM $BaCl_2$ with either 1mM TEA; 10 mM GLIB or 1mM 4-AP, respectively.

long period in HS has never been described. Therefore, the results of our study demonstrated that excess dietary salt significantly impairs H_2S-induced aortic relaxation, suggesting that HS diet modulate the molecular pathway of vasorelaxation mediated by H_2S.

Plasma Na^+ may be raised by 1-3 mM/L in hypertensive population and spontaneous hypertensive rats. However, increase in Na^+ concentration to more than 5 mM/L could have damaging effects on the brain (de Wardener *et al.*, 2004; Oberleithner *et al.*, 2007). Based on this evidence, we hypothesize that a small rise in Na^+ concentrations may impair H_2S induced aortic relaxation. Three doses of excess Na^+ were used to test this hypothesis. We demonstrated that dose-response aortic relaxation curve of H_2S is sensitive to changes in Na^+ concentrations. There is evidence to demonstrate that small changes in plasma Na^+ concentration have a great effect on the stiffness and elasticity of endothelial cells (Oberleithner *et al.*, 2007) that could inactivate NOS (Li et al., 2009; Oberleithner et al., 2010). Therefore, it is important to recognize that this study is the first attempt to confirm that a small rise in plasma Na^+ concentration will impair vascular activity of H_2S.

It is well documented that muscle contractility and vascular tone are principally regulated by K^+ channels (Nelson and Quayle, 1995). A rise in K^+ permeability normally hyperpolarizes cell membrane and thus inhibits Ca^{++} influx through voltage-gated Ca^{++} channels, resulting in muscle relaxation (Callera *et al.*, 2004). To test whether or not H_2S can increase K^+ permeability, the relaxation response to H_2S in aortic rings taken from HS rats were treated with high K^+ Krebs solution, non-selective K^+ channels blocker (Tsang *et al.*, 2003). The vascular tone induced by high K^+ concentration was comparable to that of PE. Although, the relaxation response to H_2S was smaller in rings receiving K^+ than those receiving PE, suggesting that H_2S dilates aorta at least through activation of K^+ channels. It is difficult to compare the results since no data are available on the effect of PE and KCl on the relaxation response of aorta to H_2S in HS rats. However, more or less the same type response was reported for rats fed normal diet (Kiss *et al.*, 2008).

Based on the above results, we further studied the possible role of K_{IR} channels in H_2S-induced aortic relaxation mechanism in HS rats. K_{IR} channels are unique among the classes of K^+ channels in that an increase in external K^+, favours the opening of the channel, allowing the ion to flow out of the cell and produce hyperpolarization and relaxation (Loeb *et al.*, 2000). In VSMCs , the K_{IR} channels are characterised by a current that is rectified at potentials positive to the K^+ equilibrium potential (Edwards and Hirst, 1988) and might contribute to the resting membrane potential (Ko *et al.*, 2008). The first important finding of this study is that pre-treatment with $BaCl_2$, a blocker of K_{IR} channels strongly abolished aortic relaxation induced by cumulative doses of Na_2S. This finding indicates that H_2S may exert a vasodilatory effect possibly by activation of K_{IR} channels in HS rats.

One of the interesting results we would like to point out is simultaneous incubation of K_{IR} with either K_{ATP} or K_V channels blockers enhanced the reduction of aortic relaxations induced by cumulative doses of Na_2S. These data clearly demonstrate the important role of K_{IR} channels in the vasodilatory effect of H_2S in HS rats.

Generally, some of the vasodilatory actions of H_2S have been linked to K_{ATP} channels activation. The first clear connection between H_2S and K_{ATP} channels was demonstrated by Zhao *et al.* (2001) through a series of *in vivo* and *in vitro* experiments. In this study, previous inhibition of the K_{ATP} channel by GLIB did not alter H_2S-induced vasorelaxation. This effect has best been explained by Whidden *et al.* (2011) who demonstrated that HS alters K_{ATP} channels function and affects the mediation of dilator stimuli.

A wide range of K_V channels are expressed in VSMCs, they open to allow an efflux of K^+ in response to depolarization of the membrane, resulting in repolarization and maintenance of resting vascular tone (Ko *et al.*, 2008). K_V channels may also be a part of the mechanism of action of both vasodilators and vasoconstrictors (Jackson, 2000). The results of this study demonstrated that K_V channels have a minor role in the relaxation mechanism of H_2S, suggesting that K_V channels might not be responsible for the H_2S-induced aortic relaxation in HS rats. Although, there was no previous set of data to explain the relation between K_V channels and H_2S in salt-sensitive hypertensive rats, according to Ko *et al.* (2010) and Tajada *et al.* (2012) studies, the functional expression of different

K_V channels are decreased and downregulated in essential hypertension and decreased K_V current intensity were observed in spontaneously hypertensive rats, implying that these channels are small enough to be induced by H_2S.

Opening of K_{Ca} channels are induced by cytosolic Ca^{++} ions (Faber and Sah, 2003). It hyperpolarizes the membrane, promoting closure of Ca^{++} channels and thus opposing vasoconstriction (Ledoux *et al.*, 2006). Different changes in the K_{Ca} channels expression and current have been described in different animal models of hypertension (Liu *et al.*, 1998; Tajada *et al.*, 2012; Joseph *et al.*, 2013) as a negative feedback response to the increased vascular tone (Pinterova *et al.*, 2011). However, the literature regarding the effects of H_2S on K_{Ca} channels is not as extensive. It's known that H_2S induces vasorelaxation by activating endothelial K_{Ca} channels (Mustafa *et al.*, 2011; Beltowski and Jamroz-Wisniewska, 2014). Interestingly, the second important finding of this study is that inhibition of K_{Ca} channels in HS rats enhanced the dose-dependent relaxation response to Na_2S. It has been proposed that composition of K_{Ca} channels unit changes during hypertension which leads to increased Ca^{++} sensitivity of K_{Ca} channels and increase in vascular tonality (Amberg *et al.*, 2003). This indicates that K_{Ca} channels play a negative role in contributing to the vasodilating effect of H_2S in HS rats.

Conclusion

In conclusion, the results of this study demonstrated that the mechanism of H_2S-induced aortic relaxation differs from those of normal rats and HS rats. The H_2S-induced relaxation in aorta measured in rats that remained in excess sodium diet is mainly mediated by the stimulation of K_{IR} channels. In addition, H_2S-induced aortic relaxation in HS rats significantly enhanced by blocking K_{Ca} channels; this can be considered as a future choice for treatment of salt-sensitive hypertension. Furthermore, cell signal transduction pathways of the vasorelaxation mediated by exogenous H_2S in different animal models to study endothelial dysfunction and hypertension should be further investigated in order to understand its molecular mechanism.

Acknowledgment

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Conflict of Interest

There is no conflict of interest.

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