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$_2$S) 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$_2$S-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$_2$S 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$_2$S, an H$_2$S 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$_2$S-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$_2$S-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$_2$S-induced relaxation.

Conclusion: These results revealed that HS partially impairs aortic relaxation caused by H$_2$S, 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; Relaxation; Aorta; High salt diet

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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 (H₂S) (Moore et al., 2003). H₂S 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). H₂S 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⁺ (KATP) channels in VSMCs (Zhao et al., 2001; Tang et al., 2005), and Calcium (Ca²⁺)-activated K⁺ (KCa) channels in endothelium (Cheng et al., 2004). VSMCs express at least four different functional types of K⁺ channels, including KATP, KCa, inward rectifier K⁺ (Kir), and voltage-dependent K⁺ (KV) channels (Jackson, 2000). Opening of K⁺ channels and increase in K⁺ efflux in VSMCs cause membrane potential hyperpolarization and decrease Ca²⁺ entry via closing of voltage activated Ca²⁺ channels (Nelson and Quayle, 1995). This leads to stimulation of electrogenic sodium (Na⁺)-K⁺ pump and/or activation of Kir 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 KV (Joseph et al., 2013), Kir (Sobey, 2001) and KATP channels (Sobey, 2001; Blanco-Rivero et al., 2008) are impaired in several models of hypertension. Meanwhile, the density of membrane KCa 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 H₂S, regulated gene expression and enzymatic activity of CSE (Dombkowski et al., 2004; Lowicka and Beltowski, 2007) and the vascular H₂S synthase/H₂S 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 H₂S-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 H₂S-induced vascular relaxation in HS- incubated rats, with focus on Kir 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 Xylazine (10mg/Kg) intraperitoneally (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, NaHCO₃-23.8, MgCl₂-1, CaCl₂-1.5, and EDTA-0.003). It was brought to equilibration with 95% O₂ and 5% CO₂.
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$_2$S; 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$_2$S (1-6 mM) was constructed against contractions induced with PE (1µM). To examine whether the Na$_2$S-induced vasorelaxation were mediated by increased K$^+$ conductance or by activation of $\alpha$1-adrenoceptor subtype, aortic rings were contracted with either potassium chloride (KCl; 60 mM) or PE (1µM) (Sun et al., 2013). Then, to test the role of different K$^+$ channels in the process of relaxation induced by Na$_2$S, the aortic rings were preincubated 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$_2$S 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$_2$S 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$_2$S 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$_2$S 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$_2$S 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$_2$S 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$_2$S-induced aortic relaxation.
The effects of extra NaCl incubation on the vasodilator activity of Na$_2$S

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$_2$S 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$_2$S on isolated aortic rings from normal rats and rats fed 8% NaCl diet for 6 weeks and precontracted with PE (1 µM).

Effect of Na$_2$S 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$_2$S 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$_2$S 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$_2$S relaxes aorta in HS rats via activation of K$^+$ channels.

The role of K$^+$ channels in the aortic effects of Na$_2$S

To identify the role of specific type of K$^+$ channels in the Na$_2$S-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$_2$S. Typical traces showing the role of K$^+$ channels in the dose-response vasodilator effects of Na$_2$S 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$_2$S-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$_2$S-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...
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Fig. 2. (A, B and C) Dose-response relationship curve to Na2S induced aortic relaxations in a medium of Krebs solution containing either 1mM (□; n=6), 3mM (○; n=4) and 5mM (△; n=4) extra NaCl, precontracted with 1 µM PE. Although extra NaCl significantly increased IC50 and shifted the dose-response curve of Na2S to the left, but the Emax 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 Na2S induced aortic relaxations in a medium of Krebs solution containing either 1mM, 3mM and 5mM extra NaCl, respectively.

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 H2S 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 H2S in rats who remained 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 Na2S with an IC50 2.64±0.16mM, and Emax was increased to 73.43±3.37%, (Fig 4A). On the other hand, preincubation of aortic rings with 4-AP had negligible effect on Na2S-induced relaxation with an IC50 (2.54±0.19mM) and Emax (44.46±2.66%), (Fig 4D).

To determine the possible role of Na2S in the activation of more than one K+ channels simultaneously, rings were incubated with a combination of BaCl2 with either GLIB, 4-AP or TEA. Typical traces showing the role of K+ channels in the dose-response vasodilator effects of Na2S on aortic rings incubated in buffer containing BaCl2 with either TEA; GLIB or 4-AP, are shown in Fig. 6D, E and F, respectively.

Combination of BaCl2 with either GLIB or 4-AP significantly (P<0.001) shifted the curve to the left and reduced Na2S-induced relaxation with IC50 8±2.72 mM and 2.25±1.1mM, and reduced the Emax to -1.37±2.1% and -4±1.1% respectively, (Fig 6B and C).

Meanwhile, Fig 6A indicates that combination of BaCl2 with TEA significantly (P<0.001) enhanced Na2S-induced relaxation with an IC50 2.53±0.18 mM, and increased the Emax to 60±3.1%. These results further clarify the role of KIR channels in Na2S-induced relaxation in HS rats.
Fig. 3. (A) Dose-response relationship curve to Na$_2$S induced relaxations in rat thoracic aortic rings precontracted with either 1 µM PE (●; n=14) or 60 mM K-Krebs buffer (∆; n=9). Na$_2$S 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$_2$S (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$_2$S 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 (∆; n=4) for 20 min and then contracted with 1 µM PE. Dose-response relaxation induced by Na$_2$S 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$_2$S. 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.
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Fig. 5. Typical traces showing the role of K⁺ channels in the dose-response vasodilator effects of Na₂S (1-6mM) on aortic rings of HS rats incubated in buffer containing (A) 1mM TEA, (B) 10 mM GLIB, (C) 1mM BaCl₂ 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 Na₂S on PE-constricted aortic rings of rats fed 8% NaCl diet. Aortic rings were first incubated in buffer containing either (A) 1mM BaCl₂ and 1mM TEA (∆; n=6); (B) 1mM BaCl₂ and 10 mM GLIB (●; n=7) or (C) 1mM BaCl₂ and 1mM 4-AP (∆; n=7) for 20 min and then contracted with 1mM PE. Combination of BaCl₂ with either GLIB or 4-AP significantly blocked Na₂S-induced aortic relaxation, while combination of BaCl₂ and TEA significantly enhanced dose-response relaxation induced by Na₂S. 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 Na₂S (1-6mM) on aortic rings incubated in buffer containing 1mM BaCl₂ 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 H2S-induced aortic relaxation, suggesting that HS diet modulate the molecular pathway of vasorelaxation mediated by H2S.

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 H2S induced aortic relaxation. Three doses of excess Na+ were used to test this hypothesis. We demonstrated that dose-response aortic relaxation curve of H2S 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 H2S.

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 H2S can increase K+ permeability, the relaxation response to H2S 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 H2S was smaller in rings receiving K+ than those receiving PE, suggesting that H2S 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 H2S 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 KIR channels in H2S-induced aortic relaxation mechanism in HS rats. KIR 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 KIR 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 BaCl2, a blocker of KIR channels strongly abolished aortic relaxation induced by cumulative doses of Na2S. This finding indicates that H2S may exert a vasodilatory effect possibly by activation of KIR channels in HS rats.

One of the interesting results we would like to point out is simultaneous incubation of KIR with either KATP or KV channels blockers enhanced the reduction of aortic relaxations induced by cumulative doses of Na2S. These data clearly demonstrate the important role of KIR channels in the vasodilatory effect of H2S in HS rats.

Generally, some of the vasodilatory actions of H2S have been linked to KATP channels activation. The first clear connection between H2S and KATP 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 KATP channel by GLIB did not alter H2S-induced vasorelaxation. This effect has best been explained by Whidden et al. (2011) who demonstrated that HS alters KATP channels function and affects the mediation of dilator stimuli. A wide range of KV 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). KV 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 KV channels have a minor role in the relaxation mechanism of H2S, suggesting that KV channels might not be responsible for the H2S-induced aortic relaxation in HS rats. Although, there was no previous set of data to explain the relation between KV channels and H2S in salt-sensitive hypertensive rats, according to Ko et al. (2010) and Tajada et al. (2012) studies, the functional expression of different
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Kv channels are decreased and downregulated in essential hypertension and decreased Kv current intensity were observed in spontaneously hypertensive rats, implying that these channels are small enough to be induced by H2S.

Opening of KCa channels are induced by cytosolic Ca^{2+} ions (Faber and Sah, 2003). It hyperpolarizes the membrane, promoting closure of Ca^{2+} channels and thus opposing vasoconstriction (Ledoux et al., 2006). Different changes in the KCa 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 H2S on KCa channels is not as extensive. It’s known that H2S induces vasorelaxation by activating endothelial KCa channels (Mustafa et al., 2011; Beltowski and Jamroz-Wisniewska, 2014). Interestingly, the second important finding of this study is that inhibition of KCa channels in HS rats enhanced the dose-dependent relaxation response to Na2S. It has been proposed that composition of KCa channels unit changes during hypertension which leads to increased Ca^{2+} sensitivity of KCa channels and increase in vascular tonality (Amberg et al., 2003). This indicates that KCa channels play a negative role in contributing to the vasodilating effect of H2S in HS rats.

**Conclusion**

In conclusion, the results of this study demonstrated that the mechanism of H2S-induced aortic relaxation differs from those of normal rats and HS rats. The H2S-induced relaxation in aorta measured in rats that remained in excess sodium diet is mainly mediated by the stimulation of KIR channels. In addition, H2S-induced aortic relaxation in HS rats significantly enhanced by blocking KCa 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 H2S in different animal models to study endothelial dysfunction and hypertension should be further investigated in order to understand its molecular mechanism.

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**Conflict of Interest**

There is no conflict of interest.

**References**


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