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Nitric Oxide Donor Dilates Aorta in Salt Loaded Rats via Activation of Inward-Rectifier Potassium Channels

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A B S T R A C T

Since the mechanism of salt impairing NO-induced vascular relaxation is not fully clear, this study was designed to investigate the role of potassium (K^+) channels in the vasodilatory effects of NO donor in salt loaded rats. Isolated thoracic aortic rings of adult male albino rats fed 8% NaCl containing diet for six weeks were used for isometric tension recording using PowerLab tissue bath system. The recorded data revealed that high salt diet (HS) did not change the relaxation responses to sodium nitroprusside (SNP, an NO donor) in rat's thoracic aortic rings. SNP-induced relaxation in salt loaded rats was significantly lower in rings contracted by high K^+ than phenylephrine (PE, a selective α1-adrenergic receptor agonist). On the other hand, incubation of aortic rings from salt loaded rats with inward-rectifier K^+ (K_{IR}) channel blockers either individually or simultaneously with other K^+ channel blockers significantly inhibited SNP-induced relaxation in PE-contracted rings; however incubation of rings with either calcium (Ca^{+2})-activated K^+ (K_{Ca}), ATP-dependent (K_{ATP}) or voltage-sensitive K^+ (K_V) channels blockers had non-significant effects on relaxation responses of SNP. These results reveal that NO donor-induced aortic relaxation from salt loaded rats is mainly mediated by the activation of K_{IR} channels.

1. INTRODUCTION

High salt diet is strongly associated with increased blood pressure and causes endothelial dysfunction (Jiang *et al.*, 2015). Impaired endothelium-dependent dilation in vessels of salt loaded rats could resulted by either because the production of vasodilator substances by the endothelium is impaired or because the vessels liberate vasoconstrictor substances in response to vasoactive stimuli that normally relax the blood vessels (Zhu *et al.*, 2004). Some studies have suggested that HS lead to increased oxidative stress in the microcirculation and to changes in the level of pro or antioxidant enzyme activities (Sofola *et al.*, 2003). The elevated level of superoxide in the vessels from salt loaded rats may contribute to reduce NO availability and impaired vascular relaxation in response to endothelium-dependent vasodilator stimuli (Raffai, 2010).

Nitric oxide is a short-lived molecule that is produced by nitric oxide synthases enzymes (NOSs), which catalyze NO and L-citrulline formation from L-arginine and NADPH (Scatena *et al.*, 2005). In the vasculature, NO formed by the endothelium diffuses across vascular smooth muscle cells (VSMCs) membranes and plays an important role in the regulation of contractile activity, prevention of platelet and monocyte adhesion, and inhibition of cell proliferation (Costa and Assreuy, 2005).

NO dilates different blood vessels via activation of the soluble guanylyl cyclase (sGC) and elevate the production of cGMP (Fleming and Busse, 2003). In VSMCs, cGMP causes relaxation by reducing intracellular Ca^{+2} concentration and by down regulating the contractile apparatus (Hampl and Herget, 2000) through opening of a variety of K^+ channels (Stankevicius *et al.*, 2011).

Vascular smooth muscle cells express at least four different functional types of K^+ channels, including K_{ATP}, K_{Ca}, K_{IR} and K_V channels (Jackson, 2000). Opening of K^+ channels and increase in K^+ efflux in VSMCs cause membrane hyperpolarization and decrease Ca^{+2} entry via closing of voltage activated Ca^{+2} channels (Nelson and Quayle, 1995) leads to stimulation of electrogenic sodium (Na^+)-K^+ pump and/or activation of K_{IR} channels and vasodilation (Haddy *et al.*, 2006). While alteration in the function and expression of K^+ channels has been observed in different models of hypertension (HT) (Callera *et al.*, 2004).

Several studies suggest that acetylcholine (ACh)-induced relaxation has been impaired in both spontaneously hypertensive rats (Kagota *et al.*, 2002) and salt-sensitive rats (Nishida *et al.*, 1998). Salt intake impairs relaxation via decrease in NO production (Vapaatalo *et al.*, 2000), or suppression of eNOS activity (Li *et al.*, 2009). Despite the reduction of NO production during elevated dietary salt intake, the mechanism of salt impairing NO-induced vascular relaxation and the role of different types of K^+ channels are not completely understood. Therefore, the aim of the current study was to investigate the contribution of different subtypes of K^+ channels in the mechanism of NO-induced vascular relaxation in salt loaded rats, with focus on K_{IR} channels.

2. MATERIALS AND METHODS

2.1. Experimental Animals

The 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 weighing about 200–300g were allocated to 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 12hr light/dark cycle.

2.2. 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 aerated with 95% O_2 and 5% CO_2.

The procedure of (Furchgott and Zawadzki, 1980) was followed to study the vascular reactivity in the isolated aortic rings 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 an organ bath (Model 166051, Radnoti, Monrovia Ca, USA) and other wire was connected to force transducer (MLT0201/RAD 5-25mg, AD instruments, Sydney, Australia) 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 Labchart 7 software for isometric tension measurement. The degree of contraction and relaxation were indicated by the tension development in the recording system and expressed in g.

2.3. Aortic relaxation studies

Rings were allowed to equilibrate for 60 min at a resting tension of 2g with changes of buffer every 15 min. When the isometric tension had stabilized, inhibitory concentration-response curve of the SNP (10^{-8}–$3X10^{-4}$M) was constructed against contractions induced with PE (1µM).

To examine whether the SNP-induced vasorelaxation were mediated by increased K^+ conductance or by activation of α1-adrenoceptor subtype, aortic rings were contracted with either potassium chloride (KCl; 60mM) or PE (1µM). Then, to test the role of different K^+ channels in the development of relaxation induced by SNP, the aortic rings were incubated for 20 min with the following K^+ channel inhibitors, tetraethylammonium (TEA; 1mM), glibenclamide (GLIB; 10µM), barium chloride ($BaCl_2$;1mM) and 4-aminopyridine (4-AP; 1mM), for K_{Ca}, K_{ATP}, K_{IR} and K_V channels inhibition. The inhibitors were applied 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 given as geometric mean. Maximum contractile responses to SNP 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 was defined as 100% relaxation.

2.4. 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 analysis were performed using GraphPad Prism 7 (GraphPad Software, San Diego, California, USA).

3. RESULTS

3.1. The vasodilator effects of SNP in rats fed high and normal diet

The results showed non-significant difference between the maximum relaxation (E_{max}) responses of aortic rings from salt loaded rats (73.54±7.34%) and control rats (84.79±4.94%) contracted by PE as shown in Figure 1A. Furthermore, the negative log of the molar concentration (PD_2) values of aortic rings from salt loaded rats (5.62±0.09mM) was similar to that of control rings (5.65±0.01mM). Also, typical traces of the dose-dependent vasodilator effects of SNP on isolated aortic rings from normal and salt loaded rats for 6 weeks and contracted with PE (1µM) are shown in Figure 1B.

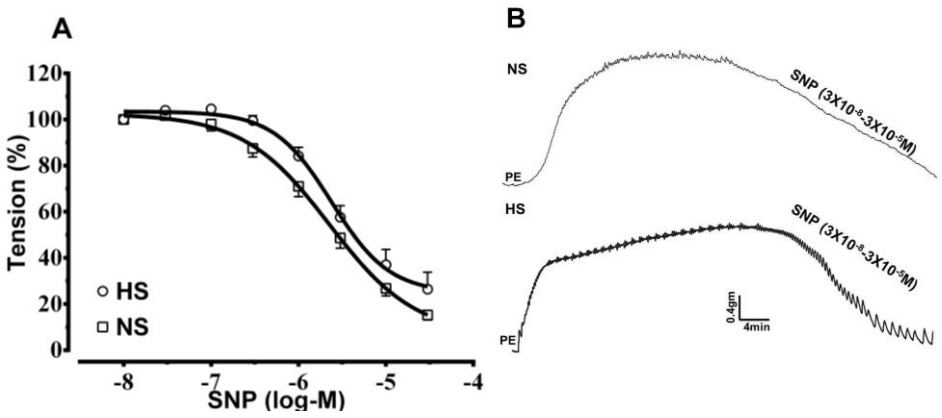

Figure 1. (**A**) Dose-response relationship curve to SNP ($3X10^{-8}$-$3X10^{-5}$M) induced relaxation in aortic rings of rats on normal salt diet (□; *n*=9) or 8% NaCl diet (○; *n*=6). Relaxation responses were not significantly changed in salt loaded rats. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE. (**B**) Typical traces showing the dose-dependent vasodilator effects of SNP on isolated aortic rings from normal and salt loaded rats for 6 weeks and contracted with PE.

3.2. Effect of SNP on aortic constriction evoked by PE or KCl

A typical traces from representative experiments on relaxing effects of SNP on rats contracted aortic rings with PE and KCl are shown in Figure 2 B. Aortic rings from salt loaded rats significantly (P<0.001) attenuated E_{max} to SNP in KCl-contracted rings (34.85±5.09%) when compared with those of PE-contracted rings. While, pD_2 was non-significantly changed in KCl (5.45±0.26mM) than PE-contracted aortic rings (5.62±0.09mM), as shown in Figure 2A.

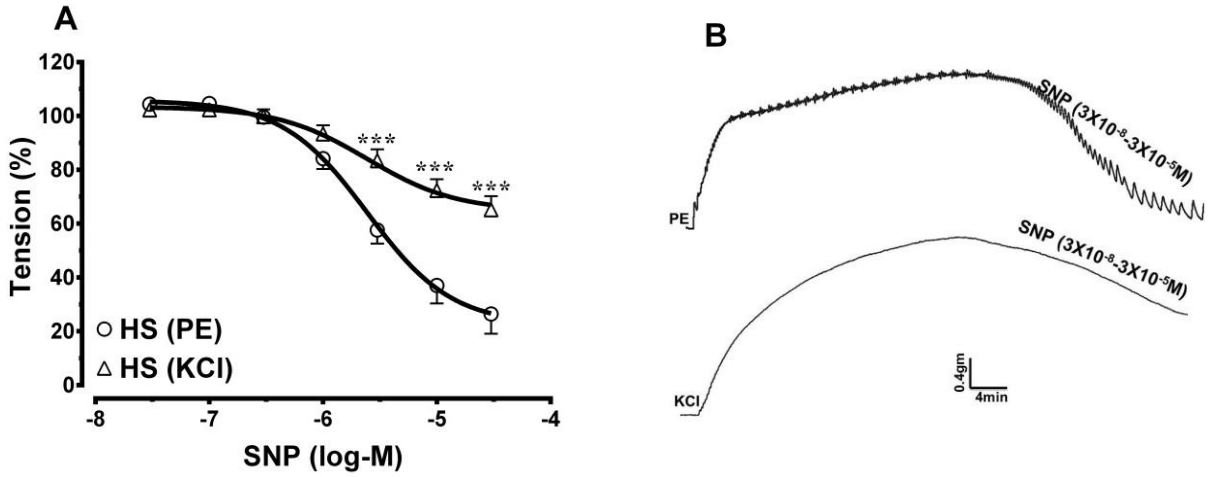

Figure 2. (**A**) Dose-response relationship curve to SNP ($3X10^{-8}$-$3X10^{-5}$M) induced relaxations in rat thoracic aortic rings contracted with either 1µM PE (○; n=6) or 60mM K-Krebs buffer (Δ; *n*=6). SNP caused a dose-dependent relaxation after 1µM PE precontraction, while relaxation completely blocked by 60 mM KCl. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE. *** P<0.001 versus control. (**B**) Typical traces showing the dose-dependent vasodilator effects of SNP on isolated aortic rings from salt loaded rats for 6 weeks, contracted with either

3.3. Role of K^+ channels in the aortic effects of SNP

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

The dose-response curve for the inhibitory effect of $BaCl_2$ on SNP-induced relaxation in salt loaded rats aortic rings contracted with PE is summarized in Figure 3C. The prior addition of individual $BaCl_2$ non-significantly changed pD_2 (5.15±0.67mM), whereas significantly (P<0.001) inhibited E_{max} to (29.92±9.4%), suggesting that K_{IR} channels are responsible for the SNP-induced relaxation in salt loaded rats. Whereas, dose-response curve taken from the rings pretreated with TEA, GLIB and 4-AP showed that pD_2 (6.07±0.27mM, 5.64±0.11mM and 5.85±0.13mM) and E_{max} (52.38±8.68%, 75.89±4.84% and 85.85±3.27%) did not differ significantly in comparison to rings of salt loaded rats (Figure 3A, B and D).

To determine the possible role of SNP 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 SNP on aortic rings incubated in

Krebs solution containing $BaCl_2$ with either TEA; GLIB or 4-AP, are shown in Figure 5 D, E and F, respectively.

Combination of $BaCl_2$ with either TEA, GLIB and 4-AP significantly ($P<0.001$) reduced E_{max} of SNP-induced relaxation from (36.68±4.26%, 49.13±5% and 42.28±7.21%) with pD_2 (5.66±0.18mM, 5.4±0.19mM and 5.5±0.23 mM), as shown Figure 5 A, B and C respectively.

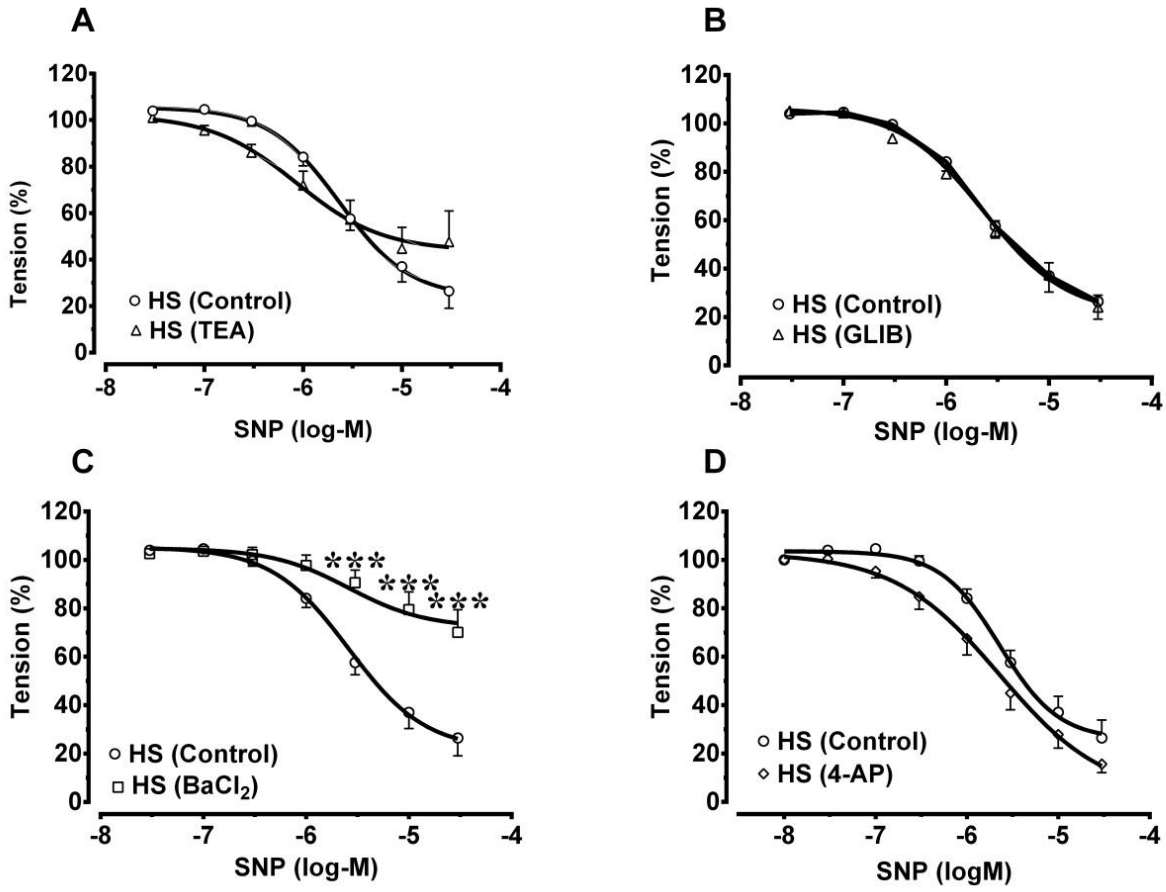

Figure 3. Role of K^+ channels in the vasodilator effects of SNP ($3X10^{-8}$-$3X10^{-5}$M) on PE-constricted aortic rings of salt loaded rats. Aortic rings were first incubated in buffer containing (**A**)1mM TEA (Δ; n=9), (**B**) 10µM GLIB (Δ; n=5), (**C**) 1mM $BaCl_2$ (□; n=7) and (**D**)1mM 4-AP (◊; n=6) for 20 min and then contracted with 1µM PE. Dose-response relaxation induced by SNP significantly blocked by $BaCl_2$ incubation. While, incubation by TEA, GLIB and 4-AP did not change significantly dose-response relaxation induced by SNP. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE. * $P<0.05$ versus control; *** $P<0.001$ versus control.

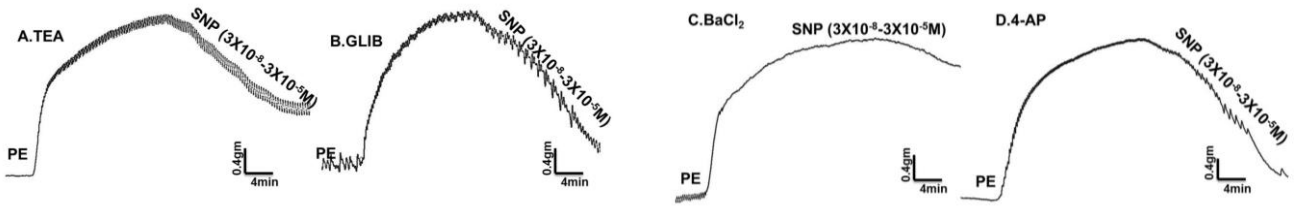

Figure 4. Typical traces showing the role of K^+ channels in the dose-response vasodilator effects of SNP ($3X10^{-8}$-$3X10^{-5}$M) on aortic rings of salt loaded rats incubated in buffer containing (**A**) 1mM TEA, (**B**) 10µM GLIB, (**C**) 1mM $BaCl_2$ and (**D**) 1mM 4-AP for 20 min and then contracted with 1µM PE.

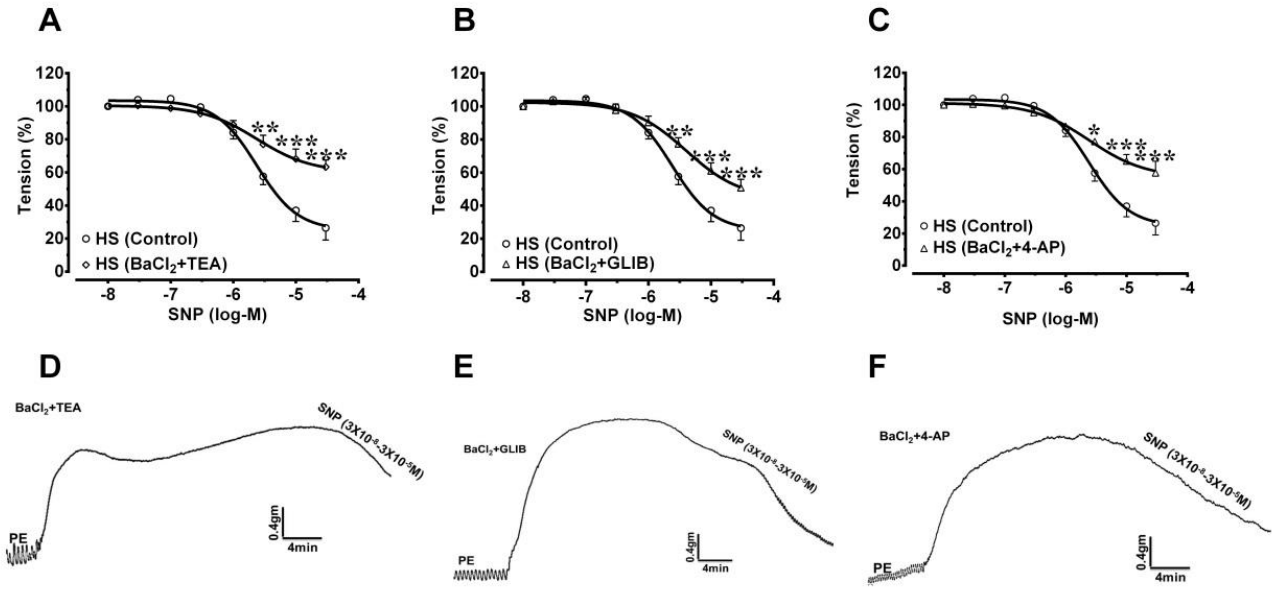

Figure 5. Role of K^+ channels combination in the vasodilator effects of SNP ($3X10^{-8}$-$3X10^{-5}$M) on PE-constricted aortic rings of salt loaded rats. Aortic rings were first incubated in buffer containing either (**A**) 1mM $BaCl_2$ and 1mM TEA (◊; n=5); (**B**) 1mM $BaCl_2$ and 10µM GLIB (Δ; n=5) or (**C**) 1 mM $BaCl_2$ and 1mM 4-AP (◊; n=6) for 20 min and then contracted with 1µM PE. Combination of $BaCl_2$ with other K^+ channels blockers significantly blocked SNP-induced aortic relaxation. All data are expressed as % of relaxation of PE-induced aortic tone and are represented as the mean±SE. * 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 SNP on aortic rings incubated in buffer containing $BaCl_2$ with either TEA; GLIB or 4-AP, respectively.

4. DISCUSSION

Hypertension is a major risk factor for cardiovascular disease and dietary salt is a contributing factor to high blood pressure (Whidden et al., 2011), 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) through inhibition of NO release (Sylvester et al., 2002). Furthermore, dietary salt impairs the myogenic responsiveness of normotensive rats aortic rings to H2S (Salihi, 2016) and impair the relaxation response NO in spontaneously hypertensive rats (Kagota et al., 2002), but not in normal rats (Sofola et al., 2003). Moreover, the lack of a difference in SNP-induced aortic dilation between rats fed normal and HS diet in the current study does not support impairment of relaxation pathway induced by NO in salt loaded rats.

The data of the present study demonstrates that aortic relaxation in salt loaded rats in response to NO was attenuated when they were contracted with KCl, more than PE, suggests that NO may activate K+ channels in aortic tissue. Decreased relaxation to NO was not due to a difference in the degree of the contractions because they were similar with each contractile agent. On the other hand, high extracellular K+ concentration abolish the K+ gradient across the cell membrane, attenuate intracellular K+ efflux and decrease VSMCs relaxation (Cheng et al., 2013). It is difficult to compare the results since no data are available on the effect of PE and KCl on the relaxation responses of rat aortic rings to NO. Bracamonte et al. (1999) reported that canine femoral veins fed normal diet had a weak vasodilatory response to NO donor in a high K+ solution .

It is well known that in normotensive animals NO provoke vasodilation via the

activation of sGC/cGMP pathway and/ or activation of different K+ channels, including KCa channels (Chitaley and Webb, 2002), KIR channels (Schubert et al., 2004), KATP channels (Murphy and Brayden, 1995) and KV channel at least partially by KV2.1 subunit (Tanaka et al., 2006). On the other hand, conflicting results were recorded regarding the contribution of K+ channels in the NO-induced vascular relaxation in different models of HT in animals. Bonaventura et al. (2011), have shown that the K+ channels do not play important role in the relaxation induced by the NO donors in aortic rings from HT rats. In contrast, Araujo et al. (2013) reported that the vascular relaxation induced by NO involves the activation of K+ channels sensitive to TEA in normotensive and renal hypertensive rat mesenteric resistance arteries.

The results of the present study confirm that NO-mediated relaxation of salt loaded rats aortic rings is depend on the flux of K+ ion through the activation of KIR channels. This observation is confirmed by attenuation of SNP relaxation by $BaCl_2$. The best mechanism which supports our results is explained by (Nakahata et al., 2006). They indicated that vasodilation of cerebral arterioles via KIR channels is augmented in chronic HT. Although, others showed that chronic HT decrease expression and/or function of vascular KIR channels (Sobey, 2001), especially in renal and mesenteric arteries (Tajada et al., 2012) but not in aorta. Moreover, simultaneous incubation of $BaCl_2$ with either TEA, GLIB or 4-AP K_{IR} highly reduced relaxations induced by cumulative doses of SNP, suggesting a dual activation of K+ channels in rings of salt loaded rats by NO donor.

Since it has been reported that properties of KCa, KATP and KV channels are altered in different types of HT, we have evaluated the involvement of these channels in the NO pathway in salt loaded rats. In the present study, the blockade of KCa, KATP and KV channels did not change SNP-induced vasorelaxation supporting that in salt loaded rats NO relax aortic rings through a pathway did not include these channels. Despite increase in KCa channel expression in salt loaded rats (Cordaillat et al., 2007), there are major change in unit composition and its sensitivity to Ca+2 has been decreased (Tajada et al., 2013). Moreover, decreased expression and function of KATP channels (Tajada et al., 2012) and KV channels were observed in different models of HT (Ko et al., 2010). Therefore, these results consider that HS is associated with diminished activity of KCa, KATP and KV channels, and these channels do not participate in NO donor-induced relaxation pathway in salt loaded rats.

Taken together, these results show that NO donor-induced relaxation in aortic rings taken from rats remained on HS is mainly mediated by the activation of KIR channels. This can be considered as a future choice for treatment of salt-sensitive HT. Beside this, cell signal transduction pathways of the vasorelaxation mediated by exogenous NO in different animal models of endothelial dysfunction and HT should be further studied in order to understand its molecular mechanism.

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Conflict of Interest

There is no conflict of interest.

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