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The vasodilator mechanism of sulfur dioxide on isolated aortic rings of rats: Involvement of the K^+ and Ca^{2+} channels

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ABSTRACT

The present study was designed to investigate vasodilator effect of endogenous gaseous sulfur dioxide (SO_2) and roles of Ca^{2+} channels and K^+ channels in relaxations of SO_2 in isolated rat aortic rings. Isolated rat aortic rings were perfused in organ baths and changes in isometric tension were recorded. The results showed that: (1) SO_2 could relax isolated aortic rings contracted by norepinephrine in a dose-dependent manner (EC_{50}, 1247.38±98.32 μM). The vasorelaxant effect of SO_2 at basal (110.34±35.22 μM) and low concentrations (<450 μM) was endothelium-dependent, while it was endothelium-independent at high concentrations (>500 μM). (2) The vasorelaxation of 1500 μM SO_2 on both endothelium-intact and endothelium-denuded aortic rings was partially inhibited by nifedipine, an L-type calcium-channel blocker. (3) The vasoconstriction responses induced by $CaCl_2$ were inhibited by 1500 μM SO_2 on both endothelium-intact and endothelium-denuded aortic rings. (4) The initial fast vasoconstriction induced by intracellular Ca^{2+} release was enhanced by 1500 μM SO_2, but the sustained vasoconstriction evoked by extracellular Ca^{2+} influx was inhibited by 1500 μM SO_2. (5) Pretreated by 1500 μM SO_2, the vasoconstriction responses induced by norepinephrine or KCl were enhanced at low concentrations and inhibited at high concentrations. (6) The SO_2-induced vasorelaxation was partially inhibited by tetraethylammonium (TEA) and glibenclamide for both endothelium-intact and endothelium-denuded rings. For the endothelium-intact rings, the vasorelaxant effects induced by 30 and 300 μM SO_2 were partially inhibited by iberiotoxin. These results led to the conclusions that endogenous gaseous SO_2 could cause vasorelaxation on rat aortic rings in a dose-dependent manner. The vasorelaxant effects of SO_2 at basal and low concentrations were endothelium-dependent, which might be partly related to big-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel. The mechanism of SO_2-induced vasorelaxation at high concentrations was shown to be endothelium-independent, which might be related to ATP-sensitive K^+ (K_{ATP}) channel and L-type calcium-channel as well as possible alterations in Ca-influx and release pathways.

1. Introduction

Sulfur dioxide (SO_2) is the main product from the combustion of sulfur compounds and is of significant environmental concern. Since coal and petroleum often contain sulfur compounds, their combustion generates SO_2. It or its conjugate base bisulfite is produced biologically as an intermediate in both sulfate-reducing organisms and sulfur-oxidizing bacteria. As we know, SO_2 is toxic in large amounts, and it causes a wide variety of health risks and environmental hazards because of the way it reacts with other substances in the air. Some studies showed that SO_2 inhalation could affect the respiratory system and cardiovascular system in sensitive groups, especially the children and elderly people.

In our previous studies, it was shown that SO_2 was a systemic toxin and might have many kinds of biological and toxicological roles in multiple organs of mammals (Meng, 2003). SO_2 derivatives could cause the increases of sodium and potassium currents in the rat hippocampal CA1 neurons and dorsal root ganglion neurons (Du and Meng, 2004a,b; Meng and Nie, 2005a,b) and modulation of L-type calcium current in rat cardiac myocytes (Nie and Meng, 2006). The epidemiological investigations had revealed that SO_2 was correlative with the cardiovascular diseases (Mar et al., 2000; Hong et al., 2002). Recently, we had found that rat blood pressure could be lowered by SO_2 and its derivatives (Meng et al., 2003; Meng and Zhang, 2007; Meng et al., 2007a,b). Our another study was carried out to identify the involvement of various signal transduction pathways in the vascular effect of endogenous gaseous SO_2, which demonstrated that the vasorelaxant effect of SO_2 at basal and low concentrations might be mediated by the NO (nitric oxide) and/or cGMP pathway (unpublished observation). NO mediates vasorelaxation by increasing the cellular cGMP activity and/or stimulating K_{Ca} channels in vascular SMCs. So the mechanism of SO_2-induced vasorelaxation should partially involve the contribution of K_{Ca} channel. However, the mechanism involved of K^+ and Ca^{2+} channels by which SO_2 produces this effect is unknown. Therefore, the purpose of the present study was to investigate roles of

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Ca^{2+} channels and K^+ channels in relaxations of endogenous gaseous SO_2 in isolated rat aortic rings.

2. Materials and methods

2.1. Chemicals and solution preparation

Acetylcholine, norepinephrine, glibenclamide, 4-aminopyridine (4-AP), nifedipine, iberiotoxin, apamin, and tetraethylammonium (TEA) were purchased from Sigma (St. Louis, MO, USA). Krebs solution contains (mM) NaCl 120.6, KCl 5.9, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, $CaCl_2$ 2.5, and glucose 11.5. The components of Ca^{2+}-free Krebs solution were similar to those of Krebs solution but with EGTA at 0.2 mM instead of $CaCl_2$. High K^+, Ca^{2+}-free Krebs solution (Polster et al., 1990) consisted of (mM) NaCl 17, KCl 100, and other components was similar to those of Ca^{2+}-free Krebs solution. When the concentration of KCl in Krebs solution was increased to 20 or 100 mM in some experiments, the osmolality of solution was adjusted by equimolarly decreasing NaCl concentration.

For SO_2 solution preparation, pure SO_2 gas (purity: 99.99%) obtained from Beijing He-Pu-Bei-Fen Gas Company, Ltd. (Beijing, China) was used in this study. SO_2 solution was freshly prepared before each experiment by bubbling saline with pure SO_2 gas to achieve a solution containing SO_2 at concentrations ranging from 0–2000 μM.

2.2. Preparation of isolated rat thoracic aorta rings

Animal care and experimental protocols complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (Documentation 55, 2001) and the Animal Care Committee of Shanxi University. Male Wistar rats weighting about 220–250 g were obtained from Heibei Medical University (Shijiazhuang, China). Rats were killed by anesthetic overdose (intraperitoneal injection of pentobarbital sodium). The thoracic aorta rings were prepared carefully so that the endothelial cells were not damaged. The thoracic aorta was removed immediately and dissected fat and connective tissues, then cut into rings about 3 mm long. The rings were placed in a bath of Krebs solution at pH 7.4, 37 °C under a resting optimal tension of 1.5 g; 95% O_2 and 5% CO_2 were bubbled through the solution. Tension was recorded with a MedLab Biological Signal Collection System (Medease Science and Technology, Nanjing, China) during the experiment. The aortic rings in bath tubes were allowed to equilibrate for 1 h before experiment and the Krebs solution was changed every 15 min. The viability of the ring preparation was assessed by contracting vessels with 60 mM KCl before each experiment. The artery endothelium viability and integrity were checked by dilatory response of the ring to acetylcholine (10^{-6} M) as described by Furchgott and Zawadzki (1980) and Fiscus et al. (1991). All rings with endothelium tested had the dilatory response enough to acetylcholine.

2.3. Vasodilator effects of SO_2 on the thoracic aorta rings

To study the vasodilator effects of SO_2, isolated rat thoracic aorta rings were contracted by 10^{-6} M norepinephrine, when the vasoconstriction curves of rings reached the plateau phase of the maximum tension, SO_2 at 0–2000 μM was added, and the tensions were recorded. At the plateau phase of contraction, the cumulative concentration–response curves of SO_2 were built and fitted with a Hill equation (Weiss, 1997). Percentage of dilation was calculated when the vasodilator curve reached the plateau phase of the minimum tension. The maximum constriction caused by 10^{-6} M norepinephrine was about 80% of the maximum constriction response induced by 10^{-4} M norepinephrine (Fig. 5). In this study, norepinephrine at 10^{-6} M was used, therewith vasodilator effect of SO_2 was expressed as a percentage of relaxation to maximum constriction induced by 10^{-6} M norepinephrine. Saline was used as a control group.

To investigate the role of endothelium in the relaxation response, endothelium was removed by gently scraping with a cotton ball. Verification of endothelium removal was confirmed by failure of the vessel to relax >20% in response to 10^{-6} M acetylcholine (Furchgott and Zawadzki, 1980; Fiscus et al., 1991).

2.4. Involvement of L-type calcium-channel in the vascular effects of SO_2

To elucidate whether an L-type calcium-channel was the target of SO_2 vascular action, rings with intact endothelium and denuded endothelium were pre-incubated with nifedipine (1 μM), an L-type calcium-channel blocker, for 10 min before contraction with norepinephrine. Then SO_2 at 30, 300, or 1500 μM was added, respectively, and the tensions were recorded.

2.5. Effects of SO_2 on vasoconstriction induced by $CaCl_2$

To determine if Ca^{2+} channels are involved in the vasorelaxant effect of SO_2, aortic rings with intact endothelium and denuded endothelium were equilibrated in Ca^{2+}-free Krebs solution and washed three times at 20 min intervals at 37 °C, then equilibrated in high K^+,Ca^{2+}-free Krebs solution for 15 min to depolarize the membrane, and $CaCl_2$ was added accumulatively from 0.01 mM to 4.44 mM to observe the normal concentration–effect curve. Then, after SO_2 at 30, 300, or 1500 μM or nifedipine at 1 μM was added and bathed for 10 min, respectively, the concentration–effect curves of $CaCl_2$ at 0.01 to 4.44 mM were derived as already mentioned in the presence of SO_2 or nifedipine at various concentrations.

2.6. Effects of SO_2 on vasoconstrictions of two components

This experiment was performed in order to study effects of SO_2 on vasoconstrictions of two components: the initial fast vasoconstriction (induced by intracellular Ca^{2+} release caused by norepinephrine) and the sustained vasoconstriction (evoked by extracellular Ca^{2+} influx caused by $CaCl_2$). In this experiment, briefly, nifedipine (1 μM) or SO_2 (30, 300, or 1500 μM) was added to the rings with intact endothelium and denuded endothelium bathed in Ca^{2+}-free Krebs solution, respectively, and 10 min later norepinephrine (1 μM) was added to observe the initial fast vasoconstriction. When the initial fast vasoconstriction reached its peak, immediately $CaCl_2$ (2.5 mM) was added to observe the sustained vasoconstriction (Meng and Zhang, 2007).

2.7. Effects of SO_2 on vasoconstriction induced by KCl or norepinephrine

Vasoconstriction effects induced by norepinephrine or KCl were measured by the method described by Kwan et al. (1992) and Rossum (1963). Briefly, after the aortic rings with intact endothelium and denuded endothelium equilibrated in Krebs solution (37 °C), norepinephrine from 10^{-8} to 10^{-4} M or KCl from 10 to 100 mM was accumulatively added to obtain the normal concentration–effect curves of norepinephrine and KCl, respectively. Then the ring preparations were washed to normal tension and bathed in Krebs solution (37 °C) for 30 min. After that SO_2 at 30, 300, or 1500 μM or nifedipine at 1 μM was added, respectively. And 10 min later, concentration–effect curves of norepinephrine or KCl were derived as mentioned earlier, in the presence of SO_2 or nifedipine at various concentrations, respectively.

2.8. Studies to investigate the involvement of K^+ channels

To examine whether the SO_2-induced vasorelaxation was mediated by the increased potassium conductance, aortic rings were contracted with either 20 or 100 mM KCl and the vasorelaxant effect of SO_2 was then examined.

To determine the involvement of K^+ channels in the effects of SO_2, rings with intact endothelium and denuded endothelium were pre-incubated with TEA (10 mM), 4-AP (2.5 mM), iberiotoxin (100 nM), apamin (50 nM) and glibenclamide (10 μM) for 10 min before contraction with norepinephrine, respectively (Leung et al., 2007). Then SO_2 at 30, 300 or 1500 μM was added, respectively, and the tensions were recorded.

2.9. Statistical analysis

All values were expressed as mean±standard deviation, and the data were analyzed using one-way analysis of variance (ANOVA) for significant differences between two groups. A level of $P<0.05$ was accepted as statistically significant.

3. Results

3.1. The vasodilator effects of SO_2

Fig. 1 shows that SO_2 caused relaxation of isolated rat aortic rings in a dose-dependent manner for both endothelium-intact (EC_{50}, 1247.38±98.32 μM) and endothelium-denuded aortic rings (EC_{50}, 1321.89±89.67 μM). Fig. 1 also shows that SO_2 at basal and low (<450 μM) concentrations caused relaxation of endothelium-intact aortic rings, but not for endothelium-denuded aortic rings. At high concentrations (>500 μM) SO_2 caused relaxation of both endothelium-intact and endothelium-denuded aortic rings. These results indicate that the vasorelaxation caused by SO_2 at basal and low concentrations was endothelium-dependent, but at high concentrations was endothelium-independent.

3.2. Involvement of L-type calcium-channel in the vascular effects of SO_2

From Fig. 2 we can see that the vasorelaxant effects of 1500 μM SO_2 on both endothelium-intact and endothelium-denuded rings were partially inhibited by nifedipine, an L-type calcium-channel blocker. But the vasorelaxant effects of 30 or 300 μM SO_2 on the endothelium-intact rings was not affected by nifedipine.

3.3. Effects of SO_2 or nifedipine on vasoconstriction evoked by $CaCl_2$

For both endothelium-intact and endothelium-denuded rings, pretreatments with 1500 μM SO_2 or nifedipine produced an inhibition of vasoconstrictions induced by $CaCl_2$. SO_2 or nifedipine shifted these concentration–effect curves to the right, and the vasoconstrictions induced by $CaCl_2$ were depressed (Fig. 3).

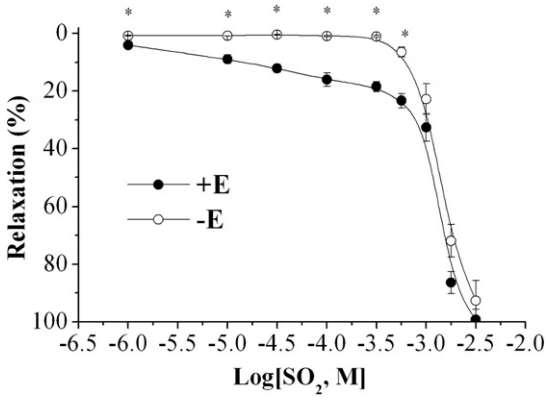

Fig. 1. Vasorelaxant effects of SO_2 on the endothelium-denuded or endothelium-intact rat aortic rings contracted by norepinephrine. The relaxation effect was expressed as a percentage of decrement to the maximum tension caused by norepinephrine. Compared with the SO_2 group of endothelium-intact rat aortic rings, *$P<0.05$.

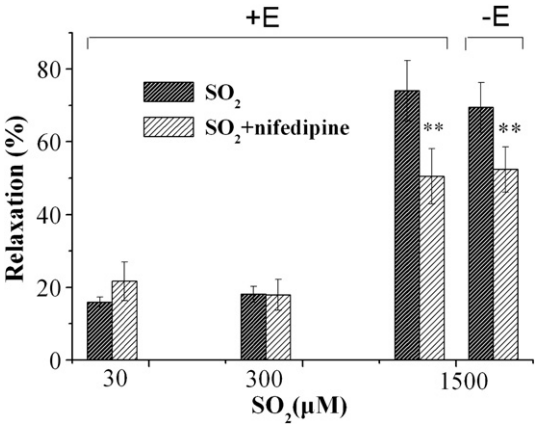

Fig. 2. Inhibitory effect of nifedipine (1 μM) on the SO_2-induced relaxation on the endothelium-denuded or endothelium-intact rat aortic rings. The rings with intact endothelium and denuded endothelium were pre-incubated with nifedipine (1 μM), an L-type calcium-channel blocker, for 10 min before contraction with norepinephrine. "% Relaxation" of "SO_2+nifedipine" was expressed as a percentage of relaxation to maximum constriction induced by 10^{-6} M norepinephrine in the presence of nifedipine. Compared with corresponding control group, **$P<0.01$.

3.4. Effects of SO_2 or nifedipine on vasoconstrictions of two components

In this experiment, briefly, nifedipine or SO_2 was added in advance to the rings bathed in Ca^{2+}-free Krebs solution, respectively, and 10 min

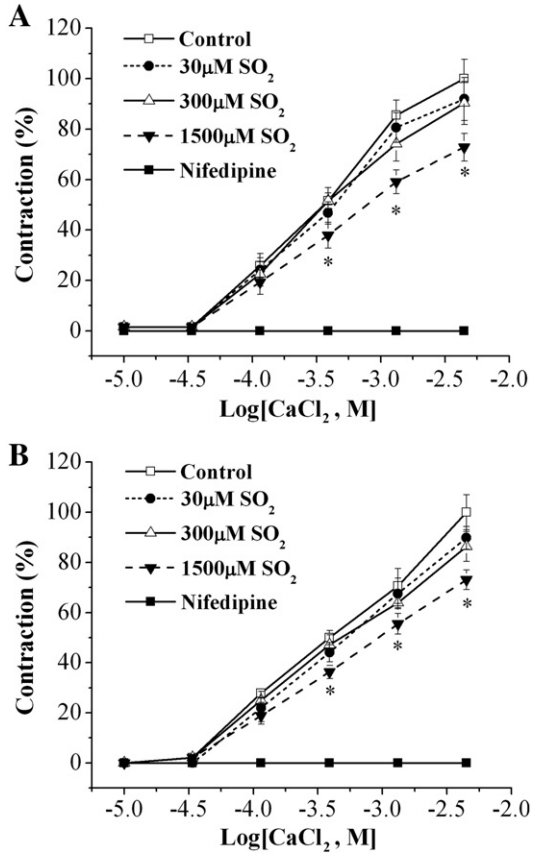

Fig. 3. Effect of SO_2 or nifedipine on $CaCl_2$-induced contractions on the endothelium-intact (A) or endothelium-denuded (B) rat aortic rings incubated in Ca^{2+}-free buffer primed with KCl (100 mM). The rings were pre-incubated with 30, 300, or 1500 μM SO_2 or 1 μM nifedipine for 10 min, respectively, and the subsequent cumulative administration of $CaCl_2$. Constrictions were expressed as a percentage of constriction to maximum constriction induced by $CaCl_2$ in control group, which was taken as 100%. Compared with corresponding control group, *$P<0.05$.

Table 1
Effect of SO_2 or nifedipine on the vasoconstriction of two components in rat thoracic aorta rings

Chemicals	No. of rings	Initial fast vasoconstriction		Sustained vasoconstriction	
		+E	−E	+E	−E
Control	8	39.30±6.56	43.16±4.77	60.70±6.56	56.84±4.77
Nifedipine	8	25.55±3.28[b]	22.14±4.21[b]	12.86±2.68[c]	16.86±3.24[c]
Control	8	34.22±7.06	41.31±3.98	64.59±7.06	58.69±3.98
10 μM SO_2	8	33.16±4.28	38.29±4.28	55.16±8.56	53.37±7.35
Control	8	35.41±5.10	39.55±6.26	64.59±5.10	60.45±6.26
300 μM SO_2	8	36.78±5.48	37.37±6.38	59.87±11.35	57.96±8.37
Control	8	37.05±7.33	41.87±4.15	62.95±7.33	58.13±4.15
1500 μM SO_2	8	51.63±6.89[b, d]	48.23±4.38[b, d]	41.56±8.68[b]	42.37±7.31[b]

Values are the vasoconstriction ratio (%) (mean±SD). In this experiment, briefly, nifedipine (1 μM) or SO_2 was added to the rings bathed in Ca^{2+}-free Krebs solution, respectively; 10 min later norepinephrine (1 μM) was added to observe the initial fast vasoconstriction. When the initial fast vasoconstriction reached peak, immediately $CaCl_2$ (2.5 mM) was added to observe the sustained vasoconstriction. One-way ANOVA test: compared with corresponding control group, significance indicated by [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$; compared with SO_2 the above lower concentration group, [d]$P<0.05$.

later norepinephrine (1 μM) was added to observe the initial fast vasoconstriction. When the initial fast vasoconstriction reached peak, immediately $CaCl_2$ (2.5 mM) was added to observe the sustained vasoconstriction. For both endothelium-intact and endothelium-denuded rings, the initial fast vasoconstriction induced by norepinephrine (intracellular Ca^{2+} release) in Ca^{2+}-free Krebs solution were significantly enhanced by 1500 μM SO_2, but were significantly inhibited by 1 μM nifedipine. The sustained vasoconstriction evoked by extracellular Ca^{2+} influx caused by $CaCl_2$ was inhibited significantly by 1 μM nifedipine or 1500 μM SO_2 (Table 1).

3.5. Effects of SO_2 or nifedipine on vasoconstriction evoked by KCl or norepinephrine

For both endothelium-intact and endothelium-denuded rings, pretreatments with 1500 μM SO_2 produced a significant enhancement of vasoconstrictions induced by a low concentration (20 mM) KCl and a significant inhibition of vasoconstriction of high concentrations KCl. However, pretreatments with nifedipine produced a complete inhibition of vasoconstrictions induced by KCl for both rings with and without endothelium (Fig. 4).

Similarly, for both endothelium-intact and endothelium-denuded rings, pretreatments with 1500 μM SO_2 produced a significant enhancement of vasoconstrictions induced by a low concentration (10^{-7} M) norepinephrine and a significant inhibition of vasoconstriction of high concentrations norepinephrine. However, pretreatments with nifedipine produced a significant inhibition of vasoconstrictions induced by norepinephrine for both rings with and without endothelium, nifedipine shifted these concentration–effect curves to the right (Fig. 5).

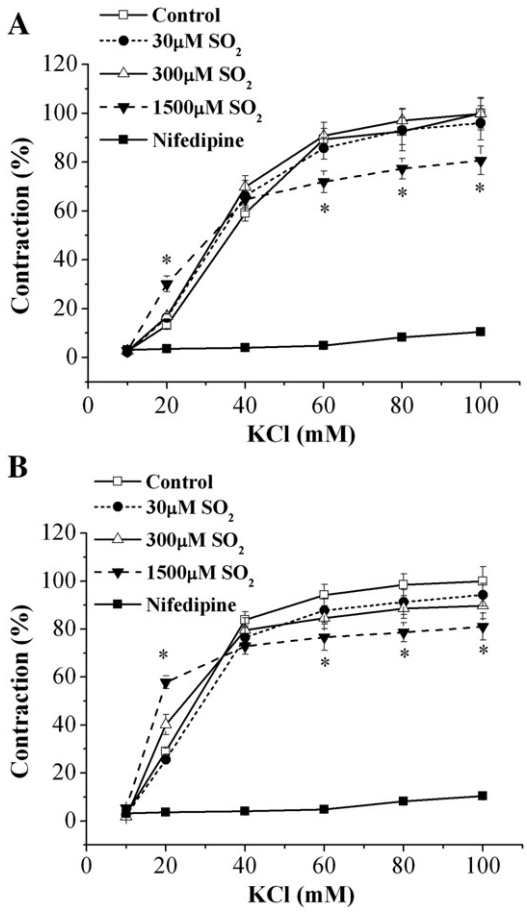

Fig. 4. Effect of SO_2 or nifedipine on vasocontraction induced by KCl on the endothelium-intact (A) or endothelium-denuded (B) rat aortic rings. The rings were pre-incubated with 30, 300, or 1500 μM SO_2 or 1 μM nifedipine for 10 min, respectively, and the subsequent cumulative administration of KCl. Constrictions were expressed as a percentage of constriction to maximum constriction induced by KCl in control group, which was taken as 100%. Compared with corresponding control group, *$P<0.05$.

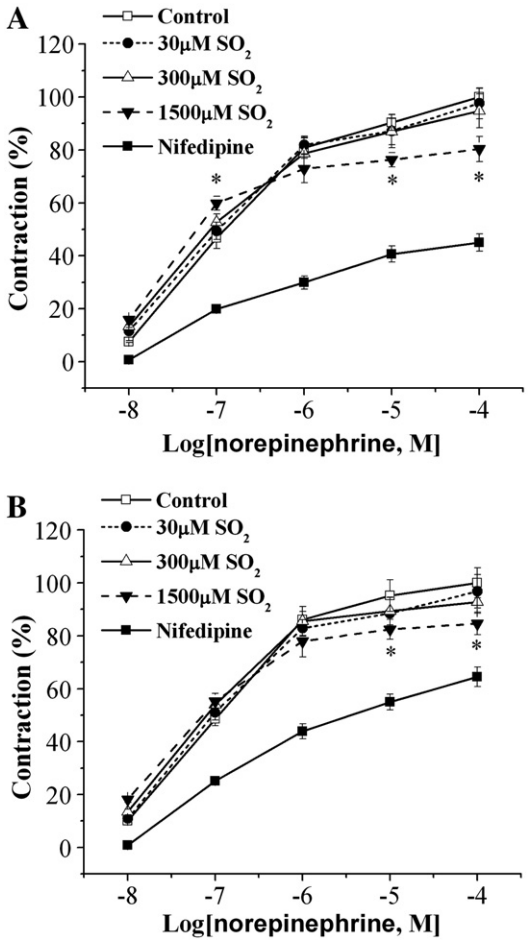

Fig. 5. Effect of SO_2 or nifedipine on vasocontraction induced by norepinephrine on the endothelium-intact (A) or endothelium-denuded (B) rat aortic rings. The rings were pre-incubated with 30, 300, or 1500 μM SO_2 or 1 μM nifedipine for 10 min, respectively, and the subsequent cumulative administration of norepinephrine. Constrictions were expressed as a percentage of constriction to maximum constriction induced by norepinephrine in control group, which was taken as 100%. Compared with corresponding control group, *$P<0.05$.

3.6. Different relaxation potencies of SO_2 on rings precontracted by high or low concentration of KCl

The contraction forces induced by 20 and 100 mM KCl were 0.56±0.06 and 1.94±0.08 g, respectively. Fig. 6 shows that the vasorelaxant effect of SO_2 on the rings contracted by 20 mM KCl was more potent than that on the rings contracted by 100 mM KCl. For example, the vasorelaxant effect induced by 1000 μM SO_2 was 52.65±4.77% and 13.85±1.87% when the rings were contracted with 20 and 100 mM KCl, respectively.

3.7. Involvement of Ca^{2+}-activated K^+ (K_{Ca}) channel in the vascular effects of SO_2

In order to identify the role of specific types of K^+ channels in the SO_2-induced relaxation, aortic rings were incubated with either 10 mM TEA, 50 nM apamin (small-conductance K_{Ca} channel inhibitor) or 100 nM iberiotoxin (big-conductance K_{Ca} channel inhibitor) for 20 min prior to the application of SO_2. In the presence of TEA, the SO_2-induced vasorelaxation was partially inhibited for both endothelium-intact and endothelium-denuded rings (Fig. 7A). At this high concentration, TEA is known to block many different types of K^+ channels (Nelson and Quayle, 1995). The vasorelaxant effects induced by 30 and 300 μM SO_2 were partially inhibited by iberiotoxin for the endothelium-intact rings (Fig. 7B). The SO_2-induced vasorelaxation was not affected by apamin (data not shown), suggesting that small-conductance K_{Ca} channels might not be responsible for the SO_2-induced vasorelaxation.

3.8. Involvement of voltage-dependent K^+ (K_v) channel in the vascular effects of SO_2

A previous study has shown that 4-AP specifically inhibited the K_v channel with an EC_{50} of 1.4 mM (Remillard and Leblanc, 1996). In the present study, 2.5 mM 4-AP was used to treat aortic rings 20 min before the application of SO_2. The relaxant effect of SO_2 was not affected by 4-AP, eliminating the involvement of K_v channels.

3.9. Involvement of ATP-sensitive K^+ (K_{ATP}) channel in the vascular effects of SO_2

To elucidate whether K_{ATP} channel was the target of SO_2, the interaction of SO_2 with known K_{ATP} channel modulator was examined. For both endothelium-intact and endothelium-denuded rings, the vasorelaxant effects induced by 1500 μM SO_2 were partially inhibited by glibenclamide, suggesting that K_{ATP} channel might be involved in the SO_2-induced vasorelaxation at high concentrations (Fig. 7C). The vasorelaxant effect of 30 or 300 μM SO_2 on the endothelium-intact rings was not affected by glibenclamide.

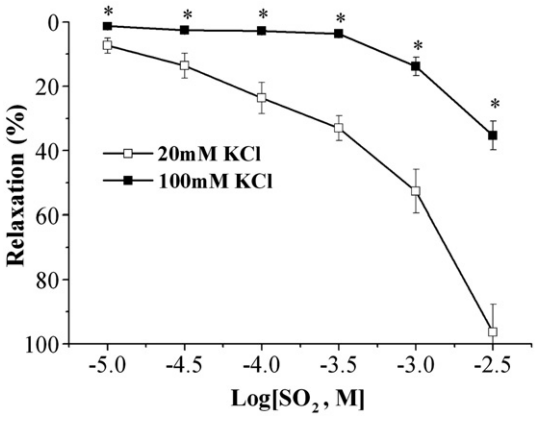

Fig. 6. The relaxant effect of SO_2 on the rat aortic rings contracted with 20 or 100 mM KCl. Aortic rings were contracted with either 20 or 100 mM KCl and the vasorelaxant effect of SO_2 was then examined. "% Relaxation" of SO_2 on the rings contracted by 20 mM KCl or 100 mM KCl was expressed as a percentage of relaxation to maximum constriction induced by 20 mM KCl or 100 mM KCl, respectively. Compared with the 20 mM KCl group, $^*P<0.05$.

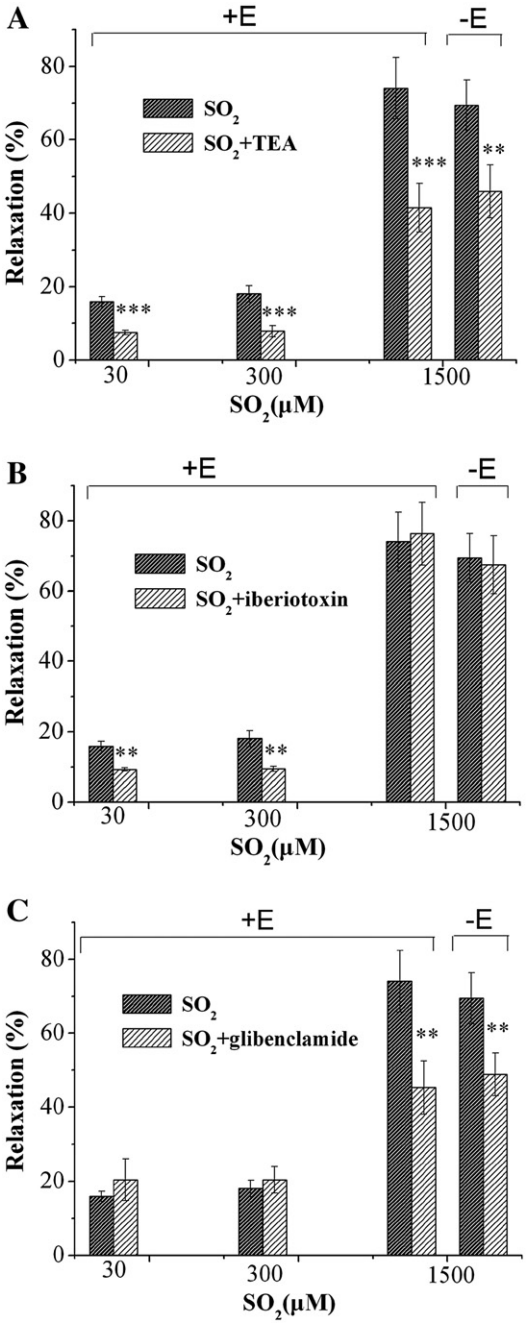

Fig. 7. Inhibitory effect of TEA (A), iberiotoxin (B), glibenclamide (C) on the SO_2-induced relaxation on the endothelium-denuded or endothelium-intact rat aortic rings. The rings with intact endothelium and denuded endothelium were pre-incubated with TEA (10 mM), iberiotoxin (100 nM), and glibenclamide (10 μM) for 10 min before contraction with norepinephrine, respectively. Compared with corresponding control group, $^{**}P<0.01$, $^{***}P<0.001$.

4. Discussion

Since the late 1980s, NO and carbon monoxide (CO) have been demonstrated to be gasotransmitters with a variety of vital functions including vasorelaxation, suppression of cell proliferation, inhibition of platelet aggregation, and so on. Recently, some studies have suggested that hydrogen sulfide (H_2S) might be another possible gasotransmitter

(Wang, 2002). We note that all these three gases have been generally considered as toxic gases found in the contaminated environmental atmosphere. In recent years, our studies also indicated that SO_2 and its derivatives (sulfite and bisulfite, 3:1 M/M) were systemic toxic agents that could cause many kinds of toxic effects (Meng, 2003), including induction of chromosomal aberrations (CA), micronuclei (MN) and sister chromatid exchanges (SCE) (Meng et al., 2002; Meng and Zhang, 2002), DNA damage (Meng et al., 2005b), gene mutagenesis (Meng and Zhang, 1999), lipid peroxidation (Meng, 2003), changes of cytokine levels (Meng et al., 2005a) and genome expressions (Meng et al., 2007a,b). In addition, our investigation has discovered that SO_2 and its derivatives could cause a decrease of rat blood pressure (Meng et al., 2003). However, the mechanism by which SO_2 produces this effect is unknown. We wonder whether SO_2 might be also a vasoactive substance which could cause changes of blood pressure and vascular tension. Therefore, the purpose of the present study was to investigate roles of Ca^{2+} channels and K^+ channels in relaxations of endogenous gaseous SO_2 in isolated rat aortic rings.

In the present study, norepinephrine-contracted rings were directly exposed to gaseous SO_2 or its solution. In SO_2 solution, SO_2 exists in water mostly in the chemical state SO_2–nH_2O, and only a little HSO_3^- is found (Meng, 2008). Therefore, vasorelaxation caused by exposure to SO_2 solution was actually the effect of SO_2 on the vascular rings. Our results showed that the basal levels of SO_2 in plasma and aortic tissues of Wistar rats were 12.59±9.03 μM and 110.34±35.22 μM, respectively (unpublished observation). So 30 μM SO_2 selected in our study was within a basally relevant concentration range. The present study demonstrated that the removal of functional endothelium abolished the relaxation response to SO_2 at basal and low concentrations, indicating that vasorelaxation was endothelium-dependent. However, SO_2 at higher concentrations caused vasorelaxation of both endothelium-intact and endothelium-denuded rings, indicating that the vasorelaxation was endothelium-independent. These results implied that the vasorelaxation mechanism of SO_2, within a basally relevant concentration range, was different from that of SO_2 at higher concentrations.

Generally, calcium influx plays an important physiological role in mediating the contraction of vascular smooth muscle cells. During sarcolemmal membrane depolarization, the L-type calcium channel will open to permit Ca^{2+} influx and trigger intracellular calcium-induced calcium release, leading to cell contraction (Kruse et al., 1994). Therefore, we observed the role of L-type calcium channels in SO_2-induced vasorelaxation. The result showed that the vasorelaxant effects of 1500 μM SO_2 on both endothelium-intact and endothelium-denuded rings were partially inhibited by nifedipine, an L-type calcium-channel blocker, which suggested the possible involvement of L-type Ca^{2+} channel and other mechanisms during the vasorelaxant effects of SO_2 at high concentrations. However, Du et al. (2008) reported that nicardipine, an L-type calcium-channel blocker, completely inhibited the vasorelaxant response of SO_2 derivatives (sodium sulfite: sodium bisulfite, 3:1, M/M) at 6 mM. It is known that SO_2 derivatives and SO_2 are different chemicals and their biological effects are different. For example, Du et al. (2008) and our present study reported the EC_{50} values of vasorelaxation effects induced by SO_2 derivatives and SO_2 were 7.28± 0.12 mM and 1.25±0.10 mM, respectively. In addition, our previous study (Meng et al., 2007a,b) showed that the vasorelaxation of SO_2 derivatives was mediated in part by the signal transduction pathway of prostacyclin (PGI_2)-adenylyl cyclase (AC)-adenosine 3′,5′-cyclic monophosphate (cAMP)-protein kinase (PKA). Therefore, the vasorelaxation of SO_2 derivatives might involve other mechanisms besides L-type calcium channel. Further study is necessary to demonstrate whether or not the vasorelaxation of SO_2 derivatives can be completely inhibited by the blocker of L-type calcium channel.

It is well known that the vasoconstriction of vascular smooth muscle is initiated by an increase of intracellular calcium level. The vasoconstriction may be achieved by two ways: extracellular Ca^{2+} influx via the voltage-dependent calcium channel evoked by depolarization with high potassium concentration (high K^+) or via the receptor-operating calcium channel evoked by norepinephrine and release of intracellular Ca^{2+} (Broekaert and Godfraind, 1979; Saida and van Breemen, 1983). Our results showed that, for both endothelium-intact and endothelium-denuded rings, pretreatments with 1500 μM SO_2 produced a significant enhancement of vasoconstrictions induced by low concentrations norepinephrine or KCl and a significant inhibition of vasoconstriction by high concentrations norepinephrine or KCl. Our results also showed that 1500 μM SO_2 could cause an inhibition of the vasoconstriction induced by $CaCl_2$, just like nifedipine (Fig. 3).

On the other hand, our paper studied the effects of SO_2 on vasoconstrictions of two components: the initial fast vasoconstriction (induced by intracellular Ca^{2+} release caused by norepinephrine) and the sustained vasoconstriction (evoked by extracellular Ca^{2+} influx caused by $CaCl_2$). Our results showed that, for both endothelium-intact and endothelium-denuded rings, the initial fast vasoconstriction induced by intracellular Ca^{2+} release was enhanced by 1500 μM SO_2, but the sustained vasoconstriction evoked by extracellular Ca^{2+} influx was inhibited by 1500 μM SO_2 (Table 1). Pretreatments with 1500 μM SO_2 produced an enhancement of vasoconstrictions induced by low concentrations of norepinephrine or KCl and the initial fast vasoconstriction induced by intracellular Ca^{2+} release, which indicated that SO_2 could enhance the Ca^{2+} release from sarcoplasmic reticulum. Further studies are required to understand the exact mechanism of the enhancement effect of SO_2 on the Ca^{2+} release. It was shown that the enhancement effect was smaller than the inhibition effect of SO_2 for the Ca^{2+} influx and the total effect was inhibition for the increase of intracellular calcium level in this study. These results demonstrated that SO_2 could depress Ca influx through a mechanism (s) that might reflect actions on L-type or receptor-operated pathways, but further experimentation is required.

Opening of K^+ channels caused membrane potential depolarization in vascular smooth muscle cells (SMCs), providing an important mechanism in vasodilation (Nelson and Quayle, 1995; Farouque et al., 2004). Our study showed that the vasorelaxant effect of SO_2 on the rings contracted by 20 mM KCl was more potent than that on the rings contracted by 100 mM KCl. To determine the potential involvement of K^+ channels, aortic rings were pre-incubated with TEA, 4-AP, iberiotoxin, apamin and glibenclamide for 10 min before contraction with norepinephrine, respectively. The results showed that (1) the SO_2-induced vasorelaxation was inhibited by TEA that blocked many K^+ channels in vascular SMCs, including K_{Ca}, K_v and K_{ATP} channels. Blocker of K_v channel failed to affect the vascular effects of SO_2. (2) Iberiotoxin inhibited the 30, 300 μM SO_2-induced vasorelaxation for the endothelium-intact rings, suggesting that BK_{Ca} channels might be responsible for the basal and low concentrations SO_2-induced vasorelaxation. (3) Glibenclamide inhibited the 1500 μM SO_2-induced vasorelaxation for both endothelium-intact and endothelium-denuded rings, showing that K_{ATP} channels might be responsible for the high concentrations SO_2-induced vasorelaxation. This was consistent with a previous study which suggested that SO_2 might be an endothelium-derived hyperpolarizing factor that caused relaxation of vascular smooth muscle presumably by opening of K_{ATP} channels (Balazy et al., 2003). The SO_2-induced vasorelaxation was not affected by apamin (data not shown), suggesting that small-conductance K_{Ca} channels might not be responsible for the SO_2-induced vasorelaxation. These results suggested that opening of K^+ channels (BK_{Ca} and K_{ATP}) might contribute to the vasorelaxant effect of SO_2.

In conclusion, we demonstrated that SO_2 was a gaseous vasorelaxant substance. NO and CO mediate vasorelaxation by increasing the cellular cGMP activity and/or stimulating K_{Ca} channels in vascular SMCs. The H_2S-induced vasorelaxation is mediated mainly by the opening of K_{ATP} channels in vascular SMCs and partially through a K^+ conductance in endothelial cells (Zhao et al., 2001). The mechanism of SO_2-induced vasorelaxation differs from those of NO, CO or H_2S. The mechanism of endogenous gaseous SO_2-induced vasorelaxation was

shown to be endothelium-independent at high concentrations and endothelium-dependent at basal and low concentrations, in part, involved the contribution of BK_{Ca} and K_{ATP} channels as well as possible alterations in Ca-influx and release pathways.

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