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ARTICLE *in* EUROPEAN JOURNAL OF PHARMACOLOGY · DECEMBER 2008

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## Cardiovascular Pharmacology

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

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## ARTICLE INFO

## Article history:

Received 10 July 2008

Received in revised form 14 November 2008

Accepted 17 November 2008

Available online 25 November 2008

## Keywords:

Sulfur dioxide

Thoracic aorta ring

Vasorelaxation

 $K^+$  channel $Ca^{2+}$  channel

(Rat)

## 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 ( $EC_{50}$ ,  $1247.38 \pm 98.32 \mu M$ ). The vasorelaxant effect of  $SO_2$  at basal ( $110.34 \pm 35.22 \mu M$ ) and low concentrations ( $< 450 \mu M$ ) was endothelium-dependent, while it was endothelium-independent at high concentrations ( $> 500 \mu M$ ). (2) The vasorelaxation of  $1500 \mu M$   $SO_2$  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  $1500 \mu M$   $SO_2$  on both endothelium-intact and endothelium-denuded aortic rings. (4) The initial fast vasoconstriction induced by intracellular  $Ca^{2+}$  release was enhanced by  $1500 \mu M$   $SO_2$ , but the sustained vasoconstriction evoked by extracellular  $Ca^{2+}$  influx was inhibited by  $1500 \mu M$   $SO_2$ . (5) Pretreated by  $1500 \mu M$   $SO_2$ , 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  $300 \mu M$   $SO_2$  were partially inhibited by ibuprofen. 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.

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## 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<sup>2+</sup> channels and K<sup>+</sup> channels in relaxations of endogenous gaseous SO<sub>2</sub> 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<sub>2</sub>PO<sub>4</sub> 1.2, MgCl<sub>2</sub> 1.2, NaHCO<sub>3</sub> 15.4, CaCl<sub>2</sub> 2.5, and glucose 11.5. The components of Ca<sup>2+</sup>-free Krebs solution were similar to those of Krebs solution but with EGTA at 0.2 mM instead of CaCl<sub>2</sub>. High K<sup>+</sup>, Ca<sup>2+</sup>-free Krebs solution (Polster et al., 1990) consisted of (mM) NaCl 17, KCl 100, and other components was similar to those of Ca<sup>2+</sup>-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<sub>2</sub> solution preparation, pure SO<sub>2</sub> gas (purity: 99.99%) obtained from Beijing He-Pu-Bei-Fen Gas Company, Ltd. (Beijing, China) was used in this study. SO<sub>2</sub> solution was freshly prepared before each experiment by bubbling saline with pure SO<sub>2</sub> gas to achieve a solution containing SO<sub>2</sub> 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<sub>2</sub> and 5% CO<sub>2</sub> were bubbled through the solution. Tension was recorded with a MedLab Biological Signal Collection System (Medase 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<sup>−6</sup> 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<sub>2</sub> on the thoracic aorta rings

To study the vasodilator effects of SO<sub>2</sub>, isolated rat thoracic aorta rings were contracted by 10<sup>−6</sup> M norepinephrine, when the vasoconstriction curves of rings reached the plateau phase of the maximum tension, SO<sub>2</sub> at 0–2000 μM was added, and the tensions were recorded. At the plateau phase of contraction, the cumulative concentration–response curves of SO<sub>2</sub> 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<sup>−6</sup> M norepinephrine was about 80% of the maximum constriction response induced by 10<sup>−4</sup> M norepinephrine (Fig. 5). In this study, norepinephrine at 10<sup>−6</sup> M was used, therewith vasodilator effect of SO<sub>2</sub> was expressed as a percentage of relaxation to maximum constriction induced by 10<sup>−6</sup> 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<sup>−6</sup> M acetylcholine (Furchgott and Zawadzki, 1980; Fiscus et al., 1991).

### 2.4. Involvement of L-type calcium-channel in the vascular effects of SO<sub>2</sub>

To elucidate whether an L-type calcium-channel was the target of SO<sub>2</sub> 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<sub>2</sub> at 30, 300, or 1500 μM was added, respectively, and the tensions were recorded.

### 2.5. Effects of SO<sub>2</sub> on vasoconstriction induced by CaCl<sub>2</sub>

To determine if Ca<sup>2+</sup> channels are involved in the vasorelaxant effect of SO<sub>2</sub>, aortic rings with intact endothelium and denuded endothelium were equilibrated in Ca<sup>2+</sup>-free Krebs solution and washed three times at 20 min intervals at 37 °C, then equilibrated in high K<sup>+</sup>, Ca<sup>2+</sup>-free Krebs solution for 15 min to depolarize the membrane, and CaCl<sub>2</sub> was added accumulatively from 0.01 mM to 4.44 mM to observe the normal concentration–effect curve. Then, after SO<sub>2</sub> 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<sub>2</sub> at 0.01 to 4.44 mM were derived as already mentioned in the presence of SO<sub>2</sub> or nifedipine at various concentrations.

### 2.6. Effects of SO<sub>2</sub> on vasoconstrictions of two components

This experiment was performed in order to study effects of SO<sub>2</sub> on vasoconstrictions of two components: the initial fast vasoconstriction (induced by intracellular Ca<sup>2+</sup> release caused by norepinephrine) and the sustained vasoconstriction (evoked by extracellular Ca<sup>2+</sup> influx caused by CaCl<sub>2</sub>). In this experiment, briefly, nifedipine (1 μM) or SO<sub>2</sub> (30, 300, or 1500 μM) was added to the rings with intact endothelium and denuded endothelium bathed in Ca<sup>2+</sup>-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<sub>2</sub> (2.5 mM) was added to observe the sustained vasoconstriction (Meng and Zhang, 2007).

### 2.7. Effects of SO<sub>2</sub> 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<sup>−8</sup> to 10<sup>−4</sup> 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<sub>2</sub> 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<sub>2</sub> or nifedipine at various concentrations, respectively.

### 2.8. Studies to investigate the involvement of K<sup>+</sup> channels

To examine whether the SO<sub>2</sub>-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<sub>2</sub> 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  $\mu$ M) for 10 min before contraction with norepinephrine, respectively (Leung et al., 2007). Then  $SO_2$  at 30, 300 or 1500  $\mu$ M was added, respectively, and the tensions were recorded.

### 2.9. Statistical analysis

All values were expressed as mean  $\pm$  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 $SO_2$

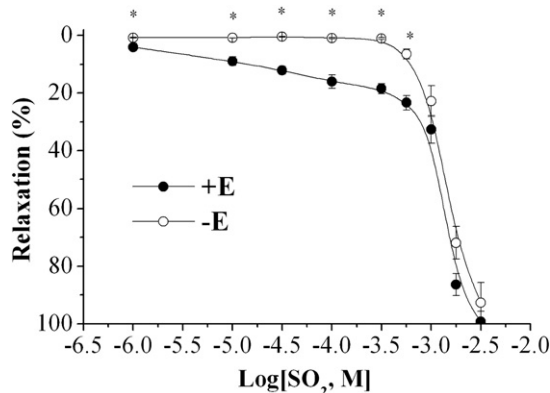
Fig. 1 shows that  $SO_2$  caused relaxation of isolated rat aortic rings in a dose-dependent manner for both endothelium-intact ( $EC_{50}$ ,  $1247.38 \pm 98.32$   $\mu$ M) and endothelium-denuded aortic rings ( $EC_{50}$ ,  $1321.89 \pm 89.67$   $\mu$ M). Fig. 1 also shows that  $SO_2$  at basal and low (<450  $\mu$ M) concentrations caused relaxation of endothelium-intact aortic rings, but not for endothelium-denuded aortic rings. At high concentrations (>500  $\mu$ M)  $SO_2$  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 $SO_2$

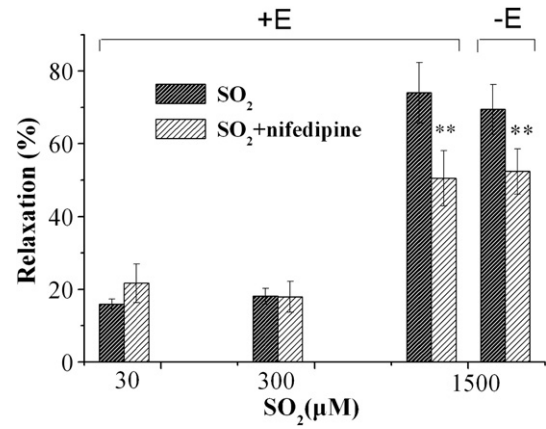
From Fig. 2 we can see that the vasorelaxant effects of 1500  $\mu$ M  $SO_2$  on both endothelium-intact and endothelium-denuded rings were partially inhibited by nifedipine, an L-type calcium-channel blocker. But the vasorelaxant effects of 30 or 300  $\mu$ M  $SO_2$  on the endothelium-intact rings 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  $\mu$ M  $SO_2$  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).



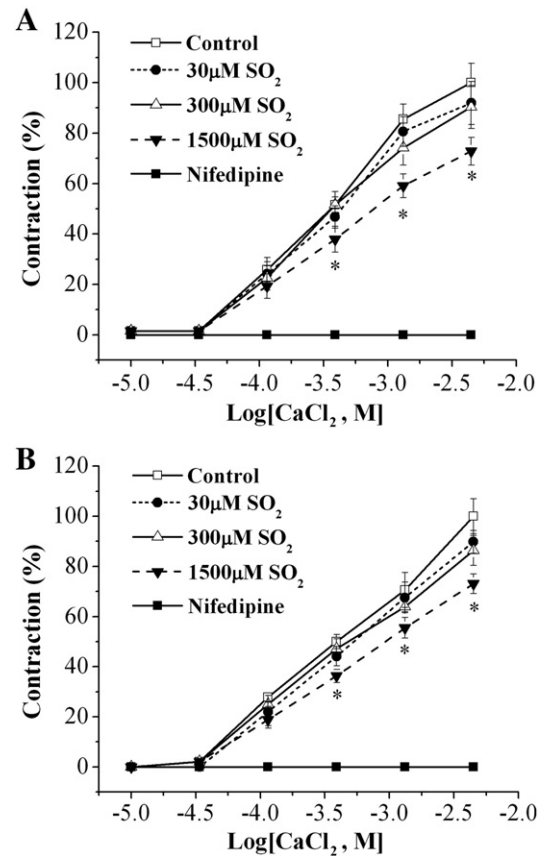
**Fig. 1.** Vasorelaxant effects of  $SO_2$  on the endothelium-denuded or endothelium-intact rat aortic rings contracted by norepinephrine. The relaxation effect was expressed as a percentage of decrement to the maximum tension caused by norepinephrine. Compared with the  $SO_2$  group of endothelium-intact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



**Fig. 2.** Inhibitory effect of nifedipine (1  $\mu$ M) 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  $\mu$ M), 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  $\mu$ M  $SO_2$  or 1  $\mu$ M nifedipine for 10 min, respectively, and the subsequent cumulative administration of  $CaCl_2$ . Contractions 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<sub>2</sub> 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 <sup>b</sup>	22.14±4.21 <sup>b</sup>	12.86±2.68 <sup>c</sup>	16.86±3.24 <sup>c</sup>
Control	8	34.22±7.06	41.31±3.98	64.59±7.06	58.69±3.98
10 μM SO <sub>2</sub>	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 <sub>2</sub>	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 <sub>2</sub>	8	51.63±6.89 <sup>b, d</sup>	48.23±4.38 <sup>b, d</sup>	41.56±8.68 <sup>b</sup>	42.37±7.31 <sup>b</sup>

Values are the vasoconstriction ratio (%) (mean±SD). In this experiment, briefly, nifedipine (1 μM) or SO<sub>2</sub> was added to the rings bathed in Ca<sup>2+</sup>-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<sub>2</sub> (2.5 mM) was added to observe the sustained vasoconstriction. One-way ANOVA test: compared with corresponding control group, significance indicated by <sup>a</sup>*P*<0.05, <sup>b</sup>*P*<0.01, <sup>c</sup>*P*<0.001; compared with SO<sub>2</sub> the above lower concentration group, <sup>d</sup>*P*<0.05.

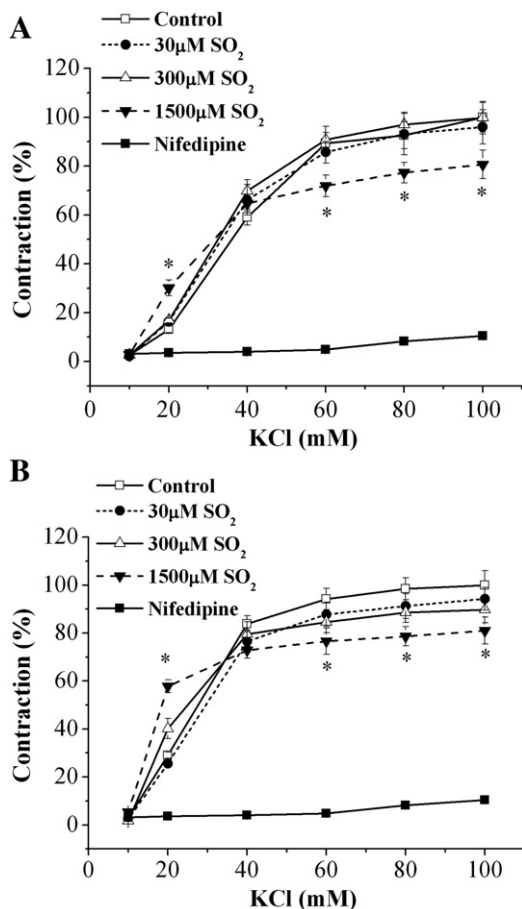
later norepinephrine (1 μM) was added to observe the initial fast vasoconstriction. When the initial fast vasoconstriction reached peak, immediately CaCl<sub>2</sub> (2.5 mM) was added to observe the sustained vasoconstriction. For both endothelium-intact and endothelium-denuded rings, the initial fast vasoconstriction induced by norepinephrine (intracellular Ca<sup>2+</sup> release) in Ca<sup>2+</sup>-free Krebs solution were

significantly enhanced by 1500 μM SO<sub>2</sub>, but were significantly inhibited by 1 μM nifedipine. The sustained vasoconstriction evoked by extracellular Ca<sup>2+</sup> influx caused by CaCl<sub>2</sub> was inhibited significantly by 1 μM nifedipine or 1500 μM SO<sub>2</sub> (Table 1).

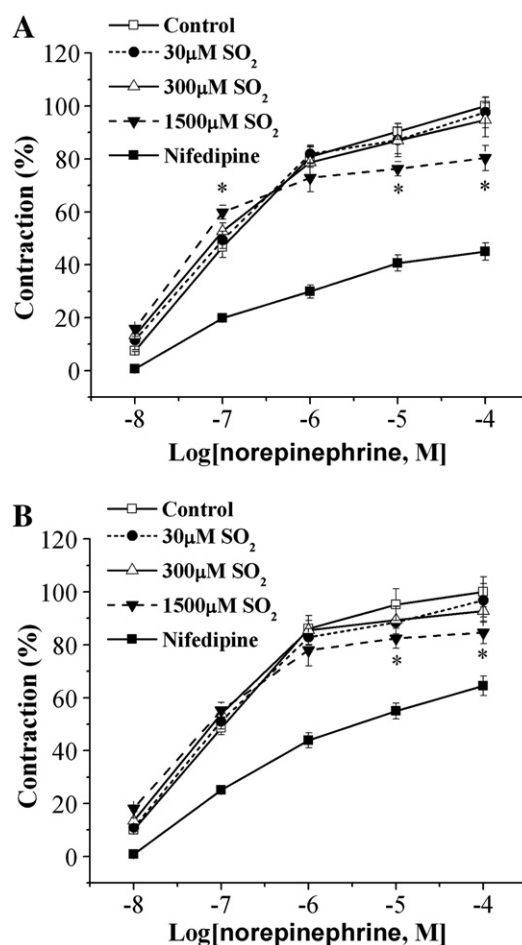
### 3.5. Effects of SO<sub>2</sub> or nifedipine on vasoconstriction evoked by KCl or norepinephrine

For both endothelium-intact and endothelium-denuded rings, pretreatments with 1500 μM SO<sub>2</sub> 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<sub>2</sub> produced a significant enhancement of vasoconstrictions induced by a low concentration (10<sup>-7</sup> 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<sub>2</sub> or nifedipine on vasoconstriction 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<sub>2</sub> 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<sub>2</sub> or nifedipine on vasoconstriction 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<sub>2</sub> 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 $\text{SO}_2$ on rings precontracted by high or low concentration of KCl

The contraction forces induced by 20 and 100 mM KCl were  $0.56 \pm 0.06$  and  $1.94 \pm 0.08$  g, respectively. Fig. 6 shows that the vasorelaxant effect of  $\text{SO}_2$  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  $\mu\text{M}$   $\text{SO}_2$  was  $52.65 \pm 4.77\%$  and  $13.85 \pm 1.87\%$  when the rings were contracted with 20 and 100 mM KCl, respectively.

### 3.7. Involvement of $\text{Ca}^{2+}$ -activated $\text{K}^+$ ( $\text{K}_{\text{Ca}}$ ) channel in the vascular effects of $\text{SO}_2$

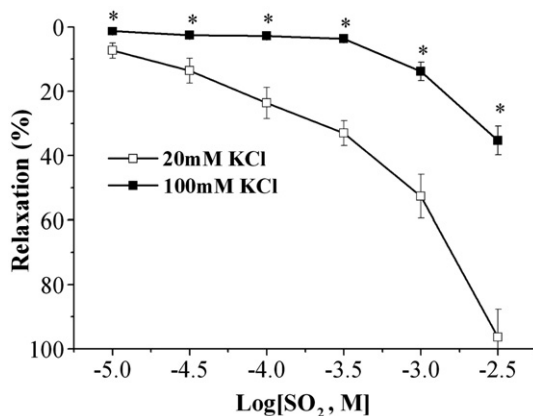
In order to identify the role of specific types of  $\text{K}^+$  channels in the  $\text{SO}_2$ -induced relaxation, aortic rings were incubated with either 10 mM TEA, 50 nM apamin (small-conductance  $\text{K}_{\text{Ca}}$  channel inhibitor) or 100 nM iberiotoxin (big-conductance  $\text{K}_{\text{Ca}}$  channel inhibitor) for 20 min prior to the application of  $\text{SO}_2$ . In the presence of TEA, the  $\text{SO}_2$ -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  $\text{K}^+$  channels (Nelson and Quayle, 1995). The vasorelaxant effects induced by 30 and 300  $\mu\text{M}$   $\text{SO}_2$  were partially inhibited by iberiotoxin for the endothelium-intact rings (Fig. 7B). The  $\text{SO}_2$ -induced vasorelaxation was not affected by apamin (data not shown), suggesting that small-conductance  $\text{K}_{\text{Ca}}$  channels might not be responsible for the  $\text{SO}_2$ -induced vasorelaxation.

### 3.8. Involvement of voltage-dependent $\text{K}^+$ ( $\text{K}_{\text{v}}$ ) channel in the vascular effects of $\text{SO}_2$

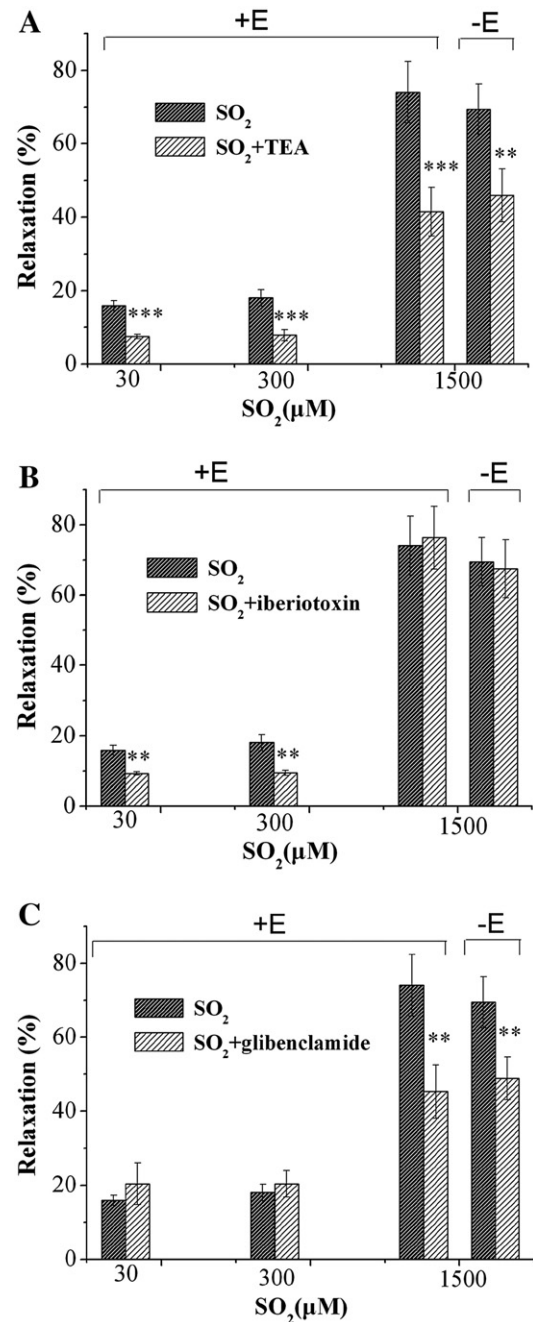
A previous study has shown that 4-AP specifically inhibited the  $\text{K}_{\text{v}}$  channel with an  $\text{EC}_{50}$  of 1.4 mM (Remillard and Leblanc, 1996). In the present study, 2.5 mM 4-AP was used to treat aortic rings 20 min before the application of  $\text{SO}_2$ . The relaxant effect of  $\text{SO}_2$  was not affected by 4-AP, eliminating the involvement of  $\text{K}_{\text{v}}$  channels.

### 3.9. Involvement of ATP-sensitive $\text{K}^+$ ( $\text{K}_{\text{ATP}}$ ) channel in the vascular effects of $\text{SO}_2$

To elucidate whether  $\text{K}_{\text{ATP}}$  channel was the target of  $\text{SO}_2$ , the interaction of  $\text{SO}_2$  with known  $\text{K}_{\text{ATP}}$  channel modulator was examined. For both endothelium-intact and endothelium-denuded rings, the vasorelaxant effects induced by 1500  $\mu\text{M}$   $\text{SO}_2$  were partially inhibited



**Fig. 6.** The relaxant effect of  $\text{SO}_2$  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  $\text{SO}_2$  was then examined. “% Relaxation” of  $\text{SO}_2$  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  $\text{SO}_2$ -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  $\mu\text{M}$ ) for 10 min before contraction with norepinephrine, respectively. Compared with corresponding control group,  $**P < 0.01$ ,  $***P < 0.001$ .

by glibenclamide, suggesting that  $\text{K}_{\text{ATP}}$  channel might be involved in the  $\text{SO}_2$ -induced vasorelaxation at high concentrations (Fig. 7C). The vasorelaxant effect of 30 or 300  $\mu\text{M}$   $\text{SO}_2$  on the endothelium-intact rings was not affected by glibenclamide.

## 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 ( $\text{H}_2\text{S}$ ) 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<sub>2</sub> 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<sub>2</sub> and its derivatives could cause a decrease of rat blood pressure (Meng et al., 2003). However, the mechanism by which SO<sub>2</sub> produces this effect is unknown. We wonder whether SO<sub>2</sub> 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<sup>2+</sup> channels and K<sup>+</sup> channels in relaxations of endogenous gaseous SO<sub>2</sub> in isolated rat aortic rings.

In the present study, norepinephrine-contracted rings were directly exposed to gaseous SO<sub>2</sub> or its solution. In SO<sub>2</sub> solution, SO<sub>2</sub> exists in water mostly in the chemical state SO<sub>2</sub>·nH<sub>2</sub>O, and only a little HSO<sub>3</sub><sup>-</sup> is found (Meng, 2008). Therefore, vasorelaxation caused by exposure to SO<sub>2</sub> solution was actually the effect of SO<sub>2</sub> on the vascular rings. Our results showed that the basal levels of SO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> at basal and low concentrations, indicating that vasorelaxation was endothelium-dependent. However, SO<sub>2</sub> 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<sub>2</sub>, within a basally relevant concentration range, was different from that of SO<sub>2</sub> 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<sup>2+</sup> 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<sub>2</sub>-induced vasorelaxation. The result showed that the vasorelaxant effects of 1500 μM SO<sub>2</sub> 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<sup>2+</sup> channel and other mechanisms during the vasorelaxant effects of SO<sub>2</sub> at high concentrations. However, Du et al. (2008) reported that nifedipine, an L-type calcium-channel blocker, completely inhibited the vasorelaxant response of SO<sub>2</sub> derivatives (sodium sulfite: sodium bisulfite, 3:1, M/M) at 6 mM. It is known that SO<sub>2</sub> derivatives and SO<sub>2</sub> are different chemicals and their biological effects are different. For example, Du et al. (2008) and our present study reported the EC<sub>50</sub> values of vasorelaxation effects induced by SO<sub>2</sub> derivatives and SO<sub>2</sub> 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<sub>2</sub> derivatives was mediated in part by the signal transduction pathway of prostacyclin (PGI<sub>2</sub>)-adenylyl cyclase (AC)-adenosine 3',5'-cyclic monophosphate (cAMP)-protein kinase (PKA). Therefore, the vasorelaxation of SO<sub>2</sub> derivatives might involve other mechanisms besides L-type calcium channel. Further study is necessary to demonstrate whether or not the vasorelaxation of SO<sub>2</sub> 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<sup>2+</sup> influx via the voltage-dependent calcium channel evoked by depolarization with high

potassium concentration (high K<sup>+</sup>) or via the receptor-operating calcium channel evoked by norepinephrine and release of intracellular Ca<sup>2+</sup> (Broekaert and Godfraind, 1979; Saïda and van Breemen, 1983). Our results showed that, for both endothelium-intact and endothelium-denuded rings, pretreatments with 1500 μM SO<sub>2</sub> 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<sub>2</sub> could cause an inhibition of the vasoconstriction induced by CaCl<sub>2</sub>, just like nifedipine (Fig. 3).

On the other hand, our paper studied the effects of SO<sub>2</sub> on vasoconstrictions of two components: the initial fast vasoconstriction (induced by intracellular Ca<sup>2+</sup> release caused by norepinephrine) and the sustained vasoconstriction (evoked by extracellular Ca<sup>2+</sup> influx caused by CaCl<sub>2</sub>). Our results showed that, for both endothelium-intact and endothelium-denuded rings, the initial fast vasoconstriction induced by intracellular Ca<sup>2+</sup> release was enhanced by 1500 μM SO<sub>2</sub>, but the sustained vasoconstriction evoked by extracellular Ca<sup>2+</sup> influx was inhibited by 1500 μM SO<sub>2</sub> (Table 1). Pretreatments with 1500 μM SO<sub>2</sub> produced an enhancement of vasoconstrictions induced by low concentrations of norepinephrine or KCl and the initial fast vasoconstriction induced by intracellular Ca<sup>2+</sup> release, which indicated that SO<sub>2</sub> could enhance the Ca<sup>2+</sup> release from sarcoplasmic reticulum. Further studies are required to understand the exact mechanism of the enhancement effect of SO<sub>2</sub> on the Ca<sup>2+</sup> release. It was shown that the enhancement effect was smaller than the inhibition effect of SO<sub>2</sub> for the Ca<sup>2+</sup> influx and the total effect was inhibition for the increase of intracellular calcium level in this study. These results demonstrated that SO<sub>2</sub> 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<sup>+</sup> 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<sub>2</sub> 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<sup>+</sup> channels, aortic rings were pre-incubated with TEA, 4-AP, ibertoxin, apamin and glibenclamide for 10 min before contraction with norepinephrine, respectively. The results showed that (1) the SO<sub>2</sub>-induced vasorelaxation was inhibited by TEA that blocked many K<sup>+</sup> channels in vascular SMCs, including K<sub>Ca</sub>, K<sub>V</sub> and K<sub>ATP</sub> channels. Blocker of K<sub>V</sub> channel failed to affect the vascular effects of SO<sub>2</sub>. (2) Iberitoxin inhibited the 30, 300 μM SO<sub>2</sub>-induced vasorelaxation for the endothelium-intact rings, suggesting that BK<sub>Ca</sub> channels might be responsible for the basal and low concentrations SO<sub>2</sub>-induced vasorelaxation. (3) Glibenclamide inhibited the 1500 μM SO<sub>2</sub>-induced vasorelaxation for both endothelium-intact and endothelium-denuded rings, showing that K<sub>ATP</sub> channels might be responsible for the high concentrations SO<sub>2</sub>-induced vasorelaxation. This was consistent with a previous study which suggested that SO<sub>2</sub> might be an endothelium-derived hyperpolarizing factor that caused relaxation of vascular smooth muscle presumably by opening of K<sub>ATP</sub> channels (Balazy et al., 2003). The SO<sub>2</sub>-induced vasorelaxation was not affected by apamin (data not shown), suggesting that small-conductance K<sub>Ca</sub> channels might not be responsible for the SO<sub>2</sub>-induced vasorelaxation. These results suggested that opening of K<sup>+</sup> channels (BK<sub>Ca</sub> and K<sub>ATP</sub>) might contribute to the vasorelaxant effect of SO<sub>2</sub>.

In conclusion, we demonstrated that SO<sub>2</sub> was a gaseous vasorelaxant substance. NO and CO mediate vasorelaxation by increasing the cellular cGMP activity and/or stimulating K<sub>Ca</sub> channels in vascular SMCs. The H<sub>2</sub>S-induced vasorelaxation is mediated mainly by the opening of K<sub>ATP</sub> channels in vascular SMCs and partially through a K<sup>+</sup> conductance in endothelial cells (Zhao et al., 2001). The mechanism of SO<sub>2</sub>-induced vasorelaxation differs from those of NO, CO or H<sub>2</sub>S. The mechanism of endogenous gaseous SO<sub>2</sub>-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<sub>Ca</sub> and K<sub>ATP</sub> channels as well as possible alterations in Ca-influx and release pathways.

## Acknowledgements

This research was supported by grants 20477023 and 20677035 from the National Natural Science Foundation of China.

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