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Endogenously generated sulfur dioxide and its vasorelaxant effect in rats[1]

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Key words

calcium channel; L-type; aspartate aminotransferase; sulfur dioxide; vasorelaxant effect

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Abstract

Aim: The present study was designed to explore the endogenous production and localization of the sulfur dioxide (SO_2)/aspartate aminotransferase pathway in vascular tissues of rats and to examine its vasorelaxant effect on isolated aortic rings, as well as the possible mechanisms. **Methods:** The content of SO_2 in the samples was determined by using high performance liquid chromatography with fluorescence detection. Aspartate aminotransferase activity and its gene expression were measured by an enzymatic method and quantitative RT–PCR, respectively. Aspartate aminotransferase mRNA location in aorta was detected by *in situ* hybridization. The vasorelaxant effect of SO_2 on isolated aortic rings of the rats was investigated *in vitro*. L-type calcium channel blocker, nicardipine, and L-type calcium channel agonist, Bay K8644, were used to explore the mechanisms by which SO_2 relaxed the aortic rings. **Results:** Aorta had the highest SO_2 content among the vascular tissues tested ($P<0.01$). The aortic aspartate aminotransferase mRNA located in endothelia and vascular smooth muscle cells beneath the endothelial layer. Furthermore, a physiological dose of the SO_2 derivatives (Na_2SO_3/$NaHSO_3$) relaxed isolated artery rings slightly, whereas higher doses (1–12 mmol/L) relaxed rings in a concentration-dependent manner. Pretreatment with nicardipine eliminated the vasorelaxant response of the norepinephrine-contracted rings to SO_2 completely. Incubation with nicardipine or SO_2 derivatives successfully prevented vasoconstriction induced by Bay K8644. **Conclusion:** Endogenous SO_2 and its derivatives have a vasorelaxant function, the mechanisms of which might involve the inhibition of the L-type calcium channel.

Introduction

Sulfur dioxide (SO_2) is a common air pollutant that resulted from increased industrial activity over the past several decades. Inhaled SO_2 can easily be hydrated to produce sulfurous acid in the respiratory tract, which subsequently dissociates to form its derivatives, sulfite and bisulfite (3:1 mole ratios in neutral fluids). The toxic effects of SO_2 have been extensively studied[1–5]. However, SO_2 was recently found to exert various biological effects on both animals and plants. For example, SO_2 inhalation was found to cause changes in oxidative stress and antioxidative status in various organs of mice[6], and the serum sulfite level increased under physiopathological conditions[7]. Endogenous SO_2 was reported to be generated in the liver during the normal processing of sulfur-containing amino acids such as *L*-cysteine[8]. *L*-cysteine is first oxidized to *L*-cysteine sulfinate, and the latter can develop through transamination by aspartate aminotransferase into β-sulfinylpyruvate, which decomposes spontaneously to pyruvate and SO_2. SO_2 is further hydrated to sulfite *in vivo*, oxidized by sulfite oxidase to sulfate and excreted in urine[9].

Up until now, the distribution of the endogenous SO_2/aspartate aminotransferase pathway and its physiological and pathophysiological roles of endogenous SO_2 in the regulation of cardiovascular system are unclear. In this study, therefore, we explored the characterization of endogenous

SO_2 production and the expression of aspartate aminotransferase mRNA in rat vascular tissues, and observed the influence of SO_2 on vascular function.

Materials and methods

Reagents Sodium sulfite and sodium bisulfite (Na_2SO_3/$NaHSO_3$, the SO_2 derivatives), *L*-aspartate-β-hydroxamate (HDX, the inhibitor of aspartate aminotransferase), glibenclamide, nicardipine, Bay K8644, and monobromobimane (mBrB) were purchased from Sigma (St Louis, MO, USA). Trizol, M-MuLV reverse transcriptase, dNTP, *Taq* DNA polymerase, and oligo $(dT)_{15}$ were bought from Promega (Madison, WI, USA). Other chemicals and reagents were of analytical grade.

Animal treatment Animal care and experimental protocols complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (2001) and the Animal Care Committee of Peking University First Hospital. Sixty male Wistar rats (body weight 250±5 g) were obtained from the Experimental Animal Center, Peking University Health Science Center. The rats were housed under special pathogen-free conditions, and kept at a temperature of 22 °C with 40% humidity and a 12-h light/12-h dark cycle.

Preparation of vascular tissue samples in rats Ten male Wistar rats were anaesthetized with 10% chloral hydrate (4 mL/kg, intraperitoneally). Vascular tissues, including aorta, pulmonary, mesenteric, renal, and tail arteries were rapidly isolated. Samples were snap frozen and stored in liquid nitrogen. The vascular tissues were homogenized in 0.1 mol/L phosphate-buffered saline (PBS; pH 7.4, 10 mL/g tissue) using a glass homogenizer, and then the homogenates were centrifuged at 12 000×*g* for 30 min at 4 °C. The supernatants obtained were stored at –70 °C for determination of SO_2 content, aspartate aminotransferase activity, and protein assay. The protein level was measured according to the Bradford assay. For *in situ* hybridization, the rats were injected with 4% polyoxymethylene into their left ventricles, and the tissues were then fixed in polyoxymethylene.

Determination of SO_2 content Sulfite is the hydrate form of SO_2 in mammalian plasma and sera[10]. Therefore, we detected the sulfite concentration in plasma and vascular tissues to represent SO_2 content indirectly. Sulfite determination was analyzed using high performance liquid chromatography with fluorescence detection (HPLC-FD)[8]. Briefly, 100 μL sample was mixed with 70 μL of 0.212 mol/L sodium borohydride in 0.05 mol/L Tris-HCl (pH 8.5) and incubated at room temperature for 30 min. The sample was then mixed with 10 μL of 70 mmol/L mBrB in acetonitrile, incubated for 10 min at 42 °C, and then mixed with 40 μL of 1.5 mol/L perchloric acid. Protein precipitate in the mixture was removed by centrifugation at 12 400×*g* for 10 min at 23 °C. The supernatant was immediately neutralized by adding 10 μL of 2 mol/L Tris-HCl (pH 3.0), and centrifuged at 12 400×*g* for 10 min. The neutralized supernatant was used for high performance liquid chromatography. The column (4.6×150 mm C18 reverse-phase column, Agilent series 1100; Agilent Technologies, Waldbronn, Karlsruhe, Germany) was first equilibrated with a buffer (methanol: acetic acid: water= 5.00:0.25:94.75 by volume, pH 3.4). The sample loaded onto the column was resolved by a gradient of methanol for 0–5 min, 3%; 5–13 min, 3%–35%; 13–30 min, 35%–62%; 30–31 min, 62%–100%; 31–39 min, 100%; 39–40 min, 100%–3%; and 40–45 min, 3% at a flow rate of 1.0 mL/min. Sulfitebimane was measured by excitation at 392 nm and emission at 479 nm. Quantification was carried out by the standardization of sodium sulfite, and the sulfite content in the tissues was expressed as μmol/mg protein.

Measurement of aspartate aminotransferase activity Aspartate aminotransferase activity in plasma and tissue homogenates were determined by a Hitachi 7600 automatic biochemistry analyzer (Tokyo, Japan). Tissue aspartate aminotransferase activity was expressed as IU/g protein.

Determination of aspartate aminotransferase 1 and aspartate aminotransferase 2 mRNA in tissues by quantitative real-time RT–PCR Total RNA in tissues was extracted by Trizol reagent and reverse transcribed by oligo $d(T)_{18}$ primer and M-MuLV reverse transcriptase. Primers and probes used are listed in Table 1. Quantitative real-time PCR was performed on an ABI PRISM 7300 instrument (ABI USA Sales Corp, Los Angeles, CA, USA). The PCR mixture contained 5 μL of 10×PCR buffer, 5 μL cDNA template or standard DNA, 4 μL of 2.5 mmol/L each dNTP, 5 U of *Taq* DNA polymerase, 1 μL 6-carboxy-X-rhodamine (ROX; category No12223-012; Invitrogen, Carlsbad, CA ,USA), 15 pmol of each forward and reverse primers, and 10 pmol *Taq*Man probe in a total volume of 50 μL. Samples and standard DNA were determined in duplicate. The PCR condition was predenaturing at 95 °C for 5 min, then 95 °C for 15 s, and 60 °C for 1 min for 40 cycles. The amount of β-actin cDNA in the sample was used to calibrate the sample amount used for the determination[11].

***In situ* hybridization of aspartate aminotransferase 1 and aspartate aminotransferase 2 mRNA levels in tissues** Digoxin-labeled oligonucleotides designed according to aspartate aminotransferase 1 and aspartate aminotransferase 2 mRNA were used as probes for the *in situ* hybridization (aspartate aminotransferase 1: 5'-CCT GTG GAA CCT GGG

Table 1. Primers and TaqMan probes used in quantitative real time RT-PCR for the measurement of aspartate aminotransferase 1, aspartate aminotransferase 2 and β-actin cDNAs in rat tissues.

cDNA	Oligonucleotide	Sequence	Product size (bp)
Aspartate aminotransferase 1	Forward primer	5'-CCAGGGAGCTCGGATCGT-3'	79
	Reverse primer	5'-GCCATTGTCTTCACGTTTCCTT-3'	
	TaqMan probe	5'-CCACCACCCTCTCCAACCCTGA-3'	
Aspartate aminotransferase 2	Forward primer	5'-GAGGGTCGGAGCCAGCTT-3'	82
	Reverse primer	5'-GTTTCCCCAGGATGGTTTGG-3'	
	TaqMan probe	5'-TTTAAGTTCAGCCGAGATGTCTTTC-3'	
β-actin	Forward primer	5'- ACCCGCGAGTACAACCTTCTT-3'	80
	Reverse primer	5'- TATCGTCATCCATGGCGAACT-3'	
	TaqMan probe	5'- CCTCCGTCGCCGGTCCACAC-3'	

TaqMan probe labeled with FAM at the 5' end and TAMRA at the 3' end

CAA AGA ATG ATG GA-3' and 5'-ACA GGC GTG GAG GAC AAA GAT GGA GAA CT-3'; and aspartate aminotransferase 2: 5'-ACC GCC ATT TCC TTC CAC TGC TCT G-3' and 5'-CTT GGC ATA GGA TTG GCA GAG GCA GAC AT-3'). The protocol was the same as that described previously[7]. Paraffin sections were treated with xylene and a series of descending ethanol and rinsed in PBS for 5 min. After being digested with proteinase K (0.05 g/L) for 12 min at 37 °C, the sections were prehybridized at 42 °C for 4 h with 20 μL hybridization mixture (70% formamide10 μL, 20×standard sodium citrate buffer 4 μL, 50% dextrin sulfate 2 μL, 50× Denhardt's solution 2 μL, 10 g/L of single strand DNA 1 μL, and 1 mol/L dithiothreitol 1 μL). Then 5 μL labeled cDNA probes were applied into the hybridization mixture, and hybridized at 42 °C for 120 min. The sections were then immersed in 1:100 horse serum for 20 min to block the nonspecific binding sites, incubated with rabbit antidigoxigenin antibody at 37 °C for 60 min, and biotin-conjugated goat antirabbit immunoglobulin G antibody at 37 °C for 20 min. After being rinsed 3 times, the sections were incubated with horseradish peroxidase-conjugated avidin at 37 °C for 30 min and developed using diaminobenzidine. The developed sections were counterstained with hematoxylin. Dark brown dots in the section represented positive signals of aspartate aminotransferase 1 or aspartate aminotransferase 2 mRNA in the tissue. At least 10 aortic arteries were assessed in each animal. Omission of cDNA probe on previously confirmed positive tissue sections was used as the negative control process in this study. And the sections of hepatic tissue were used as the positive control process in this study[12].

Measurement of rat aortic contractility The method used was as previously described with modification[13,14]. Briefly, male Wistar rats (n=30) were anesthetized, and the thoracic aorta was excised immediately and immersed in Krebs' bicarbonate buffer. The adherent adipose and connective tissues of the aorta were removed, and the aorta was cut into rings of length 2–3 mm and mounted in 20 mL organ baths containing prewarmed Krebs' bicarbonate buffer gassed with 95% O_2/5% CO_2 at 37 °C. Changes in tension were recorded using force transducers connected to a PowerLab (BL-Newcentrary, TaiMeng, Chengdu, China). The rings were first stretched passively to a tension of 9.8×10^{-3} Newt and allowed to equilibrate for 1 h before starting of the experiment. The endothelia of the rings were kept functionally undamaged and confirmed by the relaxation reactivity to acetylcholine (1 μmol/L). The aortic rings were pretreated with a submaximal dose of norepinephrine (NE, 1 μmol/L) to initiate contraction and then incubated with the SO_2 derivatives (Na_2SO_3/$NaHSO_3$ 3:1 mole ratio, final content 25 μmol/L–12 mmol/L). Following incubation with the aspartate aminotransferase inhibitor HDX (0.1 μmol/L) to inhibit endogenous SO_2, the vasorelaxant effect was observed.

To explore the effect of SO_2 on the vasoconstrictive response to NE, the concentration-dependent (10 nmol/L–1 mmol/L) vasoconstrictive effects of NE on the aortic ring were studied in the absence or in the presence of SO_2 derivatives (6 mmol/L) or aspartate aminotransferase inhibitor HDX (0.1 μmol/L) in the isolated bathed ring with a constant flow of Krebs' buffer.

Additionally, to elucidate whether a K_{ATP} or an L-type calcium channel was the target of SO_2 vascular action, the ring was pre-incubated with glibenclamide (1 μmol/L), a known K_{ATP} channel blocker, or nicardipine (1 μmol/L), a L-type calcium channel blocker, for 10 min before precontraction with NE and SO_2 derivatives.

To further study the role of the L-type calcium channel

in SO_2-induced vasorelaxation, the aortic rings were incubated with nicardipine (1 μmol/L) or SO_2 derivatives (25 μmol/L-12 mmol/L) for 10 min, then treated with Bay K8644 (final content 1, 5, and 10 μmol/L), an L-type calcium channel agonist, and the contraction of rings was observed.

Finally, endothelium was preremoved from the isolated aortic rings by rubbing with a glass stick. The absence of a functional endothelium was verified by the failure of acetylcholine (1 μmol/L) to induce relaxation of the NE-precontracted vascular tissues. The effects of the SO_2 derivatives or HDX on the aortic rings were compared between intact and nude vascular rings, respectively.

Statistics Results are expressed as mean±SD. Data are presented as either the percentage of tension reduction or the absolute value of induced tension. Statistical analysis was performed using SPSS 11.5 (SPSS, Chicago, IL, USA). Student's *t*-test for unpaired samples was used to compare results between 2 groups. For multiple group comparisons, ANOVA followed by a *post-hoc* analysis (Newman–Keuls test) was used. Statistical significance was set at $P<0.05$.

Results

Characterization of the endogenous SO_2 production in rat vascular tissues The plasma SO_2 concentration was 15.54±1.68 μmol/L. In addition, a significant amount of SO_2 was noted in the arteries. Among the arteries, the aorta had the highest concentration (5.55±0.35 μmol/g protein), followed by the pulmonary (3.27±0.21 μmol/g protein), mesenteric (2.67±0.17 μmol/g protein), tail (2.50±0.20 μmol/g protein), and renal arteries (2.23±0.19 μmol/g protein), respectively (Table 2).

The tissue distribution of aspartate aminotransferase, a key SO_2 generating enzyme, is shown in Table 2. Plasma aspartate aminotransferase activity was 87±18 U/L. Unlike the SO_2 content, aspartate aminotransferase activity in the aorta (88±11 U/g protein) was the lowest, then the pulmonary arteries (96±12 U/g protein), mesenteric arteries (112±15 U/g protein), tail arteries (143±36 U/g protein), and renal arteries (188±30 U/g protein), respectively. Using quantitative real-time RT–PCR, aspartate aminotransferase 1 and aspartate aminotransferase 2 mRNA were detected in various tissues. The relative mRNA expression of aspartate aminotransferase 1 and aspartate aminotransferase 2 paralleled that of total aspartate aminotransferase. *In situ* hybridization was used to further locate aspartate aminotransferase 1 and aspartate aminotransferase 2 mRNA in the aortic wall. The results revealed aspartate aminotransferase 1 (Figure 1A) and aspartate aminotransferase 2 (Figure 1B) mRNA expression in endothelial cells and vascular smooth muscle cells beneath the endothelial layer.

Vasorelaxant effect of SO_2 on the isolated aortic ring Upon incubation with Na_2SO_3/$NaHSO_3$ at a low concentration of 25–100 μmol/L, the NE-precontracted aortic ring showed a slight and temporary relaxation (Figure 2A). However, a high concentration of SO_2 derivatives (1–12 mmol/L), much higher than that in plasma (approximately 0–30 μmol/L), induced a remarkable and persistent relaxant response in a concentration-dependent manner (Figure 2B). To further

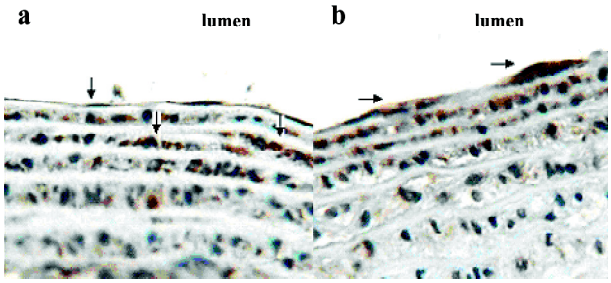

Figure 1. Distribution of glutamate oxaloacetate transaminase 1 mRNA (a) and glutamate oxaloacetate transaminase 2 mRNA (b) in rat aorta. Dark brown dots (arrows) represent positive signals of glutamate oxaloacetate transaminase 1 or glutamate oxaloacetate transaminase 2 mRNA in aorta. (DAB stained, ×400).

Table 2. Endogenous SO_2 content, aspartate aminotransferase activity, and relative amount of aspartate aminotransferase 1 and aspartate aminotransferase 2 cDNAs in rat tissues.

	SO_2 content (μmol/g protein)	Aspartate aminotransferase content (U/g protein)	Aspartate aminotransferase 1 cDNA/actin cDNA	Aspartate aminotransferase 2 cDNA/actin cDNA
Aorta	5.55±0.35	88±11	0.15±0.01	0.22±0.01
Pulmonary artery	3.27±0.21	96±12	0.50±0.08	0.29±0.01
Mesenteric artery	2.67±0.17	112±15	0.30±0.02	0.29±0.02
Tail artery	2.50±0.20	143±36	0.23±0.03	0.25±0.01
Renal artery	2.23±0.19	188±30	0.52±0.04	0.47±0.02

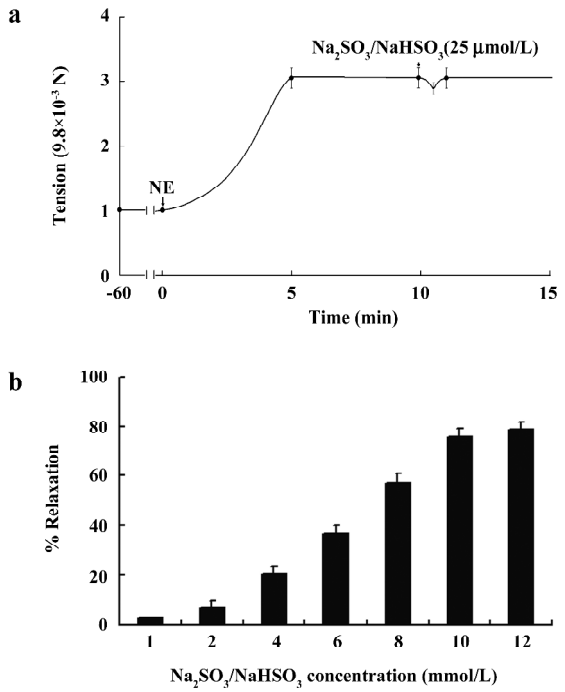

Figure 2. Effect of various concentrations of $Na_2SO_3/NaHSO_3$ in incubation buffer on NE-induced constricted aortic ring (n=8). (a) Low concentration (25 μmol/L) of $Na_2SO_3/NaHSO_3$ to the NE-induced constricted aortic ring. (b) High concentrations (1–12 mmol/L) of $Na_2SO_3/NaHSO_3$ to the NE-induced constricted aortic ring.

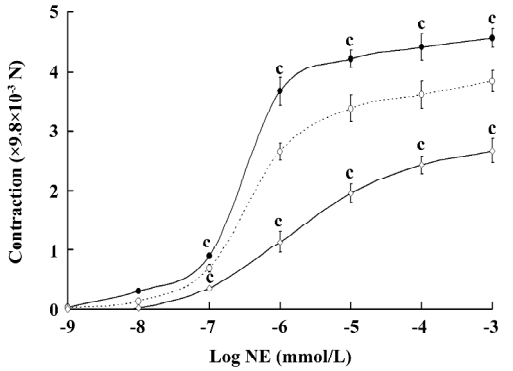

Figure 3. Effect of endogenous and exogenous SO_2 in aorta on its contraction reactivity to NE (n=8). Aortic ring was first treated with 0.1 μmol/L HDX (-•-) for 10 min to reduce the endogenous SO_2 or with 6 mmol/L $Na_2SO_3/NaHSO_3$ (-◦-) for 10 min to increase the SO_2 content in aortic ring, and then we tested its contraction reactivity to various concentrations of NE. ^cP<0.01 by Newman-Keuls test, as compared with the corresponding contraction reactivity of aorta ring without pre-treatment.

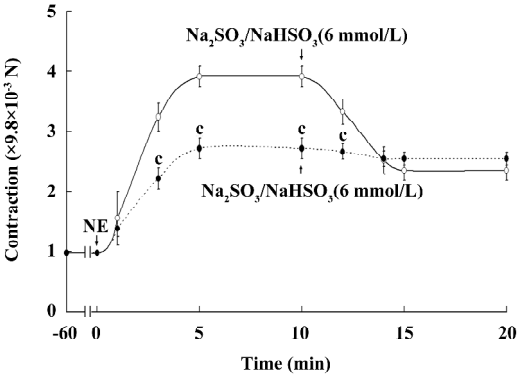

Figure 4. Role of nicardipine in the vasorelaxant effect of SO_2 on constricted aortic ring (n=8). Aortic rings were first incubated in buffer containing 1 μmol/L nicardipine (-•-) for 10 min and then in buffer containing 1 μmol/L NE to induce its constriction. These aortic rings lost the vasorelaxation response to 6 mmol/L $Na_2SO_3/NaHSO_3$. ^cP<0.01 by Newman-Keuls test, as compared with the corresponding contraction reactivity of aorta ring without pre-treatment of nicardipine (-◦-) .

explore the vasorelaxant effect of SO_2, HDX (0.1 μmol/L), a blocker for endogenous SO_2 generation, was used. In this experiment, the SO_2 concentration in the incubation solution was significantly lowered after HDX treatment [(1.85±0.11) *vs* (0.67±0.08) μmol/L, P<0.01]. Pretreatment with SO_2 derivatives (6 mmol/L) for 10 min shifted the contraction curve in response to NE (10 nmol/L–1 mmol/L) to the right, whereas pretreatment with HDX for 10 min shifted the contraction curve in response to NE to the left (Figure 3).

Involvement of L-type calcium channel in the vascular effects of SO_2 After pretreatment with the L-type calcium channel blocker, nicardipine (1 μmol/L), the vasorelaxant response of the NE-contracted rings to SO_2 derivatives (6 mmol/L) was eliminated almost completely (Figure 4). Bay K8644, an L-type calcium channel agonist, contracted the aortic ring in a concentration-dependent manner. Meanwhile, pre-incubation with SO_2 derivatives eliminated the vasoconstriction induced by Bay K8644. The SO_2-induced vasorelaxant response was also mimicked by nicardipine (Figure 5).

Role of K_{ATP} channel in the vasorelaxation induced by SO_2 In the present study, glibenclamide, a known K_{ATP} channel blocker, was used to treat aortic rings 10 min before the application of SO_2 derivatives. The relaxant effect of SO_2 was not affected by glibenclamide, which did not indicate the involvement of K_{ATP} channels (Figure 6).

Role of endothelium in the SO_2-induced vasorelxation Removing endothelium from the aortic tissue did not alter the SO_2-induced vasorelaxation curve (P>0.05). HDX enhanced the vasoconstrictive response of either intact or nude aortic rings to NE to a similar extent (P>0.05; Figure 7).

Discussion

Recently, increasing experiments have demonstrated SO_2

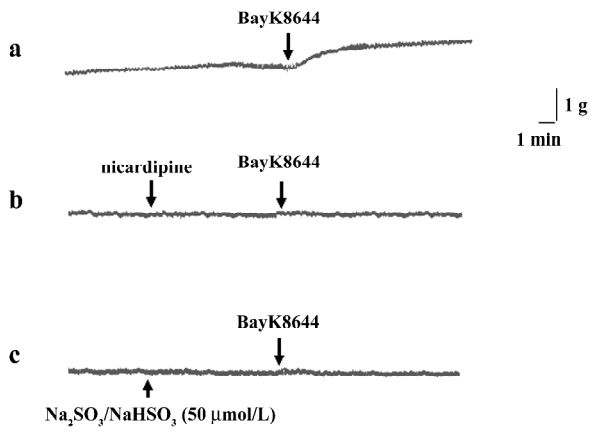

Figure 5. Role of Bay K8644 in the vasorelaxant effect of SO_2 on aortic ring (n=8). Bay K8644 alone induced constriction of aortic ring in a concentration-dependent manner. The constriction activity of Bay K8466 was blocked by the pre-treatment of nicardipine as well as the pre-treatment of 50 μmol/L Na_2SO_3/$NaHSO_3$.

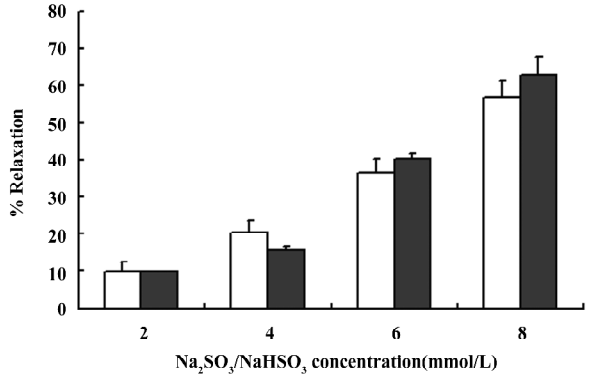

Figure 6. Effect of pre-incubation of glibenclamide on the vasorelaxant effect of Na_2SO_3/$NaHSO_3$ to NE-induced contracted aortic rings. Aortic rings were first incubated in 1 μmol/L glibenclamide (-■-) for 10 min, and then in 1 μmol/L NE to induce their contraction. Various concentrations of Na_2SO_3/$NaHSO_3$ were then applied to these aortic rings to observe their relaxant effect. P>0.05 by Newman-Keuls test, as compared with the corresponding contraction reactivity of aortic ring without pre-treatment with glibenclamide (-□-).

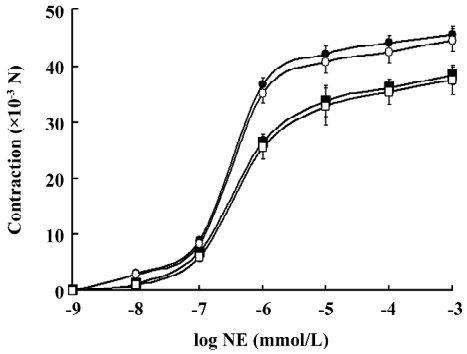

Figure 7. Effect of HDX on vasocontraction reactivity to NE in aortic rings either with or without endothelium (n=8). (-●-) stands for various concentrations of NE-induced contraction of intact aortic ring pre-treated with 0.1 μmol/L HDX, and (-■-) stands for various concentrations of NE-induced contraction of intact aortic ring without pre-treatment with 0.1 μmol/L HDX. (-○-) stands for various concentrations of NE-induced contraction of nude aortic ring pre-treated with 0.1 μmol/L HDX, and (-□-) stands for various concentrations of NE-induced contraction of nude aortic ring without pre-treatment with 0.1 μmol/L HDX.

or sulfite with multiple biological actions. The culture of human peripheral lymphocytes with a chemically-defined, protein-free medium revealed that sulfite was an essential metabolite[15], and Na_2SO_3 is a modulator of cytokine production, but does not alter either chemotaxis or cell surface expression of the tested molecules[16]. The inhalation of exogenous SO_2 was reported to reduce mean blood pressure in rats[17], but the endogenous SO_2 production in the vascular system and its bioactivity on vessels have not been made clear yet. Our present study, therefore, was undertaken to provide direct experimental evidence of endogenously-generated SO_2 in tissues, including vessels, and explore the vasorelaxant effect of SO_2 in rats.

According to a previous report, total serum sulfite in normal volunteer was found to be 0–10 μmol/L as measured by the HPLC-FD method[18]. In our study, we found the production of endogenous SO_2 in various vascular tissues. The aortic SO_2 content was the highest among the arteries, then the pulmonary, mesenteric, tail, and kidney arteries.

SO_2 and its hydrated form, sulfite/bisulfite, are generated during the normal processing of sulfur-containing amino acids, such as *L*-cysteine[8]. *L*-cysteine is first oxidized via cysteine dioxygenase to *L*-cysteine sulfinate. Then *L*-cysteine sulfinate undergoes transamination to form β-sulfinylpyruvate catalyzed by aspartate aminotransferase. β-Sulfinylpyruvate, the putative product, decomposes spontaneously to pyruvate and SO_2[18]. Thus aspartate aminotransferase could be regarded as a key enzyme in controlling endogenous SO_2 production.

Therefore, we further studied the aspartate aminotransferase activity and gene expression. We noticed that the aspartate aminotransferase activity was lowest in aorta, then pulmonary artery, mesenteric artery, tail, and renal arteries. Aspartate aminotransferase has two isoenzymes named for their intracellular location. Aspartate aminotransferase 1 is located in the cell cytoplasm, whereas aspartate aminotransferase 2 is located in the cell mitochondria. There were some functional differences between aspartate aminotransferase

1 and aspartate aminotransferase 2. For example, aspartate aminotransferase 1 and aspartate aminotransferase 2 are both were involved in the malate–aspartate shuttle. However, α-ketoglutarate and aspartate reacted together to form glutamate and oxaloacetate in a reaction catalyzed by aspartate aminotransferase 1, while glutamate and oxaloacetate reacted together to form α–ketoglutarate and aspartate in a reaction catalyzed by aspartate aminotransferase 2[19]. In this study, we explored the gene expressions of aspartate aminotransferase 1 and aspartate aminotransferase 2 in the various arteries. The data showed that the mRNA level of aspartate aminotransferase 1 and aspartate aminotransferase 2 was lowest in aorta, and that of renal artery was high. In the aorta, aspartate aminotransferase 1 mRNA and aspartate aminotransferase 2 mRNA were located in endothelia and vascular smooth muscle cells beneath the endothelial layer. No significant difference was found in the gene expression and localization in vascular tissues between the 2 isoenzymes.

The above data suggested that endogenous SO_2 and aspartate aminotransferase comprise the SO_2/aspartate aminotransferase pathway in the arteries. These observations implied the potential physiological functions of SO_2 in the cardiovascular system.

To explore the possible vasoactive effect of SO_2, we identified SO_2-induced vasorelaxation in NE precontracted aortic rings, although it was temporal and slight under the physiological plasma concentration. Furthermore, we found that SO_2 derivatives attenuated the NE-induced concentration-dependent vasoconstriction, whereas HDX, an aspartate aminotransferase inhibitor, enhanced the NE-induced vasoconstriction. The above *in vitro* data demonstrated that SO_2 exerted a vascular regulatory function as a vasorelaxant factor.

Based on the above findings, we further examined the possible mechanisms responsible for SO_2-induced vasorelaxation. Generally, calcium influx plays important physiological roles in mediating the contraction of vascular smooth muscle cells. During sarcolemmal membrane depolarization, the L-type calcium channel will open to permit calcium ion influx and trigger intracellular calcium-induced calcium release, leading to cell contraction[20]. Therefore, we observed the role of the L-type calcium channel in SO_2-induced vasorelaxation. Pretreatment with an L-type calcium channel blocker, nicardipine, almost eliminated the vasorelaxant effect induced by SO_2 (even at a higher concentration of 6 mmol/L). Furthermore, SO_2 antagonized the vasoconstriction induced by Bay K8644, an L-type calcium channel agonist, which could mimic the action of nicardipine. All these findings suggested that SO_2 might exert a vasorelaxant effect possibly by blocking the L-type calcium channel.

Potassium ion channels in the cell membrane are well known to participate in the vascular relaxation response[21]. In our study, pre-inhibition of the K_{ATP} channel by glibenclamide did not alter SO_2-induced vasorelaxation. These data do not support the involvement of the K_{ATP} channel in the SO_2-induced vasorelaxation.

Carbonyl sulfide and SO_2, which were detected by GC/MS analysis, were found in porcine artery rings and cardiac muscle tissue[22]. The authors suggested that the release of these 2 gases was related to a endothelium-derived hyperpolarizing factor. However, we observed that vascular endothelium and medial smooth muscles both generated endogenous SO_2. Moreover, the vasorelaxant effect of SO_2 was similar in intact and nude aortic rings, and HDX augmented the NE-induced vasoconstriction of either intact or nude aortic rings similarly. The results suggested that SO_2-induced vasorelaxation is endothelium independent.

In summary, we demonstrated that SO_2 could be endogenously generated in vascular tissues and could relax aortic rings, at least in part, through an L-type calcium channel, suggesting that it might act as a vasoactive molecule.

Author contributions

Shu-xu DU, Hong-fang JIN, Chao-shu TANG, and Jun-bao DU designed research; Shu-xu DU, Hong-fang JIN, Ding-fang BU, and Xia ZHAO performed research; Ding-fang BU and Bin GENG contributed new analytical reagents and tools; Shu-xu DU, Hong-fang JIN, and Bin GENG analyzed data; Shu-xu DU, Hong-fang JIN, Chao-shu TANG, and Jun-bao DU wrote the paper.

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