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Review

Sulfur dioxide: a physiologic endothelium-derived relaxing factor

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Summary. The gasotransmitter nitric oxide was classified as the first endothelium-derived relaxant factor, and opened a new era in cardiovascular research. Another small gas, sulfur dioxide (SO_2), can also be generated endogenously in mammals. Recent studies have shown that SO_2 may play important roles in the cardiovascular system. At low concentrations, the vasodilatory effect of SO_2 is endothelium-dependent. The vasodilation induced by an endothelium-derived relaxant factor is achieved by the opening of potassium channels, and hyperpolarization of the membranes of vascular smooth muscle cells. This feature is in accordance with that of SO_2. The vasodilatory effect of SO_2 is related to the opening of adenosine triphosphate-sensitive potassium channels and high-conductance calcium-activated potassium channels. The 3'-5'-cyclic guanosine monophosphate pathway and activation of nitric oxide synthase are also involved in the endothelium-derived relaxant factor effect of SO_2. The vasodilatory effect of gaseous SO_2 is much stronger than that of its derivatives (bisulfite and sulfite). It is suggested that SO_2 may be a candidate endothelium-derived relaxant factor, which could lead to a new era of research into cardiovascular disease in mammals.

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Introduction

Endothelial dysfunction plays important roles in the pathology of vascular diseases such as diabetes mellitus, atherosclerosis, and hypertension. Endothelial cells regulate basic vascular tone and reactivity by releasing a series of relaxing and contracting factors.

Nitric oxide (NO) and prostacyclin are believed to be endothelium-derived relaxing factors (EDRFs) (Radomski et al., 1987). However, abolition of the production of NO and PGI_2 does not prevent the endothelium-dependent relaxing effect (Scotland et al., 2005). Therefore, an unknown substance termed "endothelium-derived hyperpolarizing factor" (EDHF) might be present in blood vessels. Several candidates have been proposed to be EDHF: potassium (K^+) channels, epoxyeicosatrienoic acids, carbon monoxide (CO), hydrogen sulfide (H_2S), hydrogen peroxide, anandamide, citrulline, and ammonia (Feletou and Vanhoutte, 2007, 2009). However, the nature of EDHF is incompletely understood.

NO, CO and H_2S have been considered to be EDRF or EDHF, and all are "gasotransmitters". Gasotransmitters are small molecules that: can pass freely across cell membranes; are generated endogenously; can be regulated; have special biologic functions at physiologic concentrations; have specific biologic targets (Wang, 2002).

Besides NO, CO and H_2S, another small gas

molecule, sulfur dioxide (SO_2) can also be generated endogenously in mammalian cells, and pass freely across cell membranes (Stipanuk, 1986, 2004). SO_2 can be generated endogenously from the metabolism of sulfur-containing amino acids in mammals (Stipanuk, 1986; 2004). Balazy et al. (2003) found that levels of carbonyl sulfide and SO_2 could be enhanced by acetylcholine and calcium ionophore (A23187). The vasodilatory effect of opening adenosine triphosphate-sensitive potassium (K_{ATP}) channels might be another candidate for EDHF (Balazy et al., 2003).

For a long time, SO_2 was considered to be a toxic gas and air pollutant. SO_2 is detrimental to many organs (Meng, 2003; Meng and Bai, 2004). Recently, the cardiovascular effects of SO_2 have attracted considerable interest. SO_2 is generated in different tissues (stomach, intestine, myocardium, brain, pancreas, lung, kidney, spleen, and liver) (Luo et al., 2011). SO_2 has physical effects on the cardiovascular system: vasorelaxation, regulation of cardiac function and inhibition of activity of L-type calcium channels (Zhang et al., 2011). In addition, the pathophysiologic effects of SO_2 have been documented: amelioration of pulmonary hypertension (Jin et al., 2008; Sun et al., 2010; Luo et al., 2013); reduction of the myocardial injury induced by isoproterenol (Liang et al., 2011); inhibition of the development of atherosclerotic lesions (Li et al., 2011); reduction of myocardial ischemia–reperfusion injury (Wang et al., 2011b; Huang et al., 2013; Luo et al., 2013; Zhao et al., 2013); reduction of lung injury (Chen et al., 2015; Zhao et al., 2015); inhibition of vascular smooth muscle cells (VSMCs) proliferation (Liu et al., 2014); protection of neurons from the toxicity caused by febrile seizures (Han et al., 2014). Therefore, SO_2 is considered to be another novel gasotransmitter in mammals (Wang et al., 2010, 2011a, 2014, 2015; Chen et al., 2011; Huang et al., 2016a,b). This review indicates that SO_2 may be an EDRF, which could lead to a new era of research into cardiovascular disease in mammals.

Endothelial production of SO_2

Endogenous SO_2 is generated from sulfur-containing amino acids such as L-cysteine and then oxidized to L-cysteinesulfinate by cysteine dioxygenase. L-cysteinesulfinate is converted to β-sulfinylpyruvate due to transamination by aspartate aminotransferase, and decomposes spontaneously to pyruvate and SO_2 (Shapiro, 1977; Stipanuk et al., 1990). SO_2 dissociates to its derivatives (bisulfite and sulfite [$NaHSO_3/Na_2SO_3$] at 1:3 m/m in plasma), and is oxidized to a sulfate, then is excreted in urine (Stipanuk, 1986) (Fig. 1).

As a key SO_2-generating enzyme, the location of aspartate aminotransferase has been detected using *in situ* hybridization in samples of rat aorta. Expression of aspartate aminotransferase-1 mRNA and aspartate aminotransferase-2 mRNA in endothelial cells is much greater than those of VSMCs. The SO_2 concentration is different among different tissues and arteries (Du et al., 2008; Luo et al., 2011). The highest concentration of SO_2 is in the aorta (5.55±0.35 μmol/g protein), followed by the pulmonary, mesenteric, tail and renal arteries (3.27±0.21, 2.67±0.17, 2.50±0.20 and 2.23±0.19 μmol/g protein, respectively) (Du et al., 2008). The SO_2 concentration in the plasma and aortic tissues of rats is 16.77±8.24 μM and 127.76±31.34 μM, respectively (Meng et al., 2009).

In cultured vascular endothelial cells and smooth muscle cells (SMCs), SO_2 generation in endothelial cells is much higher than that in SMCs (Meng et al., 2009). SO_2 production can be stimulated by acetylcholine fourfold compared with that treated with ethanol (solvent for acetylcholine) in incubated porcine coronary artery rings. In addition, calcium ionophore can enhance SO_2 production by 1.5-fold (Balazy et al., 2003). In male Wistar rats, acetylcholine can increase the generation of endogenous SO_2, whereas noradrenaline inhibits these effects in thoracic aortic rings (Meng et al., 2009).

Endothelium-dependent vasodilation induced by SO_2

SO_2 can dose-dependently relax endothelium-intact or endothelium-denuded aortic rings in rats. SO_2 and its derivatives have vasodilatory effects, but the relaxation effects and their mechanism of action are different (Meng et al., 2009). Median effective concentrations (EC_{50}) that induce a half-maximal vasodilation response for SO_2 are (1247.38±98.32) μM in endothelium-intact

Table 1. Mechanisms of endothelium-dependent vasodilation induced by SO_2.

	Concentration	Mechanisms	Models
Gaseous SO_2	< 450 μM (Li and Meng, 2009, Zhang and Meng, 2009)	cGMP (Li and Meng, 2009)	Rat aortic rings
		BK_{Ca} (Zhang and Meng, 2009)	Rat aortic rings
		K_{ATP} (Zhang et al., 2016)	Rat aorta
		L-Ca^{2+} (Zhang et al., 2016)	Rat aorta
		sGC/cGMP/PKG pathway (Yao et al., 2016)	Rat aortic rings
SO_2 derivative	<2 mM (Wang et al. , 2009)	NOS (Wang et al., 2009)	Rat aortic rings
		cGMP (Meng et al. , 2012)	Rat aortic rings
		BK_{Ca} (Meng et al., 2012)	Rat aortic rings

BK_{Ca} channel, big calcium activated potassium channel; cGMP, 3′–5′-cyclic guanosine monophosphate; K_{ATP}, ATP-sensitive potassium channel; NOS, nitric oxide synthase; PKA, protein kinase A; and L-Ca^{2+}, L type of calcium channel; sGC, guanylate cyclase; PKG, protein kinase G

and (1321.89±89.67) μM in endothelium-denuded rat aortic rings (Zhang and Meng, 2009). EC_{50} of SO_2 derivatives is 7.28±0.12 mM (Zhang and Meng, 2009). Therefore, the vasodilatory effect of gaseous SO_2 is much stronger than that of its derivatives.

Gaseous SO_2 exerts a vasodilatory effect in an endothelium-dependent manner at low concentrations (<450 μM, Table 1), and in an endothelium-independent manner at high concentrations (>500 μM) (Meng et al., 2009; Zhang and Meng, 2009). The vasodilatory effect of SO_2 gas or gas solution is similar. SO_2 dissolved in water is present mainly as SO_2 molecules (Akhmetov et al., 1983; Feletou and Vanhoutte, 2006; Zhang and Meng, 2009).

In male Wistar rats treated with SO_2 (3.5, 7, 14 mg/m^3) 4 h every day for 30 consecutive days, the SO_2 concentrations described above could increase the expression of high-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) subunits alpha and beta-1 in rat aortas in vivo. Exposure of rats to a concentration of 14 mg/m^3 of SO_2 can up-regulate expression of K_{ATP} channel subunits Kir6.1, Kir6.2, and the sulfonylurea 2B receptor in rat aortas, with no effects on levels of the sulfonylurea 2A receptor (Zhang et al., 2016). Simultaneously, SO_2 downregulates expression of L-type calcium channel subunits Cav1.2 and Cav1.3 (Zhang et al., 2016). However, vasodilation induced by SO_2 (30 or 300 μM) can be inhibited by iberiotoxin (selectively inhibits current through BK_{Ca}) in endothelium-intact aortic rings, but this phenomenon is not affected by apamin (selectively inhibits current through low-conductance Ca^{2+}-activated K^+ channels) (Zhang and Meng, 2009). These data suggest that the vasodilation caused by SO_2 at low concentrations might be mediated by BK_{Ca} but not by low-conductance Ca^{2+}-activated K^+ channels. A recent study also showed that soluble guanylate cyclase (sGC), cyclic guanosine monophosphate (cGMP), and protein kinase G (PKG) pathways were also involved in the vasorelaxation of SO_2 (1–300 μM). Thiol reductants dithiothreitol could reverse the dimerization of sGC and PKG and vasodilation induced by SO_2. These data indicate that dimerization of sGC and PKG was related to the vasorelaxtion effect of SO_2 (Yao et al., 2016). The vasorelaxation induced by SO_2 (1500 μM) could be partially inhibited by nifedipine (L-type calcium channel blocker). Additionally, the vasorelation could also be inhibited by tetraethylammonium or glibenclamide. These data indicate the vasodilation of SO_2 was related to the K_{ATP} channel, L-type calcium channel and calcium-influx and release pathway (Zhang and Meng,

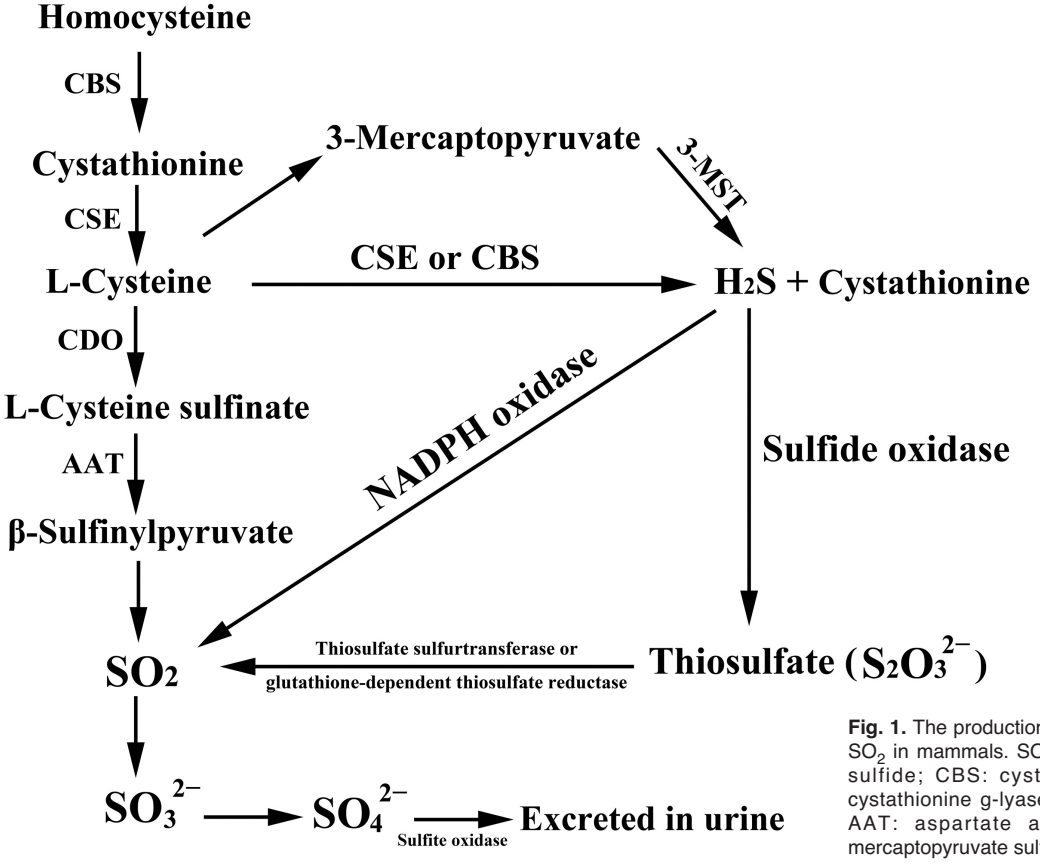

Fig. 1. The production and metabolism of endogenous SO_2 in mammals. SO_2: sulfur dioxide; H_2S: hydrogen sulfide; CBS: cystathionine ß-synthase; CSE: cystathionine g-lyase; CDO: cysteine dioxygenase; AAT: aspartate aminotransferase; 3MST: 3-mercaptopyruvate sulfurtransferase.

2009).

The relaxation wrought by SO_2 derivatives (0.5 and 1 mM) in endothelium-intact rings is stronger than that in endothelium-denuded rings. The NO synthase (NOS) inhibitor NG-nitro-L-arginine methyl ester (100 μM) can abolish the effects of SO_2 derivatives described above, but has no effect at higher doses (2–8 mM) (Wang et al., 2009). These data suggest that the NOS pathway is involved in the endothelium-dependent vasodilation caused by SO_2 derivatives. In addition, the vasodilatory effect of SO_2 derivatives is mediated by the 3'-5'-cyclic guanosine monophosphate (cGMP) pathway (Li and Meng, 2009). Interestingly, there are some differences in the SO_2 derivatives Na_2SO_3 and $NaHSO_3$ in terms of dilation of aortic rings (Meng et al., 2012). $NaHSO_3$ can cause dilation of rat aortic rings in a concentration-dependent manner (100–4000 μM), whereas Na_2SO_3 exerts a contractive effect at 500–1000 μM and relaxant effect at high concentrations (2000–4000 μM). The EC_{50} of $NaHSO_3$ and Na_2SO_3 on aortic rings is 2326±78.56 μM and 4100±87.68 μM, respectively. Vasodilation caused by $NaHSO_3$ is endothelium-dependent at low concentrations (≤500 μM) but endothelium-independent at high concentrations (≥1000 μM). At low concentrations, the vasodilatory effect of $NaHSO_3$ is mediated (at least in part) by the cGMP pathway and is related to BK_{Ca} channels, but not to the actions of prostaglandins, protein kinase C or the 3'-5'-cyclic adenosine monophosphate pathway (Meng et al., 2012). These data suggest that the vasorelaxant effect of sodium bisulfite was much stronger than that of sodium sulfite. The endothelium-dependent vasorelaxant effect of sodium bisulfite was related to the cGMP pathway and BK_{Ca} channels at low concentrations.

Resemblance of SO_2 to EDHF

The vasodilation caused by EDHF is through open potassium channels, and involves hyperpolarization of the membranes VSMCs by close voltage-dependent calcium channels (Shimokawa and Morikawa, 2005). The effect of EDHF is mainly caused by small-conductance Ca^{2+}-activated K^+ channel and partially by intermediate-conductance Ca^{2+}-activated K+ channel. SO_2 and EDHF have some common characteristics.

(1) Studies have shown that SO_2 induces vasodilation by opening K_{ATP} channels and might be EDHF (Balazy et al., 2003). In rat aortas, SO_2 can increase the expression of K_{ATP} channel subunits Kir6.1, Kir6.2, and the sulfonylurea 2B receptor (Zhang et al., 2016). These data indicate the relaxation effect of SO_2 was related to the opening K_{ATP} channel.

(2) In health, Ca^{2+} influx contributes to the contraction of VSMCs. At high concentration, SO_2 can inhibit expression of the subunits of the L-type calcium channels Cav1.2 and Cav1.3, which inhibit the contraction mediated by Ca^{2+} during depolarization of sarcolemmal membranes (Zhang et al., 2016). SO_2 can also elicit vasodilation by opening BK_{Ca} channels. Expression of BK_{Ca} channel subunits alpha and beta-1 can be up-regulated by SO_2 in rat aortas *in vivo* (Zhang et al., 2016).

Interaction of SO_2 and other EDRFs

The gasotransmitters NO and H_2S were considered as EDRFs in mammals (Fleming and Busse, 1999; Wang, 2009). Both of them play important roles in vascular tone regulation. Recent studies showed that there is some crosstalk between SO_2, NO and H_2S.

The vasodilatory effect of SO_2 can be enhanced under the presence of the NO donor sodium nitroprusside (3 or 5 nM), the EC50 of which are 598 μM and 217 μM SO_2, respectively (Li and Meng, 2009). In healthy rat, the EC_{50} of the relaxing effect induced by SO_2 is 1247.38±98.32 μM, so the vasodilatory effect of SO_2 is enhanced by NO by nearly six-fold. Interestingly, the vasodilatory effect of NO can also be enhanced by low concentrations of SO_2. In the presence of 3 μM SO_2, the relaxant effect of NO is enhanced at various concentrations. The EC_{50} of the vasodilatory effect induced by NO is 210 nM in the absence of SO_2, whereas it is 34 nM in the presence of 3 μM SO_2. The NOS inhibitor NG-nitro-L-arginine methyl ester (100 μM) can reverse the relaxant effect induced by SO_2 derivatives (0.5 and 1 mM) in endothelium-intact rings (Wang et al., 2009). In spontaneously hypertensive rats, SO_2 affects the NO level in aortic tissues. The vasodilatory effect of SO_2 can be enhanced by the presence of NO in isolated aortic rings (Lu et al., 2012). These data suggest that the actions of NO mediate the vasodilatory effect of SO_2 to some extent.

In the pulmonary hypertension induced by high pulmonary blood flow, H_2S production as well as the mRNA and protein expression of cystathionine-γ-lyase (CSE) is increased in pulmonary tissues in SO_2-exposed rats. These data suggest that SO_2 can up-regulate an endogenous H_2S pathway (Luo et al., 2013). In a model of atherosclerosis in rats, SO_2 treatment can significantly increase H_2S levels in plasma and aortic tissues and is associated with a reduction in the number of atherosclerotic lesions. Therefore, SO_2 can enhance H_2S production in rats with atherosclerosis (Li et al., 2011). These data suggest that SO_2 has some crosstalk with other EDRFs.

Perspectives and challenges

Accumulating data of the vascular effect of SO_2 suggest that SO_2 may be a new EDRF or EDHF. This hypothesis is based on five major pieces of evidence. Firstly, SO_2 can be generated in cultured normal endothelial cells and SMCs, and the level of SO_2 in endothelial cells is much higher than that in SMCs (Du et al., 2008). Secondly, at physiologic concentrations, SO_2 has a endothelium-dependent vasodilatory effect. The vasodilatory effect of SO_2, like that of NO, is mediated by a cGMP pathway and related to high-

conductance Ca^{2+}-activated channels (Li and Meng, 2009; Zhang and Meng, 2009). Thirdly, SO_2 production can be evoked by acetylcholine in endothelial cells and vascular tissues, which conforms to an EDHF being released by acetylcholine and other compounds from arteries (Feletou et al., 2003; Meng et al., 2009). Finally, SO_2 derivatives increase the intracellular concentration of free Ca^{2+} in rat ventricular myocytes (Nie and Meng, 2006), which might activate big conductance Ca^{2+}-activated channels, induce hyperpolarization of cells, and cause relaxation.

In conclusion, SO_2 could be endogenously generated in vascular tissues. It could be enhanced by acetylcholine and calcium ionophore. At physiological, or low, concentrations of SO_2, the vasodilation effect was endothelium dependent. The mechanism was related to BK_{Ca}, L-type calcium channel, K_{ATP} channel and cGMP pathway. At the same time, K_{ATP} channel subunits Kir6.1, Kir6.2, and the sulfonylurea 2B receptor, and L-type calcium channel subunits Cav1.2 and Cav1.3 may contribute to the vasorelaxation effect of SO_2 (Zhang et al., 2016). Additionally, the sGC/cGMP/PKG pathway, in association with sulfhydryl-dependent dimerization, was also involved in the vasodilatory effect of SO_2 (Yao et al., 2016). Further study showed that endogenous SO_2 was involved in vascular remodeling (Sun et al., 2010). Endogenous SO_2 could alleviate collagen remodeling and vascular calcification by inhibiting the TGF-beta/Smad pathway (Huang et al., 2016a,b; Li et al., 2016). These data indicate that SO_2 plays important roles in the regulation of vascular activities.

As an EDRF, SO_2 plays an important part in the regulation of endothelium-dependent vasoactive activities. Qualification of SO_2 as an EDHF will hinge on electrophysiologic changes in membrane potential in vascular endothelial cells and SMCs by patch-clamp studies. A deficiency in SO_2 will also probably affect the pathologies of vascular-related diseases, such as atherosclerosis, coronary vascular diseases and diabetes mellitus. More extensive studies are expected to further clarify SO_2 as a new EDRF/EDHF. In this way, the importance of SO_2 in the regulation of cardiovascular function will be better appreciated. Full understanding of the vasodilator effect of SO_2 carries considerable importance for further study on its pharmacologic effects.

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