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REVIEW ARTICLE

Sulfur-containing gaseous signal molecules, ion channels and cardiovascular diseases

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Sulfur-containing gaseous signal molecules including hydrogen sulphide and sulfur dioxide were previously recognized as toxic gases. However, extensive studies have revealed that they can be generated in the cardiovascular system *via* a sulfur-containing amino acid metabolic pathway, and have an important role in cardiovascular physiology and pathophysiology. Ion channels are pore-forming membrane proteins present in the membrane of all biological cells; their functions include the establishment of a resting membrane potential and the control of action potentials and other electrical signals by conducting ions across the cell membrane. Evidence has now accumulated suggesting that the sulfur-containing gaseous signal molecules are important regulators of ion channels and transporters. The aims of this review are (1) to discuss the recent experimental evidences in the cardiovascular system regarding the regulatory effects of sulfur-containing gaseous signal molecules on a variety of ion channels, including ATP-sensitive potassium, calcium-activated potassium, voltage-gated potassium, L- and T-type calcium, transient receptor potential and chloride and sodium channels, and (2) to understand how the gaseous signal molecules affect ion channels and cardiovascular diseases.

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Abbreviations

3MPST, 3-mercaptopyruvate sulfurtransferase; AAT, aspartate aminotransferase; BK_{Ca}, big-conductance calcium-activated potassium channel; CBS, cystathionine-β-synthase; CFTR, cystic fibrosis transmembrane conductance regulator; CSE, cystathionine-γ-lyase; $I_{Ca,L}$, L-type calcium channel currents; IK_{Ca}, intermediate-conductance calcium-activated potassium channel; K_{ATP}, ATP-sensitive potassium channel; K_{Ca}, calcium-activated potassium channel; K_{ir}, inwardly rectifying potassium channel; K_v, voltage-gated potassium channel; NCX, sodium calcium exchanger; SHR, spontaneously hypertensive rat; SK_{Ca}, small-conductance calcium-activated potassium channel; SUR, sulfonylurea receptor; TRP, transient receptor potential; TRPA1, transient receptor potential ankyrin 1; WKY, Wistar–Kyoto

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Introduction

NO was the first gaseous signal molecule to be identified in the cardiovascular system and its pivotal roles in many physiological and pathological processes have been elucidated (Ignarro *et al.,* 1987; Palmer *et al.,* 1987). Later on, sulfur-containing gaseous signal molecules, **hydrogen sulphide (H_2S)** and sulfur dioxide (SO_2), were identified (Hosoki *et al.,* 1997; Wang, 2002; Du *et al.,* 2008; Jin *et al.,* 2010; Liu *et al.,* 2010). Subsequent studies revealed that H_2S and SO_2 had a wide range of biological functions as well as broader effects on the cardiovascular system under both physiological and pathophysiological conditions (Wang, 2003; Du *et al.,* 2008; Liu *et al.,* 2012; Polhemus and Lefer, 2014; Meng *et al.,* 2015). The sulfur-containing gaseous signal molecules collectively have the following basic characteristics: (1) endogenous generation *via* a controllable enzymatic reaction by metabolism of sulfur-containing amino acids in the body; (2) small molecular weight, so can freely pass through the cell membrane and rapidly diffuse; (3) clear cellular and molecular targets; and (4) widely involved in the homeostatic control and regulation of the cardiovascular system as well as the pathogenesis of cardiovascular diseases (Barañano *et al.,* 2001; Li and Moore, 2007; Wang *et al.,* 2010). Recent studies demonstrated that sulfur-containing gaseous signal molecules regulate a variety of ion channels, which has a great impact on the physiology and pathology of the cardiovascular system.

Ion channels represent a family of important membrane proteins that conduct ions across the membrane, thereby controlling the intracellular ion content, membrane potential and action potentials. The abnormal function and structure of ion channels can lead to a variety of cardiovascular diseases (Ackerman, 1998; Navedo *et al.,* 2010; Hsiao *et al.,* 2013; George, 2014; Wang *et al.,* 2016). More importantly, endogenous sulfur-containing gaseous signal molecules play an important role in the pathogenesis of cardiovascular diseases by targeting ion channels (Wang, 2003; Brampton and Aaronson, 2016).

This review discusses the recent experimental evidences for the regulatory effects of H_2S and SO_2 on ion channels and their significance for cardiovascular diseases.

Hydrogen sulphide

Generation and metabolism of hydrogen sulphide in cardiovascular tissue

H_2S is a colourless gas smelling of rotten eggs. At the end of the 1990s, H_2S was found to be endogenously produced in the sulfur-containing amino acid metabolic pathway in mammals. Subsequently, H_2S was found to participate in regulating the physiological functions of the circulatory system (Zhao and Wang, 2002; Ji *et al.,* 2008; Wei *et al.,* 2010; Li *et al.,* 2013; Quan *et al.,* 2015; Wei *et al.,* 2015). Exploring the biological effects of endogenous H_2S has become a hot issue in life science and medicine (Wang, 2003; Tang *et al.,* 2006; Skovgaard and Olson, 2012; Vandiver and Snyder, 2012).

The key enzymes for producing endogenous H_2S include **cystathionine-γ-lyase (CSE)**, **cystathionine-β-synthase (CBS)** and **3-mercaptopyruvate sulfurtransferase (3MPST)**, with CBS and CSE dependent on **L-cystine** as a substrate and pyridoxal phosphate as a coenzyme to catalyse the production of H_2S. 3MPST catalyses the generation of H_2S by using β-mercapto-pyruvic acid as a substrate. CBS primarily catalyses the homocysteine and cysteine condensation reaction to form cystathionine while releasing H_2S. CSE catalyses the cleavage reaction of cystine to thiocysteine, which spontaneously degrades to cysteine and H_2S (Shibuya *et al.,* 2009; Gong *et al.,* 2011).

H_2S has good lipophilicity and hydrophilicity and freely passes through cell membranes. When H_2S is dissolved in water, it can be partially hydrated into H_2S and sulfur ions. H_2S is in two forms in mammals, namely, physical dissolution of H_2S gas and chemical forms of HS^-; the latter combined with hydrogen ions *in vivo* can generate H_2S, which forms a dynamic balance in the body. This condition is conducive to maintaining the H_2S content *in vivo* and the stability of pH in the internal environment (Liu *et al.,* 2012). Most of the endogenous H_2S is excreted in the form of thiosulfate and sulfate produced by oxidative metabolism in mitochondria, and a small portion is converted to methyl mercaptan and methyl sulfide by methylation in cytoplasmic solution. H_2S in the plasma is cleared by methaemoglobin. Its metabolites can be excreted in the kidneys, intestines and lungs within 24 h.

Hydrogen sulphide regulates the cardiovascular system by targeting the ion channels

ATP-sensitive potassium channel (K_{ATP}). **K_{ATP}** is a receptor-dependent potassium channel, and its activity can be significantly inhibited with an increase in intracellular ATP concentration. The K_{ATP} channel consists of the inwardly rectifying potassium channel (K_{ir}) and sulfonylurea receptors (SURs). The two K_{ir} subtypes, **$K_{ir}6.1$** and **$K_{ir}6.2$**, form the ion pore of the K_{ATP} channel. SUR also has two subtypes, **SUR1** and **SUR2** [SUR2A (ABCC8) and SUR2B (ABCC9)]. The combination of different K_{ir} subunits and SUR subunits forms the diversity of K_{ATP} molecular structure, which determines the complex function of the K_{ATP} channel in different tissues. In cardiomyocytes, the K_{ATP} channel consists of $K_{ir}6.2$ and SUR2A, whereas in vascular smooth muscle cells (VSMCs), it is mainly composed of $K_{ir}6.1$ and SUR2B.

The K_{ATP} channel mediates the vasodilatation induced by H_2S in cardiovascular tissues, such as the thoracic aorta (Zhao *et al.,* 2001), mesenteric artery (Cheng *et al.,* 2004) and coronary artery (Casalini *et al.,* 2014). Zhao *et al.* (2001) found that H_2S was a K_{ATP} channel opener and that H_2S (2.8 and 14 $\mu mol \cdot kg^{-1}$) induced a transient decrease in mean arterial blood pressure (12.5 ± 2.1 and 29.8 ± 7.6 mmHg) in anaesthetized rats. **Pinacidil** (2.8 $\mu mol \cdot kg^{-1}$) mimics but the K_{ATP} channel blocker **glibenclamide** inhibits the hypotensive effect. The currents of the K_{ATP} channel in VSMCs were recorded and found to be amplified by H_2S (300 μM) from 85.8 ± 12.4 to 149.0 ± 21.4 pA. H_2S hyperpolarizes the cellular membrane from –35.7 to –53.3 mV, and this effect is reversed by glibenclamide.

Furthermore, after inhibition of H_2S, whole-cell K_{ATP} currents are reduced (Tang *et al.,* 2005). Sun *et al.* (2015) found the expression of $K_{ir}6.1$ and SUR2B was significantly down-regulated in the aortas of spontaneously hypertensive rats (SHRs), and this effect was reversed by treatment with NaHS, a H_2S donor. As a K_{ATP} channel opener, pinacidil dose-dependently relaxes rat thoracic artery rings, but in aortic rings of SHRs, the vasorelaxant effects are attenuated as compared with those of Wistar–Kyoto (WKY) rats. NaHS at 100 μM improved the vasorelaxant response in aortic rings of SHR. These studies suggest that H_2S, by targeting the K_{ATP} channel, is involved in the pathogenesis of hypertension.

The protective role of H_2S was shown in ischaemia–reperfusion injury (Gross and Fryer, 1999). As a protective gaseous signal molecule, H_2S has a negative inotropic action and glibenclamide can, in part, block this effect of H_2S (Geng *et al.,* 2004). In rat models of myocardial ischaemia–reperfusion injury, Johansen *et al.* (2006) found that NaHS (1 μM) limited the infarct size from 41.0 ± 2.6 to 20.2 ± 2.1% of the risk zone; pretreatment with glibenclamide abolished this effect. More electrophysiological findings revealed that in cardiomyocytes, an H_2S donor at 40 μM increased the opening probability of the K_{ATP} channel from 0.07 ± 0.03 to 0.15 ± 0.08 and a NaHS donor at 100 μM increased the opening probability from 0.07 ± 0.03 to 0.36 ± 0.15. This effect was abolished by the K_{ATP} blocker glibenclamide (Zhang *et al.,* 2007). In addition, the mitochondrial K_{ATP} channel (mitoK_{ATP}) was shown to mediate this cardioprotective effect of H_2S, as a specific blocker of the mitoK_{ATP} channel, 5-hydroxydecanoate, inhibited this effect of H_2S (Sivarajah *et al.,* 2009). These studies suggest that H_2S, by targeting the K_{ATP} channel, is involved in protecting the myocardium against injury.

The potential mechanisms by which H_2S acts on the K_{ATP} channel have been clarified. Jiang *et al.* (2010) demonstrated that H_2S activates the rv$K_{ir}6.1$/rvSUR1 channel by sulfhydryl modification of Cys^6 and Cys^{26} of the rvSUR1 subunit; the major sulfhydration site was Cys^{43}. Mustafa *et al.* (2011) showed that H_2S promotes cell hyperpolarization and vasorelaxation and this is associated with an enhancement of $K_{ir}6.1$ activity induced by modifying sulfhydration, which subsequently reduces K_{ir} 6.1-**ATP** binding and increases $K_{ir}6.1$ and **PIP2** binding. Other studies showed that in cultured VSMCs, H_2S stimulates FOXO1 and FOXO3a nuclear translocation and promotes FOXO1 or FOXO3a binding to the $K_{ir}6.1$ and SUR2B gene promoters, thereby increasing the protein expression of the K_{ATP} channel and relaxing artery rings (Sun *et al.,* 2015).

Calcium-activated potassium channel (K_{Ca}). The **K_{Ca} channel** is a kind of voltage and calcium-sensitive channel and widely exists in excitable cell membranes of mammals. It is divided into three types according to electrophysiological properties and conductance levels: big-conductance K_{Ca} (BK_{Ca}), intermediate-conductance K_{Ca} (IK_{Ca}) and small-conductance K_{Ca} (SK_{Ca}).

Sheng *et al.* (2013) showed that under identical voltage-clamp conditions, NaHS at 100 μM reduced the peak current density of BK_{Ca}, I_{to} and I_{Kir} channels in human atrial fibroblasts to attenuate atrial fibroblast proliferation and moderate myofibroblast differentiation. Na_2S (10 μM) hyperpolarized pressurized arterioles from –30.3 to –47.9 mV, which returned to –39.0 mV after the application of **iberiotoxin**, a selective K_{Ca} channel blocker. This result suggests that the K_{Ca} channel mediates the vasodilatation induced by H_2S in cerebral arteries (Liang *et al.,* 2012).

Cheng *et al.* (2004) observed that the vasorelaxant effects of H_2S in rat mesenteric artery were blocked by **charybdotoxin/apamin**, with the EC_{50} changing from 22.5 ± 1.1 to 141.9 ± 21.5 μM. Furthermore, H_2S can hyperpolarize endothelial cells, and neither glibenclamide nor iberiotoxin can block this effect. However, the combination of blockers of the IK_{Ca}/SK_{Ca} channel, charybdotoxin/apamin, abolished this effect. Moreover, in human artery endothelial cells, the hyperpolarization induced by H_2S was diminished by **TRAM-34**, an IK_{Ca} channel blocker, but was unchanged by glibenclamide or iberiotoxin treatment (Mustafa *et al.,* 2011). These studies suggest that the IK_{Ca}/SK_{Ca} channel is the possible target of H_2S in the cardiovascular system.

L-type calcium channel. The **L-type calcium channel**, one subtype of voltage-dependent calcium channel, is widely distributed in VSMCs as well as cardiomyocytes, and has a characteristically slow activation time, high activation voltage and long opening time (Catterall, 2000). The L-type calcium channel has four subtypes – **$Ca_v1.1$**, **$Ca_v1.2$**, **$Ca_v1.3$** and **$Ca_v1.4$** – and regulates rapid depolarization and plateau formation and maintenance.

Zhao and Wang (2002) revealed that the vasorelaxation of rat aortic rings induced by H_2S (600 μM) was decreased by 59.3 ± 8.3% by treatment with **nifedipine**, an antagonist of the L-type calcium channel, which suggests that the L-type calcium channel mediates the relaxant effects of H_2S.

Sun *et al.* (2008) investigated the possible mechanism for the negative inotropic effects of H_2S in cardiomyocytes. Electrophysiological measurements were used to record isolated cardiomyocyte L-type calcium channel currents ($I_{Ca,L}$) in WKY rats and SHRs. NaHS at 100 μM decreased peak $I_{Ca,L}$ by 15.00 ± 2.08% in WKY cardiomyocytes and by 19.61 ± 2.18% in SHR cardiomyocytes, which suggests the inhibitory role of H_2S in calcium release from cardiomyocytes. Pre-incubation with NaHS (100 μM) enhanced cell survival to 78 ± 3% in H9c2 cells treated with H_2O_2 and decreased resting intracellular calcium $[Ca^{2+}]_i$ content, which was mimicked by nifedipine treatment, which suggests that H_2S inhibits the L-type calcium channel to enhance cell survival (Avanzato *et al.,* 2014).

With regard to the potential mechanisms by which H_2S targets the L-type calcium channel, Mustafa *et al.* (2009) and Li *et al.* (2011b) indicated that H_2S regulates the activity of proteins by modifying cysteine residues *via* S-sulfhydration. A whole-cell voltage-clamp technique was used to obtain more electrophysiological evidence. Negative inotropic effects by H_2S on cardiac function were attenuated by **diamide**, an oxidant sulfhydryl modifier, and the inhibitory effect on the peak amplitude of $I_{Ca,L}$ was reversed by dithiothreitol, a reducing modifier of the sulfhydryl group. These observations suggest that H_2S inhibits the cardiomyocyte L-type calcium channel to produce a negative

inotropic effect, and the main action site might be the sulfhydryl group in the L-type calcium channel (Zhang *et al.*, 2012).

T-type calcium channel. The **T-type calcium channel** has the following electrophysiological characteristics: fast activation, lower activation voltage, fast inactivation and short opening time. It includes three subtypes – **$Ca_v3.1$**, **$Ca_v3.2$** and **$Ca_v3.3$** – and participates in maintaining the ventricular action potential plateau and regulates the initiation and contraction of myocardium (Triggle, 1998). Unlike the L-type calcium channel, in the T-type calcium channel, the organic calcium antagonist and Bay k8644 are ineffective, but it can be blocked by the inorganic calcium blocker **Ni^{2+}**.

Avanzato *et al.* (2014) found that the inhibitory effect of H_2S on resting $[Ca^{2+}]_i$ content in H9c2 cells was abolished by pretreatment with nifedipine and Ni^{2+} combined as compared with nifedipine alone. This result suggests that both the T-type and L-type calcium channels are inhibited by H_2S.

Sekiguchi *et al.* (2014) demonstrated that H_2S enhanced $Ca_v3.2$ T-type calcium channel function in HEK293 cells. The $Ca_v3.2$ T-type calcium channel is also involved in the carotid body response to hypoxia mediated by H_2S, as NaHS has been shown to increase $[Ca^{2+}]_i$ content in glomus cells and enhance the activity of carotid body, and this effect was attenuated in glomus cells and carotid bodies of $Ca_v3.2$-knockout mice (Makarenko *et al.*, 2015).

Sodium calcium exchanger (NCX). **NCX** is a bidirectional transporter and consists of three subtypes: **NCX1**, **NCX2** and **NCX3**. NCX1 is the main subtype present in cardiomyocytes. The ratio of Na^+/Ca^{2+} exchangers was three Na^+ exchangers to one Ca^{2+} exchanger in cardiomyocytes. The currents of Na^+/Ca^{2+} exchangers are divided into inward Na^+/Ca^{2+}, outward Na^+/Ca^{2+} and dual-ward Na^+/Ca^{2+} exchange currents.

Moccia *et al.* (2011) used NaHS (250 μM) to stimulate Ca^{2+} inflow in 80% of rat aortic endothelial cells, which mimics the effects in adult human dermal microvascular endothelial cells. To investigate the possible role of NCX in the Ca^{2+} current induced by NaHS, the authors found that **KB-R7943** (20 μM), a selective inhibitor of the NCX, reduced the effect by 25.4%. The amplitude of the Ca^{2+} response induced by NaHS was small after the application of KB-R7943. Collectively, these results suggest a novel target of H_2S in rat aortic endothelial cells, and it plays a significant role in Ca^{2+} inflow and is involved in NaHS-induced angiogenesis.

To explore the effects of H_2S on NCX in cardiomyocytes, Pan *et al.* (2008) used **caffeine** to keep the **ryanodine receptor** open and prevent the sarcoplasmic reticulum from sequestering Ca^{2+}. The cells were pretreated with NaHS to elicit the Ca^{2+} inflow current and the half-decay time and 90% decay time (t_{50} and t_{90}) of $[Ca^{2+}]_I$ content were measured. The t_{50} and t_{90} of the decayed $[Ca^{2+}]_i$ transients were shortened by NaHS, and the effects were reversed by **chelerythrine**, a selective PKC inhibitor. The observations confirmed that H_2S promotes Ca^{2+} inflow *via* NCX in a PKC-dependent manner.

Markova *et al.* (2014) found increased mRNA and protein levels and the activity of NCX1 as well as concentration of cAMP were enhanced in **GYY4137** (a H_2S donor)-treated HeLa cells. The levels of **β_1-** and **β_3-adrenoceptors** were also up-regulated after GYY4137 treatment. This GYY4137-induced increase was eliminated when NCX1 was silenced. To test the relationship between β-adrenoceptors and NCX1 in the GYY4137-induced apoptosis, they confirmed that a mutual interaction between β_1- and β_3-adrenoceptors and NCX1 mediates the stimulant effects of H_2S on apoptosis.

Transient receptor potential (TRP) channel. The **TRP channel** widely exists in the mammalian brain, spinal cord, heart, kidney and other tissues. The channel can form functional homo-tetramers or hetero-tetramers, thus playing a role in signal transduction. According to the homology, the TRP ion channel superfamily has seven subgroups, including TRPA, TRPC, TRPML, TRPM, TRPP, TRPN and TRPV. Most of the TRP channels are nonselective cationic channels; TRPM4 and TRPM5 are monovalent cationic channels; and TRPV5 and TRPV6 have very high selectivity for calcium.

Intensive research showed that a TRP channel mediates the regulatory effects of H_2S on neuronal and synaptic activity (Abe and Kimura, 1996; Nagai *et al.*, 2004; Kimura, 2013). White *et al.* (2013) observed that the NaHS (300 μM) induced vasodilatation of small mesenteric arteries could not be blocked by a non-specific potassium-channel inhibitor, **tetraethylammonium (TEA)**, glibenclamide or **XE991**, a voltage-gated potassium channel (K_v) channel blocker. However, the chloride channel blocker **4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS)** abolished this effect, but **5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB)** and anthracere-9-carboxylic acid had no effect. To explore the potential mechanisms of this NaHS-induced vasodilatation, **capsaicin** was found to abolish this NaHS-induced vasodilatation, but it was not affected by **capsazepine** or a TRPV1 blocker, but was blocked by **HC-030031**, a **TRPA1 channel** blocker. These findings indicate that H_2S activates the TRPA1 channel, which stimulates the release of sensory neurotransmitters and then mediates the vasodilatation in rat mesenteric arteries.

Chloride channel. **Chloride** ion is the most abundant anion inside or outside cells, and its transmembrane transport is very important. The chloride channel is widely distributed in various tissues, and its functions include inhibiting cell excitability, promoting depolarization after repolarization and maintaining the resting membrane potential of cells. The chloride channel is divided into three subtypes according to its protein structure: **voltage-dependent chloride ion channel**, **cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel** and ligand-gated chloride channel.

Lee *et al.* (2007) examined whether H_2S affects the intracellular pH (pH_i) of VSMCs and found that the H_2S donor NaHS dose-dependently decreased pH_i, which was attenuated by DIDS, a blocker of the Cl^-/HCO_3^- exchanger, but was not affected by inhibition of the Na^+/H^+ exchanger or Ca^{2+}-ATPase. Then, the activity of Cl^-/HCO_3^-

exchanger was detected by NH_4Cl perfusion and was found to be enhanced by NaHS by approximately 60%. Similar results were found in rat aortic rings: DIDS blocked the diastolic effects of NaHS on aortic rings. These data suggest that H_2S decreases pH_i *via* activation of VSMC Cl^-/HCO_3^- exchangers and relaxed rat aortic rings.

A chemical hypoxia-induced cell injury model was used to investigate the possible role of CFTR in the cardioprotective effects of H_2S on cardiomyocytes. NaHS (400 μM) protected H9c2 cells against the toxicity of $CoCl_2$ and increased the cell survival rate from 48.3 ± 2.1 to 65.4 ± 0.6%. **NPPB**, an inhibitor of the CFTR chloride channel, which had no effect on myocardial viability, attenuated the protective effects of NaHS, with a decrease in survival rate of cardiomyocytes from 65.4 ± 0.6 to 56.3 ± 1.8%. These results suggest that the CFTR chloride channel might be involved in the protective effects of H_2S on cardiomyocytes against hypoxia-mediated injury (Li *et al.*, 2009b).

Electrophysiological evidence was provided by Malekova *et al.* (2009); they observed current changes of the single chloride channel in cardiomyocyte lysosomal vesicles incorporated into a bilayer lipid membrane in rats. NaHS decreased the open probability of the chloride channel by increasing the channel closing time but had no effect on the amplitude, conductance or mean opening time (Malekova *et al.*, 2009). These results reveal that H_2S has the potential to inhibit the chloride channel and this could be ones of its mechanisms of action.

Sulfur dioxide

Generation and metabolism of sulfur dioxide in cardiovascular tissues

Similar to NO, CO and H_2S, SO_2 is known to be one of the air pollutants and is present in industrial waste gases. However, SO_2 is also generated endogenously. Biochemical studies have shown that the enzymatic reaction of sulfur-containing amino acid metabolic pathways with methionine can produce SO_2 (Singer and Kearney, 1956). In recent years, a complete endogenous SO_2 pathway has been detected in the cardiovascular system and found to play an important role in cardiovascular physiology and pathophysiology (Zhao *et al.*, 2008; Sun *et al.*, 2010; Wang *et al.*, 2011; Li *et al.*, 2011a). Therefore, endogenous SO_2 is expected to be a new cardiovascular signal molecule after NO, CO and H_2S (Li *et al.*, 2009a; Liu *et al.*, 2010).

In the body, cysteine is oxidized by cysteine dioxygenase to form cysteinesulfinate, which generates β-sulfonyl pyruvate by transamination under the action of aspartate aminotransferase (AAT), and β-sulfonyl pyruvate further spontaneously decomposes into SO_2 and pyruvate. In addition, the oxidation of H_2S is one way to generate endogenous SO_2. Endogenous SO_2 is metabolized to produce sulfites, which are further oxidized by sulfite oxidase to sulfate and excreted in the urine. Endogenous SO_2 can be detected in rat plasma, myocardium and vascular tissues (Du *et al.*, 2008). As the key enzyme for endogenous SO_2 production, AAT is a pyridoxal phosphate-dependent transaminase that catalyses the transamination of aspartate to α-ketoglutarate to form oxaloacetate and glutamate and participate in its reverse reaction. Cysteinesulfinate, similar to the structure of aspartic acid, can be considered an analogue of aspartic acid and is catalysed by AAT *via* a transamination reaction to produce β-sulfonated pyruvic acid, by which SO_2 is produced. AAT is divided into two subtypes: AAT1 exists in the cytoplasm, and AAT2 exists in mitochondria. The activity of AAT and the mRNA expression of AAT1 and AAT2 is detected in the myocardium and vascular tissues of rats.

Regulatory effects of sulfur dioxide on ion channels in cardiovascular system

ATP-sensitive potassium channel (K_{ATP}). Isolated aortic rings of rats were used to examine the vascular electrophysiological changes in the presence of SO_2. The relaxant reactivity of aortic rings induced by SO_2 was observed after precontraction with noradrenaline (NA), and the SO_2 derivatives Na_2SO_3/$NaHSO_3$, with a mol ratio of 3:1, concentration-dependently relaxed aortic rings. Inhibiting the production of endogenous SO_2 by use of hydroxamate enhanced the contractile response of the aortic rings to NA. The relaxant effects of low-doses of SO_2 derivatives (≤4 mM) were inhibited by glibenclamide, a blocker of the K_{ATP} channel, so, in part, the relaxant effects of SO_2 on rat aortic rings are mediated by the K_{ATP} channel (Du *et al.*, 2006). Wang *et al.* (2009) found that SO_2 endothelium-dependently and -independently relaxed rat aortic rings. The diastolic effects induced by SO_2 derivatives at a low dose (0.5 and 1 mM) were attenuated by the removal of endothelium, and a similar phenomenon was observed in endothelium-intact rings after the application of **L-NAME**, a non-selective NOS inhibitor. Hence, the NOS pathway is likely to be involved in the endothelium-dependent diastolic effects of SO_2. However, a higher concentration of SO_2 derivatives relaxed aortic rings significantly, and this effect was not inhibited by removal of the endothelium. High K^+ (60 mM) inhibited the relaxation of endothelium-denuded aortic rings to SO_2 derivatives (2 mM), and this effect was blocked by glibenclamide (3 μM). Incubation with SO_2 or SO_2 derivative solution or exposure to SO_2 at 14 $mg{\cdot}m^{-3}$ increased the expression of the K_{ATP} channel subunits $K_{ir}6.1$, $K_{ir}6.2$ and SUR2B in rat aortas *in vitro* (Zhang *et al.*, 2014; 2016). These findings confirm that SO_2 relaxes vessels by activating the K_{ATP} channel.

The negative inotropic action of SO_2 was confirmed by the decreased left-ventricular pressure and ±dp/dtmax, heart rate and coronary flow in isolated perfused rat hearts (Zhang and Meng, 2012; Zhang *et al.*, 2015). The effects of SO_2 were blocked in part by the administration of glibenclamide (Zhang and Meng, 2012). Zhang *et al.* (2015) examined the effect of SO_2 on K_{ATP} channel expression and found that $K_{ir}6.2$ and SUR2A mRNA and protein levels were increased on exposure to SO_2 (14 $mg{\cdot}m^{-3}$). These findings indicate that the K_{ATP} channel participates in the SO_2-induced negative inotropic effects.

Calcium-activated potassium channel (K_{Ca}). While investigating the endothelium-independent mechanisms

involved in the relaxing effects of SO_2 on rat aortic rings, Wang *et al.* (2009) found that the relaxation of aortic rings, after removal of the endothelium, induced by SO_2 derivatives (2 mM) was inhibited by K^+ (60 mM). Application of TEA, a blocker of K_{Ca} channel, decreased the SO_2-induced relaxation of rings, suggesting that the K_{Ca} channel in smooth muscle cells mediates the relaxing effects of SO_2 on rat aortic rings (Wang *et al.*, 2009). TEA attenuated the vasorelaxant effects of SO_2 on endothelium-intact and -denuded rings. The endothelium-dependent vasorelaxation to low concentrations of SO_2 was blocked in part by iberiotoxin (Zhang and Meng, 2009; Meng *et al.*, 2012). Also the protein expression of the channel subunits α and β1 in the BK_{Ca} channel of the rat aorta was up-regulated by SO_2 application (Zhang *et al.*, 2014; Zhang *et al.*, 2016). These data suggest that the BK_{Ca} channel mediates the SO_2-induced relaxation of vessels.

Voltage-gated potassium channel (K_v). The **K_v channel**, the most complex subtype of the potassium channel superfamily, is essential in maintaining the physiological function of excitable cells. Nie and Meng (2005a) used whole-cell patch-clamp assay to explore the impact of SO_2 on the voltage-gated potassium current in isolated cardiomyocytes in adult rats. SO_2 (10 μM) elicited both I_{to} and I_{K1}, with increased peak current amplitude of I_{to} by approximately 37% and I_{K1} by approximately 26%, which suggests that I_{to} and I_{K1} are probably involved in the biological effects of SO_2 on cardiomyocytes.

L-type calcium channel. Du *et al.* (2006) observed that SO_2 has relaxant properties in rat aortic rings. The vasoconstrictor responses induced by NA were enhanced by inhibition of SO_2. **Nicardipine**, a blocker of the L-type calcium channel, attenuated the contractile effects of NA and significantly blocked the vasorelaxant effects of SO_2, which confirmed that the L-type calcium channel is involved in the vasorelaxant effects of SO_2. SO_2 at a physiological concentration was used to relax rat aortic rings slightly. Pretreatment with nicardipine prevented the vasoconstriction induced by Bay K8644 and blocked the SO_2-induced vasorelaxation of NA-precontracted aortic rings (Du *et al.*, 2008). $CaCl_2$ (0.01–10 mM) induced a vasoconstriction of endothelium-denuded aortic rings perfused with 60 mM K^+ and Ca^{2+}-free solution, and this effect was dose-dependently reduced by SO_2. However, the vasoconstriction of endothelium-denuded aortic rings to $CaCl_2$ was abolished by nifedipine, a blocker of the L-type calcium channel (Wang *et al.*, 2009). Similar results were observed in rat isolated aortic rings pretreated with nifedipine, and the relaxant effects of SO_2 in NA-precontracted rings were inhibited (Zhang and Meng, 2009; Meng *et al.*, 2012). Further investigations showed that the protein expression of subunits $Ca_v1.2$ and $Ca_v1.3$ was

Table 1

Biological effects of H_2S on ion channels in the cardiovascular system

Molecule	Concentration	Ion channel	Effect	Disease	Reference
H_2S gas	0.1 μM-10 mM	K_{ATP} ↑	Vasorelaxant effects	–	Cheng *et al.* (2004)
	10 μM-10 mM	K_{Ca} ↑		–	Cheng *et al.* (2004)
	600 μM	K_{ATP} ↑		–	Zhao *et al.* (2001)
	600 μM	L-type calcium channel ↑		–	Zhao and Wang (2002)
	50 μM	K_{ATP} ↑	Electrophysiological evidence	–	Cheng *et al.* (2004)
	100 μM	K_{ATP} ↑		–	Jiang *et al.* (2010)
	300 μM	K_{ATP} ↑		–	Zhao *et al.* (2001)
	300 μM	K_{ATP} ↑		–	Tang *et al.* (2005)
H_2S donor (NaHS)	90 μmol·kg^{-1}	K_{ATP} ↑	Vasorelaxant effects	Hypertension	Sun *et al.* (2015)
	100 μM	K_{ATP} ↑, IK_{Ca}↑, SK_{Ca}↑		–	Mustafa *et al.* (2011)
	300 μM	TRPA1 ↑		–	White *et al.* (2013)
	> 300 μM	K_{ATP} ↑		–	Casalini *et al.* (2014)
	1 mM	Cl^-/HCO_3^- exchangers ↑		–	Lee *et al.* (2007)
	2.8 μmol·kg^{-1}, 40 μM	K_{ATP} ↑	Negative inotropic effects	–	Geng *et al.* (2004)
	1 μM	K_{ATP} ↑	Cardioprotective effects	Myocardial ischaemia–reperfusion injury	Johansen *et al.* (2006)
	40 μM	K_{ATP} ↑		Myocardial ischaemia–reperfusion injury	Zhang *et al.* (2007)

continues

Table 1 (Continued)

Molecule	Concentration	Ion channel	Effect	Disease	Reference
	100 μM	NCX ↑		–	Pan *et al.* (2008)
	100 μM	L-type calcium channel ↓T-type calcium channel ↓		–	Avanzato *et al.* (2014)
	400 μM	CFTR chloride channel ↑		–	Li *et al.* (2009b)
	3 $mg \cdot kg^{-1}$	mitoK_{ATP} ↑		Myocardial ischaemia–reperfusion injury	Sivarajah *et al.* (2009)
	50 μM	T-type calcium channel ↑	Enhance the activity of carotid body	–	Makarenko *et al.* (2015)
	100 μM	BK_{Ca} ↓, I_{to} ↓, I_{Kir} ↓	Inhibit proliferation and differentiation of atrial fibroblasts	–	Sheng *et al.* (2013)
	250 μM	NCX ↑, K_{ATP} ↑	Promote angiogenesis	–	Moccia *et al.* (2011)
	40, 100 μM	K_{ATP} ↑	Electrophysiological evidences	–	Zhang *et al.* (2007)
	100 μM	L-type calcium channel ↓		–	Sun *et al.* (2008)
	100 μM	chloride channel ↓		–	Malekova *et al.* (2009)
	100 μM	L-type calcium channel ↓		–	Zhang *et al.* (2012)
	1.5 mM	T-type calcium channel ↑		–	Sekiguchi *et al.* (2014)
H_2S donor (GYY4137)	10 μM	NCX1 ↑	Stimulate apoptosis	–	Markova *et al.* (2014)
H_2S donor (Na_2S)	10 μM	K_{Ca} ↑	Vasorelaxant effects	–	Liang *et al.* (2012)
	0.1–0.3 mM	T-type calcium channel↑	Electrophysiological evidences	–	Sekiguchi *et al.* (2014)

I_{to}, transient outward potassium current; I_{Kir}, inward rectifier potassium current. ↑ stands for activation; ↓ stands for inhibition.

Table 2

Biological effects of SO_2 on ion channels in the cardiovascular system

Molecule	Concentration	Ion channel	Effect	Disease	Reference
SO_2 gas	14 $mg \cdot m^{-3}$	K_{ATP} ↑, BK_{Ca} ↑, L-type calcium channel ↓	Vasorelaxant effects	–	Zhang *et al.* (2016)
	30, 300 μM	BK_{Ca} ↑		–	Zhang and Meng (2009)
	1500 μM	K_{ATP} ↑		–	Zhang *et al.* (2014)
	1500 μM	K_{ATP} ↑, L-type calcium channel ↓		–	Zhang and Meng (2009)
	14 $mg \cdot m^{-3}$	K_{ATP} ↑, L-type calcium channel ↓	Negative inotropic effects	–	Zhang *et al.* (2015)
	1000 μM	K_{ATP} ↑, L-type calcium channel ↓		–	Zhang and Meng (2012)
SO_2 derivative (Na_2SO_3/$NaHSO_3$)	1500 μM	K_{ATP} ↑, BK_{Ca} ↑, L-type calcium channel↓	Vasorelaxant effects	–	Zhang *et al.* (2014)

continues

Table 2 (Continued)

Molecule	Concentration	Ion channel	Effect	Disease	Reference
	2 mM	K_{ATP} ↑, K_{Ca} ↑		–	Wang *et al.* (2009)
	≤ 4 mM	K_{ATP} ↑		–	Du *et al.* (2006)
	2–8 mM	L-type calcium channel ↓		–	Du *et al.* (2006)
	6 mM	L-type calcium channel ↓		–	Du *et al.* (2008)
	10 μM	L-type calcium channel ↑	Negative inotropic effects	–	Zhang *et al.* (2008)
	300, 1000 μM	K_{ATP} ↑, L-type calcium channel ↓		–	Zhang and Meng (2012)
	10 μM	I_{to} ↑, I_{K1} ↑	Electrophysiological evidence	–	Nie and Meng (2005a)
	10 μM	sodium channel ↑		–	Wei and Meng (2008)
	1–200 μM	sodium channel ↑		–	Nie and Meng (2005b)
	2–100 μM	L-type calcium channel ↑		–	Nie and Meng (2006)
	50, 100, 500, 1000 μM	L-type calcium channel ↓		–	Zhang *et al.* (2011)
SO_2 derivative ($NaHSO_3$)	400 μM	BK_{Ca} ↑	Vasorelaxant effects	–	Meng *et al.* (2012)
	2000, 4000 μM	K_{ATP} ↑, L-type calcium channel ↓		–	

I_{to}, transient outward potassium current; I_{K1}, inward rectifier potassium current; ↑ stands for activation, ↓ stands for inhibition.

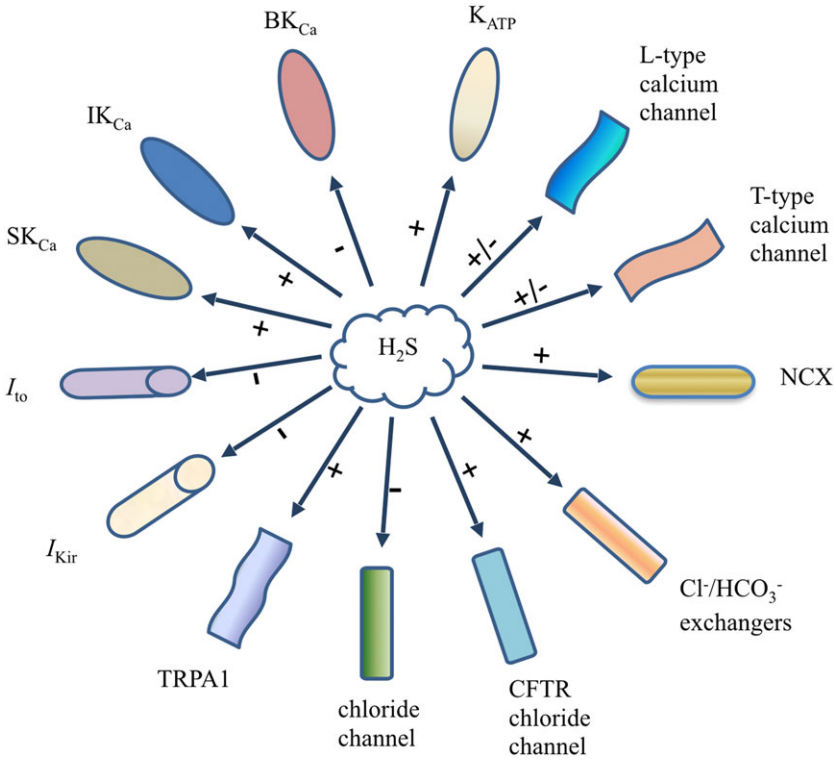

Figure 1

Effects of H_2S on ion channels in the cardiovascular system. I_{to}, transient outward potassium current; I_{Kir}, inward rectifier potassium current. + stands for activation; – stands for inhibition.

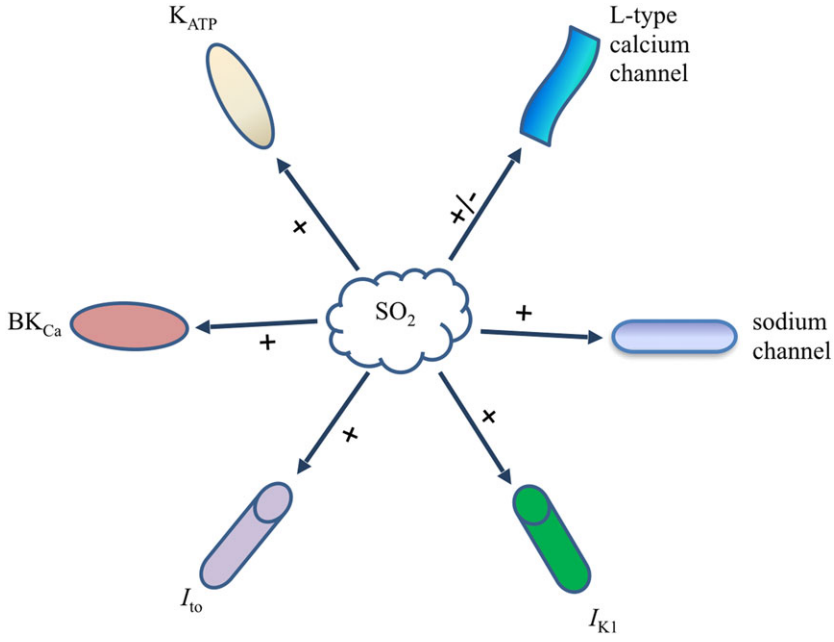

Figure 2

Effects of SO_2 on ion channels in the cardiovascular system. I_{to}, transient outward potassium current; I_{K1}, inward rectifier potassium current; + stands for activation, – stands for inhibition.

increased on exposure to SO_2 at 14 mg·m^{-3} or after incubation with SO_2 in solution (1500 μM) (Zhang *et al.*, 2014; 2016). These observations indicate that the L-type calcium channel mediates the vasorelaxant effects of SO_2 in rat aorta.

The $I_{Ca,L}$ channel in rat cardiomyocytes was measured by a whole-cell patch-clamp assay. SO_2 derivatives activated the L-type calcium channel by reducing the fast and slow time constants of inactivation, promoting the recovery of $I_{Ca,L}$ from inactivation and shifting the channel to more positive potentials (Nie and Meng, 2006). In rat isolated, perfused hearts, SO_2 inhibits the cardiac function. These negative inotropic effects of SO_2 on the myocardium were blocked by pretreatment with nicardipine (Zhang *et al.*, 2008). To investigate whether SO_2 acts on ventricular cardiomyocytes *via* the L-type calcium channel, Zhang *et al.* (2011) used the whole-cell patch-clamp method to detect the $I_{Ca,L}$ channel in rat cardiomyocytes. SO_2 derivatives depressed the peak amplitudes of $I_{Ca,L}$, and $I_{Ca,L}$ was decreased in response to SO_2, which suggests that SO_2 protects cardiomyocytes during cardiovascular diseases by inhibiting the L-type calcium channel. Zhang and Meng (2012) also confirmed the role of the L-type calcium channel in the negative inotropic action of gaseous SO_2 and its derivatives. The expression of $Ca_v1.2$ and $Ca_v1.3$ channels in rat hearts was detected after exposure to SO_2 at 14 mg·m^{-3} for 30 days. SO_2 inhibited the mRNA and protein expression of $Ca_v1.2$ and $Ca_v1.3$ in rat hearts (Zhang *et al.*, 2015). Collectively, SO_2, by up-regulating the expression and activity of the L-type calcium channel, has a negative inotropic effect on cardiomyocytes.

Sodium channel. Sodium currents (I_{Na}) in isolated cardiomyocytes were measured by a whole-cell patch-clamp assay. SO_2 derivatives activated I_{Na} dose-dependently, shortened their activation and inactivation time and accelerated their recovery (Nie and Meng, 2005b). The activating effects of SO_2 on I_{Na} were significantly enhanced by diethyldithiocarbamate (Wei and Meng, 2008). These findings suggest that SO_2 activates the sodium channel, which plays an important role in the cardiovascular system.

Conclusions and perspectives

Extensive studies have confirmed the important roles of the sulfur-containing gaseous signal molecules H_2S and SO_2, as a result of their regulatory effects on ion channels, in cardiovascular physiology and the development of cardiovascular diseases. Tables 1 and 2 and Figures 1 and 2 illustrate the important roles of H_2S and SO_2 in regulating the ion channels and their associated pathways as well as cardiovascular function. However, the molecular mechanisms by which H_2S and SO_2 target the ion channels have not been fully elucidated. Also, the interaction between H_2S and SO_2 and their possible integrated action on ion channels are unclear. Further studies are needed to reveal the mechanisms responsible for the effect of H_2S and SO_2 on ion channels. The potential integrated action of H_2S and SO_2 and even among the gaseous signal molecules on the structure and function of ion channels are also worthy of investigation. Furthermore, the effect of a single gaseous signal molecule, H_2S or SO_2, on all the ion channels merits further studies for better understanding the mechanisms of cardiovascular physiology and pathologies, which is of great importance for providing new targets and strategies for treating cardiovascular diseases.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015a,b,c,d,e).

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Conflict of interest

The authors declare no conflicts of interest.

References

Abe K, Kimura H (1996). The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci 16: 1066–1071.

Ackerman MJ (1998). The long QT syndrome: ion channel diseases of the heart. Mayo Clin Proc 73: 250–269.

Alexander SPH, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015a). The concise guide to PHARMACOLOGY 2015/16: G protein-coupled receptors. Br J Pharmacol 172: 5744–5869.

Alexander SPH, Catterall WA, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015b). The concise guide to PHARMACOLOGY 2015/16: Voltage-gated ion channels. Br J Pharmacol 172: 5904–5941.

Alexander SPH, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E *et al.* (2015c). The concise guide to PHARMACOLOGY 2015/16: Other ion channels. Br J Pharmacol 172: 5942–5955.

Alexander SPH, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015d). The concise guide to PHARMACOLOGY 2015/16: Enzymes. Br J Pharmacol 172: 6024–6109.

Alexander SPH, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E *et al.* (2015e). The concise guide to PHARMACOLOGY 2015/16: Transporters. Br J Pharmacol 172: 6110–6202.

Avanzato D, Merlino A, Porrera S, Wang R, Munaron L, Mancardi D (2014). Role of calcium channels in the protective effect of hydrogen sulfide in rat cardiomyoblasts. Cell Physiol Biochem 33: 1205–1214.

Barañano DE, Ferris CD, Snyder SH (2001). Atypical neural messengers. Trends Neurosci 24: 99–106.

Brampton J, Aaronson PI (2016). Role of hydrogen sulfide in systemic and pulmonary hypertension: cellular mechanisms and therapeutic implications. Cardiovasc Hematol Agent Med Chem 14: 4–22.

Casalini ED, Goodwill AG, Owen MK, Moberly SP, Berwick ZC, Tune JD (2014). Contribution of hydrogen sulfide to the control of coronary blood flow. Microcirculation 21: 104–111.

Catterall WA (2000). Structure and regulation of voltage-gated Ca^{2+} channels. Ann Rev Cell Dev Biol 16: 521–555.

Cheng Y, Ndisang JF, Tang G, Cao K, Wang R (2004). Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. Am J Physiol Heart Circ Physiol 287: H2316–H2323.

Du SX, Zhang CY, Jin HF, Du JB, Tang CS (2006). Vasorelaxant effect of sulfur dioxide derivatives on isolated aortic rings of rats and its mechanisms. Beijing Da Xue Xue Bao 38: 581–585.

Du SX, Jin HF, Bu DF, Zhao X, Geng B, Tang CS *et al.* (2008). Endogenously generated sulfur dioxide and its vasorelaxant effect in rats. Acta Pharmacol Sin 29: 923–930.

Geng B, Yang J, Qi Y, Zhao J, Pang Y, Du J *et al.* (2004). H_2S generated by heart in rat and its effects on cardiac function. Biochem Biophys Res Commun 313: 362–368.

George AL Jr (2014). Recent genetic discoveries implicating ion channels in human cardiovascular diseases. Curr Opin Pharmacol 15: 47–52.

Gong QH, Shi XR, Hong ZY, Pan LL, Liu XH, Zhu YZ (2011). A new hope for neurodegeneration: possible role of hydrogen sulfide. J Alzheimers Dis 24: 173–182.

Gross GJ, Fryer RM (1999). Sarcolemmal versus mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. Circ Res 84: 973–979.

Hosoki R, Matsuki N, Kimura H (1997). The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem Biophys Res Commun 237: 527–531.

Hsiao PY, Tien HC, Lo CP, Juang JM, Wang YH, Sung RJ (2013). Gene mutations in cardiac arrhythmias: a review of recent evidence in ion channelopathies. Appl Clin Genet 6: 1–13.

Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A 84: 9265–9269.

Ji Y, Pang QF, Xu G, Wang L, Wang JK, Zeng YM (2008). Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. Eur J Pharmacol 587: 1–7.

Jiang B, Tang G, Cao K, Wu L, Wang R (2010). Molecular mechanism for H(2)S-induced activation of K(ATP) channels. Antioxid Redox Signal 12: 1167–1178.

Jin HF, Du JB, Tang CS (2010). Frontiers in research reviews: the pathophysiological significance of sulfur-containing gases: introduction simple molecules play complicated roles. Clin Exp Pharmacol Physiol 37: 743–744.

Johansen D, Ytrehus K, Baxter GF (2006). Exogenous hydrogen sulfide (H_2S) protects against regional myocardial ischemia-reperfusion injury – evidence for a role of K_{ATP} channels. Basic Res Cardiol 101: 53–60.

Kimura H (2013). Physiological role of hydrogen sulfide and polysulfide in the central nervous system. Neurochem Int 63: 492–497.

Lee SW, Cheng Y, Moore PK, Bian JS (2007). Hydrogen sulphide regulates intracellular pH in vascular smooth muscle cells. Biochem Biophys Res Commun 358: 1142–1147.

Li JP, Yang CT, Yang ZL, Liao XX, Wang LC, Huang X *et al.* (2009b). Roles of CFTR Cl^- channels in hydrogen sulfide-induced cardioprotection and cell proliferation in H9c2 cells. Chin J Pathophysiol 25: 1070–1075.

Li L, Liu D, Bu D, Chen S, Wu J, Tang C *et al.* (2013). Brg1-dependent epigenetic control of vascular smooth muscle cell proliferation by hydrogen sulfide. Biochim Biophys Acta 1833: 1347–1355.

Li L, Moore PK (2007). An overview of the biological significance of endogenous gases: new roles for old molecules. Biochem Soc Trans 35: 1138–1141.

Li L, Rose P, Moore PK (2011b). Hydrogen sulfide and cell signaling. Annu Rev Pharmacol Toxicol 51: 169–187.

Li W, Tang C, Jin H, Du J (2011a). Regulatory effects of sulfur dioxide on the development of atherosclerotic lesions and vascular hydrogen sulfide in atherosclerotic rats. Atherosclerosis 215: 323–330.

Li X, Bazer FW, Gao H, Jobgen W, Johnson GA, Li P *et al.* (2009a). Amino acids and gaseous signaling. Amino Acids 37: 65–78.

Liang GH, Xi Q, Leffler CW, Jaggar JH (2012). Hydrogen sulfide activates Ca^{2+} sparks to induce cerebral arteriole dilatation. J Physiol 590: 2709–2720.

Liu D, Jin H, Tang C, Du J (2010). Sulfur dioxide: a novel gaseous signal in the regulation of cardiovascular functions. Mini Rev Med Chem 10: 1039–1045.

Liu YH, Lu M, Hu LF, Wong PT, Webb GD, Bian JS (2012). Hydrogen sulfide in the mammalian cardiovascular system. Antioxid Redox Signal 17: 141–185.

Makarenko VV, Peng YJ, Yuan G, Fox AP, Kumar GK, Nanduri J *et al.* (2015). $Ca_v3.2$ T-type Ca^{2+} channels in H S-mediated hypoxic response of the carotid body. Am J Physiol Cell Physiol 308: C146–C154.

Malekova L, Krizanova O, Ondrias K (2009). H(2)S and HS(–) donor NaHS inhibits intracellular chloride channels. Gen Physiol Biophys 28: 190–194.

Markova J, Hudecova S, Soltysova A, Sirova M, Csaderova L, Lencesova L *et al.* (2014). Sodium/calcium exchanger is upregulated by sulfide signaling, forms complex with the β1 and β3 but not β2 adrenergic receptors, and induces apoptosis. Pflugers Arch 466: 1329–1342.

Meng G, Ma Y, Xie L, Ferro A, Ji Y (2015). Emerging role of hydrogen sulfide in hypertension and related cardiovascular diseases. Br J Pharmacol 172: 5501–5511.

Meng Z, Yang Z, Li J, Zhang Q (2012). The vasorelaxant effect and its mechanisms of sodium bisulfite as a sulfur dioxide donor. Chemosphere 89: 579–584.

Moccia F, Bertoni G, Pla AF, Dragoni S, Pupo E, Merlino A *et al.* (2011). Hydrogen sulfide regulates intracellular Ca^{2+} concentration in endothelial cells from excised rat aorta. Curr Pharm Biotechnol 12: 1416–1426.

Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK *et al.* (2009). H_2S signals through protein S-sulfhydration. Sci Signal 2: ra72.

Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK *et al.* (2011). Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. Circ Res 109: 1259–1268.

Nagai Y, Tsugane M, Oka J, Kimura H (2004). Hydrogen sulfide induces calcium waves in astrocytes. FASEB J 18: 557–559.

Navedo MF, Cheng EP, Yuan C, Votaw S, Molkentin JD, Scott JD *et al.* (2010). Increased coupled gating of L-type Ca^{2+} channels during hypertension and Timothy syndrome. Circ Res 106: 748–756.

Nie A, Meng Z (2005a). Sulfur dioxide derivative modulation of potassium channels in rat ventricular myocytes. Arch Biochem Biophys 442: 187–195.

Nie A, Meng Z (2005b). Study of the interaction of sulfur dioxide derivative with cardiac sodium channel. Biochim Biophys Acta 1718: 67–73.

Nie A, Meng Z (2006). Modulation of L-type calcium current in rat cardiac myocytes by sulfur dioxide derivatives. Food Chem Toxicol 44: 355–363.

Palmer RM, Ferrige AG, Moncada S (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524–526.

Pan TT, Neo KL, Hu LF, Yong QC, Bian JS (2008). H_2S preconditioning-induced PKC activation regulates intracellular calcium handling in rat cardiomyocytes. Am J Physiol Cell Physiol 294: C169–C177.

Polhemus DJ, Lefer DJ (2014). Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. Circ Res 114: 730–737.

Quan X, Luo H, Liu Y, Xia H, Chen W, Tang Q (2015). Hydrogen sulfide regulates the colonic motility by inhibiting both L-type calcium channels and BK_{Ca} channels in smooth muscle cells of rat colon. PLoS One 10: e0121331.

Sekiguchi F, Miyamoto Y, Kanaoka D, Ide H, Yoshida S, Ohkubo T *et al.* (2014). Endogenous and exogenous hydrogen sulfide facilitates T-type calcium channel currents in $Ca_v3.2$-expressing HEK293 cells. Biochem Biophys Res Commun 445: 225–229.

Sheng J, Shim W, Wei H, Lim SY, Liew R, Lim TS *et al.* (2013). Hydrogen sulphide suppresses human atrial fibroblast proliferation and transformation to myofibroblasts. J Cell Mol Med 17: 1345–1354.

Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K *et al.* (2009). 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. Antioxid Redox Signal 11: 703–714.

Singer TP, Kearney EB (1956). Intermediary metabolism of L-cysteinesulfinic acid in animal tissues. Arch Biochem Biophys 61: 397–409.

Sivarajah A, Collino M, Yasin M, Benetti E, Gallicchio M, Mazzon E *et al.* (2009). Anti-apoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial *I*/R. Shock 31: 267–274.

Skovgaard N, Olson KR (2012). Hydrogen sulfide mediates hypoxic vasoconstriction through a production of mitochondrial ROS in trout gills. Am J Physiol Regul Integr Comp Physiol 303: R487–R494.

Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SP *et al.* (2016). The IUPHAR/BPS guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. Nucl Acids Res 44: D1054–D1068.

Sun Y, Huang Y, Zhang R, Chen Q, Chen J, Zong Y *et al.* (2015). Hydrogen sulfide upregulates K_{ATP} channel expression in vascular smooth muscle cells of spontaneously hypertensive rats. J Mol Med (Berl) 93: 439–455.

Sun Y, Tian Y, Prabha M, Liu D, Chen S, Zhang R *et al.* (2010). Effects of sulfur dioxide on hypoxic pulmonary vascular structural remodeling. Lab Invest 90: 68–82.

Sun YG, Cao YX, Wang WW, Ma SF, Yao T, Zhu YC (2008). Hydrogen sulphide is an inhibitor of L-type calcium channels and mechanical contraction in rat cardiomyocytes. Cardiovasc Res 79: 632–641.

Tang CS, Li XH, Du JB (2006). Hydrogen sulfide as a new endogenous gaseous transmitter in the cardiovascular system. Curr Vasc Pharmacol 4: 17–22.

Tang G, Wu L, Liang W, Wang R (2005). Direct stimulation of K_{ATP} channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. Mol Pharmacol 68: 1757–1764.

Triggle DJ (1998). The physiological and pharmacological significance of cardiovascular T-type, voltage-gated calcium channels. Am J Hypertens 11: 80S–87S.

Vandiver M, Snyder SH (2012). Hydrogen sulfide: a gasotransmitter of clinical relevance. J Mol Med (Berl) 90: 255–263.

Wang HG, Zhu W, Kanter RJ, Silva JR, Honeywell C, Gow RM *et al.* (2016). A novel NaV1.5 voltage sensor mutation associated with severe atrial and ventricular arrhythmias. J Mol Cell Cardiol 92: 52–62.

Wang MJ, Cai WJ, Zhu YC (2010). Mechanisms of angiogenesis: role of hydrogen sulphide. Clin Exp Pharmacol Physiol 37: 764–771.

Wang R (2002). Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? FASEB J 16: 1792–1798.

Wang R (2003). The gasotransmitter role of hydrogen sulfide. Antioxid Redox Signal 5: 493–501.

Wang XB, Huang XM, Ochs T, Li XY, Jin HF, Tang CS *et al.* (2011). Effect of sulfur dioxide preconditioning on rat myocardial ischemia/reperfusion injury by inducing endoplasmic reticulum stress. Basic Res Cardiol 106: 865–878.

Wang YK, Ren AJ, Yang XQ, Wang LG, Rong WF, Tang CS *et al.* (2009). Sulfur dioxide relaxes rat aorta by endothelium-dependent and -independent mechanisms. Physiol Res 58: 521–527.

Wei H, Meng Z (2008). Enhancing effects of diethyldithiocarbamate on increase of sodium channel by sulfur dioxide derivatives in ventricular myocytes. Cell Biol Int 32: 1143–1149.

Wei H, Zhang R, Jin H, Liu D, Tang X, Tang C *et al.* (2010). Hydrogen sulfide attenuates hyperhomocysteinemia-induced cardiomyocytic endoplasmic reticulum stress in rats. Antioxid Redox Signal 12: 1079–1091.

Wei X, Zhang B, Cheng L, Chi M, Deng L, Pan H *et al.* (2015). Hydrogen sulfide induces neuroprotection against experimental stroke in rats by down-regulation of AQP4 via activating PKC. Brain Res 1622: 292–299.

White BJ, Smith PA, Dunn WR (2013). Hydrogen sulphide-mediated vasodilatation involves the release of neurotransmitters from sensory nerves in pressurized mesenteric small arteries isolated from rats. Br J Pharmacol 168: 785–793.

Zhang Q, Bai Y, Yang Z, Tian J, Meng Z (2015). Effect of sulfur dioxide inhalation on the expression of KATP and L-Ca(2+) channels in rat hearts. Environ Toxicol Pharmacol 39: 1132–1138.

Zhang Q, Bai Y, Yang Z, Tian J, Meng Z (2016). The molecular mechanism of the effect of sulfur dioxide inhalation on the potassium and calcium ion channels in rat aortas. Hum Exp Toxicol 35: 418–427.

Zhang Q, Meng Z (2009). The vasodilator mechanism of sulfur dioxide on isolated aortic rings of rats: involvement of the K^+ and Ca^{2+} channels. Eur J Pharmacol 602: 117–123.

Zhang Q, Meng Z (2012). The negative inotropic effects of gaseous sulfur dioxide and its derivatives in the isolated perfused rat heart. Environ Toxicol 27: 175–184.

Zhang Q, Tian J, Bai Y, Lei X, Li M, Yang Z *et al.* (2014). Effects of gaseous sulfur dioxide and its derivatives on the expression of K_{ATP}, BK_{Ca} and L-Ca(2+) channels in rat aortas in vitro. Eur J Pharmacol 742: 31–41.

Zhang R, Sun Y, Tsai H, Tang C, Jin H, Du J (2012). Hydrogen sulfide inhibits L-type calcium currents depending upon the protein sulfhydryl state in rat cardiomyocytes. PLoS One 7: e37073.

Zhang RY, Du JB, Sun Y, Chen S, Tsai HJ, Yuan L *et al.* (2011). Sulfur dioxide derivatives depress L-type calcium channel in rat cardiomyocytes. Clin Exp Pharmacol Physiol 38: 416–422.

Zhang SQ, Du JB, Tian Y, Geng B, Tang CS, Tang XY (2008). Effects of sulfur dioxide on cardiac function of isolated perfusion heart of rat. Zhonghua Yi Xue Za Zhi 88: 830–834.

Zhang Z, Huang H, Liu P, Tang C, Wang J (2007). Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening K_{ATP} channels. Can J Physiol Pharmacol 85: 1248–1253.

Zhao W, Wang R (2002). H_2S-induced vasorelaxation and underlying cellular and molecular mechanisms. Am J Physiol Heart Circ Physiol 283: H474–H480.

Zhao W, Zhang J, Lu Y, Wang R (2001). The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. EMBO J 20: 6008–6016.

Zhao X, Jin HF, Tang CS, Du JB (2008). Effects of sulfur dioxide, on the proliferation and apoptosis of aorta smooth muscle cells in hypertension: experiments with rats. Zhonghua Yi Xue Za Zhi 88: 1279–1283.