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Role of sulfur-containing gaseous substances in the cardiovascular system

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1. ABSTRACT

Gaseous mediators are important signaling molecules with properties that differ from other, larger signaling molecules. Small gaseous mediators readily cross cell membranes and can access sites on target molecules that would be inaccessible to bulkier molecules. They have a variety of signaling mechanisms, some well understood, some not. The family of gasotransmitters is growing, well known members include nitric oxide (NO) and carbon monoxide (CO). Newer candidates include the sulfur containing gases hydrogen sulfide (H_2S), which has been shown to have a wide range of physiological functions, and more recently sulfur dioxide (SO_2) has been studied as a potential new gasotransmitter. This review explores the production, regulation and role of the sulfur-containing gases H_2S and SO_2 at the level of the endothelial and vascular smooth muscle cells as well as the broader effects on the cardiovascular system under both physiological and pathophysiological conditions.

2. INTRODUCTION

Gaseous mediators are important signaling molecules that regulate a variety of physiological functions. There has recently been considerable interest in the expanding field of gaseous mediators or gasotransmitter molecules. They are implicated in many physiological and pathophysiological processes including neurological, cardiovascular and inflammatory events. The most well known molecule of this class is nitric oxide (NO); its discovery marked the beginning of a whole new paradigm for cellular signaling. Since then other gaseous mediators have been identified and are involved in a broad range of physiological functions. Their involvement in the cardiovascular system is well documented and there is good evidence that pathways involving gaseous mediators may play a role in the development of a range of cardiovascular diseases. The most recent additions to the family of gaseous mediators are the sulfur containing gases H_2S and SO_2 which have drawn considerable scientific interest. The focus of this review is to examine the evidence for

Table 1. H_2S and SO_2 satisfy the criteria for gasotransmitters

Gasotransmitter credentials	H_2S	SO_2
1. Exists as a gas	✓	✓
2. Membrane permeable	✓	✓
3. Endogenous enzymatic generation	✓	✓
4. Defined functions at physiological concentrations	✓	✓
5. Specific cellular effects mimicked by exogenous gas/donor	✓	✓

physiological and pathophysiological roles of these sulfur containing gases in endothelial and vascular smooth muscle cell function, vascular regulation and in the pathology of cardiovascular disease.

3. GASEOUS MEDIATORS AS SIGNALING MOLECULES

Gaseous mediators or gasotransmitters are a relatively new class of signaling molecules, although the discovery that gases are involved in neurotransmission dates back to 1980 (1). Examples include nitric oxide, carbon monoxide, hydrogen sulfide, and possibly sulfur dioxide, carbonyl sulfide (2), nitrous oxide (3) and carbon dioxide (4). These gases share many features in their production and action but differ from classical signaling molecules. The concept that a short-lived, endogenous gas could act as a signaling molecule was discovered by Furchgott, Ignarro and Murad and was rewarded with the Nobel Prize for Physiology or Medicine in 1998. The terminology and characterization criteria for gasotransmitters were first introduced in 2002 (5). For a molecule to be considered to be a gasotransmitter, all of the following criteria should be met:

1. It must exist as a gas.
2. It is freely permeable to membranes and does not rely on a specific transporter or on a membrane receptor.
3. It is endogenously and enzymatically generated by a regulated process.
4. It has well defined and specific functions at physiologically relevant concentrations.
5. Its cellular effects may be direct or be mediated by second messengers, but it has specific cellular and molecular targets. The effects of the endogenous gas can be mimicked by exogenous application.

Advantages of gases as signaling molecules include their small size which allows easy access to a variety of target sites that would not be accessible by larger molecules. They easily cross membranes, are labile with short half-lives and are made on demand. They are not stored in their native form as they can't be constrained by vesicles and need to be bound for storage or rely upon *de novo* synthesis. They can have endocrine, paracrine, autocrine or even intracrine effects. As an endocrine regulator, gasotransmitters can enter the blood stream, be transported to remote targets by carriers and released there to modulate functions of remote target cells. As a paracrine or autocrine mediator they do not need a carrier, but the effects are short-lived. There are a number of examples of gaseous mediators acting as intracrine regulators, modulating the activity of the cell that produced it. It is also interesting that all the molecules confirmed as gasotransmitters (NO, CO, H_2S) were all considered only as toxic molecules until their endogenous production and effects were determined.

4. HYDROGEN SULFIDE

Like NO, H_2S was first considered a toxic gas and notable for its distinctive "rotten egg" odour. It now qualifies as a gasotransmitter according to the above criteria (see Table 1), which will be expanded upon below. At moderate-high concentrations it is a mitochondrial poison, which inhibits cytochrome oxidase and prevents utilization of O_2 and causes uncoupling of oxidative phosphorylation (6). Additionally it can bind to haemoglobin and interfere with oxygen transport (6). However it is now reported to be involved in a wide variety of physiological processes including memory (7), nociception (8-9), insulin secretion (10-11), liver function (12), kidney function (13), gastrointestinal function (14) as well as playing a role in the cardiovascular system (15). Another intriguing role for H_2S is in eliciting metabolic inhibition and endowing a state of suspended animation (16-17). There are a number of anti-oxidant and anti-inflammatory effects associated with H_2S which are reported to endow it with cytoprotective (18) and immunomodulatory effects (19). The field of research into the endogenous effects if H_2S is growing rapidly and promises potential targets for future therapeutic exploitation.

H_2S is a weak acid with a pKa ~6.9. Its aqueous solubility is ~80mM at 37°C. At physiological pH the ratio of HS^-:H_2S is 3:1 and this would suggest a lower capacity for transport through membrane compared to other gases (e.g. NO), although it does permeate membranes without a transporter (20). It is not known whether HS^- and H_2S have differential effects and the combination of HS^- and H_2S are considered to be the effects of hydrogen sulfide by most authors as currently it is not possible to discriminate between the two species. In most studies either dissolved H_2S gas, or a solution of NaHS is used as an H_2S donor, although newer compounds with the capacity to release H_2S are being developed.

There are three documented enzymatic pathways for H_2S production (see Figure 1). The enzymes involved have multiple enzymatic functions, with the physiological importance of their H_2S-producing ability currently uncertain, although initially CO and NO could have been considered by-products of haem and arginine metabolism, respectively. H_2S is produced by the metabolism of amino acids such as cysteine and homocysteine via the transulfuration pathway, predominantly by the pyridoxal 5'-phosphate (vitamin B_6) dependent enzymes; cystathionine-β-synthase (CBS EC 4.2.1.22) and cystathionine-γ-lyase (CSE EC 4.4.1.1) (21). The third pathway is by the catabolism of cysteine via cysteine aminotransferase (CAT EC 2.6.1.3) and 3-mercaptopyruvate sulfurtranferase (3MST EC 2.8.1.2) (21-22). CBS is expressed mainly in the brain and liver and does not seem to account for cardiovascular H_2S production

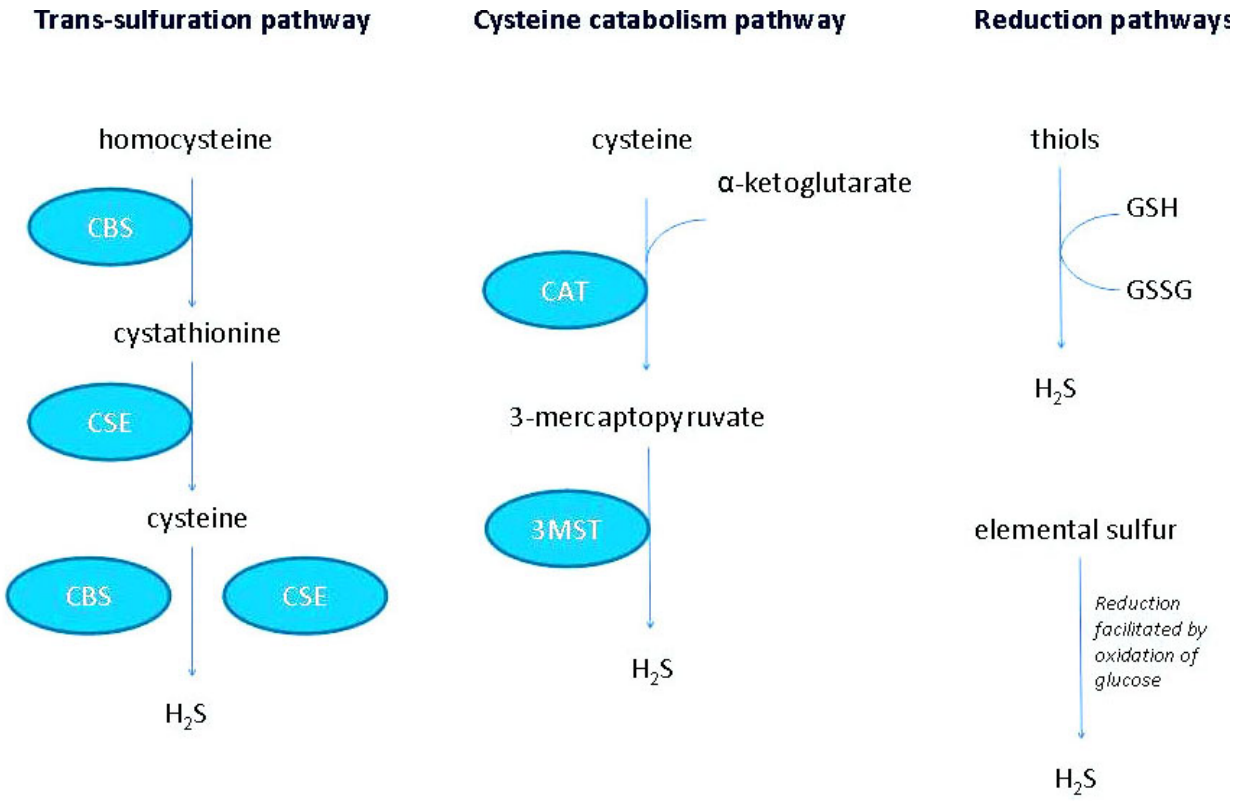

Figure 1. Pathways for endogenous production of H_2S. H_2S can be produced enzymatically via the trans-sulfuration pathway using homocysteine and cysteine as substrates (21). Cysteine can also be catabolised in mitochondria to H_2S (28-29). In addition, reduction of thiols and elemental sulfur may also be a source of endogenous H_2S. CSE: cystathionine-γ-lyase, CBS: cystathionine-β-synthase, CAT: cysteine amino transferase, 3MST: 3-mercaptopyruvate sulfurtransferase.

(23). CSE is found in large amounts in the liver, and also in the vasculature and in non-vascular smooth muscle (24). CSE is generally considered to be the cardiovascular source of H_2S although it has been localised in the brain (7, 24-26), but in much lesser quantities than CBS. 3MST is a mitochondrial enzyme and expressed in a range of tissues, including the myocardium (27), brain (28) and endothelium (29).

Other mechanisms for H_2S production include reduction of sulfate or elemental sulfur. Mammals lack the ability to reduce elemental sulfur, but this can occur via sulfur-reducing bacteria or by release of H_2S from thiols and sulfides. Notably, the mammalian gut has evolved specialised enzyme systems to degrade H_2S from bacterial sources to sulfates (30). H_2S can also be derived from inorganic sources, such as non-enzymatic reduction of elemental sulfur, achieved by utilising reducing equivalents from the oxidation of glucose (31). In addition, sulfur stores have been identified in cells, and these may release H_2S when pH varies. There are two forms of these stores, acid labile and bound sulfur (32). Acid labile sulfur is localised mainly in the mitochondria, and not considered to be a physiological source of H_2S since mitochondria are not normally acidic. Bound sulfur is localised to the cytoplasm and can release H_2S in reducing and alkaline conditions (32).

H_2S levels are tightly controlled *in vivo* and maintained at low levels through absorption and storage as bound sulfur or metabolism, thus the half-life of H_2S in plasma is short. The most important metabolic pathways for H_2S include scavenging by methaemoglobin to form sulfhaemoglobin, which may act as a sink for circulating H_2S. H_2S is scavenged by oxidised glutathione, oxidised in mitochondria or methylated in the cytosol via thiol-S-methyltransferase (30). Excretion is then via the kidney as free or conjugated sulfate (33). Endogenous circulating H_2S levels are reported anywhere from 3-300μM in the literature, however the actual tissue concentrations are probably much lower (sub-μM) as these μM order values are due to the measurement of both bound and the free sulfide together (34) and additionally without considering the balance of tissue production and metabolism (35). More recent estimates of the actual H_2S concentration in plasma suggest it is more likely to be in the nM range (35). This will be clarified as better assay techniques for H_2S become readily available.

4.1. Cardiovascular effects of H_2S

H_2S is a well documented vasorelaxant of both large and small blood vessels in animal and human models. Despite considerable effort the molecular mechanism of this effect remains unclear but seems to involve K^+ and

Ca^{2+} channels. H_2S has been reported to activate K_{ATP} channel currents (36), and more recently the molecular basis of this effect has been reported to be via sulfydration of either the K_{IR} subunit (37) or the SUR component (38) of the vascular K_{ATP} channel. These effects at the molecular level are interesting; however a closer examination of the data obtained from *in vitro* vasorelaxation studies show that the H_2S donor NaHS retains considerable vasorelaxant effect in the presence of K_{ATP} channel inhibition. For example in isolated rat aorta in the presence of 10μM glibenclamide (standard concentration used to inhibit K_{ATP} channels in vascular preparations) there remains a 40% vasorelaxation response to the H_2S donor NaHS (39) and in the isolated rat mesenteric bed 10μM glibenclamide elicited a 10-fold rightward shift and only a 20% suppression of the maximum vasodilatation elicited by H_2S-saturated solution (40). These data do not support the notion that H_2S elicits all its vasorelaxant effects via K_{ATP} channels. There is also a recent report of H_2S inhibiting another K^+ channel subtype, the BK_{Ca} channel in human cells (41) and K_V channels in rat coronary arteries (42). L-type Ca^{2+} channels are inhibited by H_2S in rat cardiomyocytes (43) and blocking vascular L-type Ca^{2+} channels with nifedipine inhibits NaHS-mediated vasorelaxation in isolated vessel experiments (44). A recent paper reports of the ability of H_2S to modulate intracellular Ca^{2+} concentrations in endothelial cells (45). Others have proposed metabolism-related mechanisms for H_2S-induced vasorelaxation, including a decrease in intracellular pH (via the Cl^-/HCO_3 exchanger) (46) or via metabolic inhibition (47). Currently it appears that there is heterogeneity in H_2S-induced vascular effects. An important advance in the field will be the actual determination of the molecular basis for vasorelaxation elicited by H_2S.

H_2S was first reported to have hypotensive effects when administration of H_2S donors *in vivo* to anaesthetised rats was found to induce a transient hypotensive effect (39). The CSE-L-cysteine pathway is downregulated in spontaneously hypertensive rats and treating them with an H_2S donor was protective, reducing blood pressure and vascular remodeling (48). The most compelling evidence for the importance of H_2S in blood pressure regulation is that mice deficient in CSE develop endothelial dysfunction and hypertension within 8 weeks of birth and that H_2S replacement decreases systolic blood pressure in both $CSE^{-/-}$ and $CSE^{+/-}$ mice (49). Further actions of H_2S are that it regulates plasma renin levels (50) and inhibits angiotensin converting enzyme activity in endothelial cells. The latter effect was caused by H_2S interaction with the Zn at the active centre of the enzyme and inhibiting its function, without affecting expression of the enzyme (51). This would enhance any hypotensive effect by reducing angiotensin II production and inhibiting bradykinin degradation and suggests a regulatory role for H_2S in the maintenance of a healthy blood pressure. Finally, H_2S regulates renal function in rats by increasing renal blood flow, glomerular filtration rate and urinary sodium and potassium secretion. Conversely, inhibiting CSE and CBS decreased all these parameters, and infusing L-cysteine had similar effects to NaHS (13), suggesting a role for endogenous H_2S in kidney function, and subsequently regulation of blood pressure.

An important consideration of H_2S biology is its relationship with fellow gaseous mediator NO. NO has been shown to increase both expression and activity of CSE in vascular smooth muscle cells (39, 52), whilst chronic nitric oxide synthase (NOS) inhibition has been shown to decrease CSE expression and activity (53). Conversely, H_2S treatment decreased NO formation, endothelial NOS (eNOS) activity and expression as well as L-arginine transport (54). Additionally, H_2S was found to directly inhibit eNOS as well as the other NOS isomers iNOS and nNOS (55). Finally, H_2S interacts directly with NO to form a novel nitrosothiol molecule *in vitro*, which was determined using a range of techniques including electron paramagnetic resonance, amperometry and measurements of nitrite concentrations (56). These studies raise the possibility that a nitrosothiol molecule may act as a store for both H_2S and NO.

H_2S is a strong reductant and reacts with many reactive oxygen and nitrogen species including peroxynitrite (57-58), superoxide (59-60), hypochlorite (61-62) hydrogen peroxide (59) and NO (56, 63), although there is chemical evidence that these anti-oxidant effects are limited by the presence of O_2 (64). In addition to scavenging reactive oxygen species H_2S has been shown to reduce accumulation of lipid peroxidation (59). H_2S downregulates NADPH oxidase expression and activity (65-66), thus reducing superoxide anion production. Additionally, H_2S can induce glutathione synthesis and cysteine uptake as well as reducing oxidised glutathione levels (67-70) thus suggesting a role for H_2S as an anti-oxidant and cytoprotective agent.

4.2. Role of H_2S in endothelial function

The endothelium is a critical regulator of vascular function. It contains the machinery to produce mediators and signaling molecules that regulate vascular function, perfusion of tissues and blood pressure including NO, prostaglandins (PGI_2) and endothelium-derived hyperpolarising factor (EDHF). The endothelium also contains the enzymes required to produce gaseous mediators including NO, CO and H_2S which are usually formed by enzymatic processes, complying with the criterion that production of gasotransmitters is regulated.

With respect to vasoregulation, the H_2S producing enzyme CSE is of particular interest as it is reported to be present in a range of vascular beds and its expression has been clearly identified in vascular smooth muscle cells, in both animal (39) and human (71) studies. It is of significant interest that CSE has recently been reported to be expressed in endothelial cells and contribute to endothelium-dependent vasorelaxation (49, 72). Indeed, L-cysteine elicits vasorelaxation in the rat mesenteric bed (40) and also in isolated mouse aorta where the response was inhibited by both the NOS inhibitor L-NAME and the soluble guanylate cyclase inhibitor, ODQ (73). Additionally we have found that in mouse aorta and mesenteric artery this response is concentration-dependent

and inhibited by endothelial removal as well as the CSE inhibitors D,L-propargylglycine (PPG) and β-cyanoalanine (Hart, unpublished observations). New evidence has now been provided to show that endothelial cells also contain the enzymatic machinery to produce H_2S via cysteine amino transferase and 3MST and although homogenates of endothelial cells can produce H_2S via this mechanism (29) this process has not been demonstrated in any *in vitro* assay. Other non-enzymatic pathways also exist, however the importance of these for endothelial H_2S production is yet to be determined.

4.3. Involvement of H_2S in cardiovascular disease

Disturbances in H_2S levels, CSE activity and expression have been reported in a number of cardiovascular diseases including ischaemia-reperfusion injury (59), chronic obstructive pulmonary disease (74), coronary heart disease (75), hypertension (48, 76), pulmonary hypertension (77), type-1 diabetes (78) and type-2 diabetes (79-80). Generally, lower levels of H_2S reflect severity of disease, suggesting that a dysfunction in H_2S production or changes in H_2S metabolism may be involved in cardiovascular pathology.

In both animal and cellular models of myocardial ischaemia-reperfusion injury there is considerable evidence that H_2S elicits cardioprotection (81-84). This area has been well reviewed recently (15). Accumulating evidence shows that H_2S preserves myocardial contractile function (85-87), limits infarct size (85-86, 88-90) and reduces arrhythmias (91). In addition, H_2S has also been shown to improve coronary microvascular function post myocardial ischemia-reperfusion (87) and inhibit platelet aggregation (92). The protective effects include reduced inflammation (93), increased glutathione production (94), inhibition of ischaemia-induced apoptosis (93, 95-97) preservation of mitochondrial function (85) and protection against Ca^{2+} overload (43, 98). In addition, exogenous and endogenous H_2S have been reported to function as pre-conditioning agents (83, 91, 99) and exogenous H_2S as a post-conditioning agent (100).

Vascular remodeling is an active process of structural alteration that involves changes in cell growth, cell death, cell migration, and production or degradation of extracellular matrix. Importantly, H_2S inhibits vascular smooth muscle cell proliferation both directly (101-102) and by modulating apoptosis, an effect which is dependent upon endogenous CSE activity and presumably endogenous H_2S production (103-104). In addition, CSE expression and activity were decreased after balloon injury in rat carotid arteries and treatment with NaHS significantly reduced neointima formation (105). Interestingly, H_2S can inhibit the increased synthesis of type-I collagen observed in spontaneously hypertensive rats. H_2S inhibited vascular smooth muscle cell proliferation *in vitro* and synthesis and secretion of collagen induced by angiotensin II (106). Additionally, H_2S can inhibit angiotensin converting enzyme (51) which would add to this effect. On the other hand, in cultured endothelial cells, NaHS stimulated cell growth and enhanced cell adhesion and cell migration via Akt phosphorylation (107), suggesting that H_2S may function to promote endothelial cell proliferation whilst inhibiting growth of vascular smooth muscle cells.

Atherosclerosis is a chronic blood vessel disease resulting in thickening of the arterial wall due to a build up of fatty deposits. The multi-factorial pathogenic process involves inflammation, endothelial cell damage, vascular smooth muscle cell migration and lipid deposition resulting in lesion formation. In accord with previous findings that H_2S plasma levels are lower in cardiovascular disease states, $ApoE^{-/-}$ mice have been shown to have lower plasma H_2S and aortic production of H_2S (108). The effects of H_2S in the pathogenesis of atherosclerosis have been recently reviewed (109). Hyperhomocysteineamia is a known risk factor for atherosclerosis (110) and mice deficient in CSE develop hyperhomocysteineamia (49), whilst H_2S inhibits homocysteine-induced vascular (111) and myocardial (60) damage. H_2S can inhibit atherogenic modification of low-density lipoprotein induced by hypochlorite which may be important in early atherogenesis (62). In addition, inhibition of NADPH oxidase activity may also be protective against atherosclerosis development (112) and there is good evidence that H_2S modulates the expression of NADPH oxidase (65, 113). In addition to this, H_2S inhibits plasma renin activity (50) and angiotensin converting enzyme (51) thereby decreasing angiotensin II production. Angiotensin II is a known activator of NADPH oxidase (114) thus H_2S may act as an inhibitor of atherogenesis through the angiotensin II-NADPH oxidase pathway. Calcification of atherosclerotic lesions is another important risk factor that affects the stability of the plaque and H_2S has been shown to interfere with the vascular calcification process (115). The mechanism of this effect of H_2S requires further investigation. Importantly, treating $ApoE^{-/-}$ mice with NaHS reduced the size of the atherosclerotic plaques (116). In addition, there are detrimental changes in adhesion molecule expression and activity in atherosclerosis, for example, in $ApoE^{-/-}$ mice there were increased plasma levels of the inter-cellular adhesion molecule ICAM-1 as well as increased ICAM-1 in aortic tissue. ICAMs facilitate the migration of inflammatory cells into the lesion. These effects were ameliorated by treatment with NaHS (116). Furthermore, H_2S has been reported to modulate inflammation mediated by leukocytes (117). H_2S inhibits platelet aggregation in human isolated platelets in a concentration-dependent manner. (92). Thus H_2S released from the endothelium could play a role in regulating platelet aggregation *in vivo* and thereby contribute to cardiovascular homeostasis and may decrease the likelihood of thrombus formation at a lesion site.

There is burgeoning evidence for a role of H_2S in the aetiology of diabetes. Plasma levels of H_2S are lower in animal models of type-1 diabetes (78-79). In addition, plasma H_2S levels were reduced in human patients with type-2 diabetes and in patients that were overweight (80). Importantly there is evidence that synthesis of H_2S in aorta declined as endothelial function decreased with the progression of diabetes in non-obese diabetic mice, a model of type-1 diabetes. This occurred due to a decrease in the activity of CSE, despite increases in both CSE and CBS

protein and mRNA production (73). Despite lower plasma levels, pancreatic production of H_2S was markedly elevated in streptozotocin-induced diabetes, as was CSE activity and mRNA expression but this effect was reversed with insulin treatment (78). Endogenous H_2S inhibits insulin release via a K_{ATP} channels (118-119) and H_2S induces beta-cell apoptosis (120) suggesting a role in diabetes pathogenesis. This is supported by further study which showed that pancreatic H_2S production was increased in Zucker diabetic fatty rats, and that treatment with the CSE inhibitor PPG increased serum insulin levels, lowered hyperglycaemia and glycated haemoglobin levels (10). Furthermore, H_2S inhibited both basal and stimulated glucose uptake in adipocytes, whilst CSE inhibitors enhanced glucose uptake into adipocytes (11), suggesting that H_2S may also act as an insulin resistance regulator.

4.4. Potential therapeutic role of H_2S

H_2S signaling pathways are implicated in the development and pathology of many cardiovascular diseases. There is now good evidence that plasma H_2S levels are inversely correlated with severity of cardiovascular disease, and that excessive H_2S production may be a pivotal point in the pathological process. There are various H_2S therapy options: H_2S gas, H_2S donors, activation or upregulation of endogenous H_2S generating enzymes. H_2S-donating non steroidal anti-inflammatory drugs have been assessed as novel anti-inflammatory agents and it would seem that the anti-oxidant and anti-inflammatory effects of H_2S may be useful in therapy for a range of conditions including cardiovascular disease (121). H_2S has been shown not only to be an effective pre-conditioning agent in myocardial infarction, but also that it has value as a post-conditioning agent (100), significantly enhancing its potential in the clinic. Finally, H_2S inhalation is under investigation for use in conjunction with therapeutic hypothermia. H_2S has been shown to protect mitochondrial integrity during the induced hypothermia used to provide organ protection following brain injury or circulatory arrest (122).

5. SULFUR DIOXIDE

Sulfur dioxide is a common atmospheric pollutant that is present in low concentrations in urban air, and at higher levels in industrial areas as it is generated by combustion of fossil fuels (123). A number of epidemiological studies have demonstrated that exposure to SO_2 can increase the risk of cardiovascular disease (124-128). When inhaled, SO_2 is hydrated to sulfurous acid which then dissociates to form the derivatives bisulfite and sulfite (1:3 M/M at neutral pH) (129). Bisulfite and sulfite are commonly consumed in food, beverages and drugs as sulfiting agents are widely used as preservatives. These SO_2 derivatives can then be distributed via the circulation. Biological or toxicological effects of SO_2 are thought to be elicited via these derivatives, which are toxic to many organs (130). Interestingly, there is now evidence that SO_2 also may qualify as a gasotransmitter (see Table 1), repeating the theme of a "toxic" gas now being seen as an important physiological regulator.

Endogenous SO_2 production has not been extensively studied. To date three main pathways for endogenous SO_2 production have been reported (see Figure 2); from normal metabolism of sulfur-containing amino acids like L-cysteine via an enzymatic pathway (131), via enzymatic reduction of thiosulfate (132) and from H_2S via NADPH oxidase in activated neutrophils (133). Endogenous bisulfite/sulfite is produced during metabolism of sulfur-containing amino acids or drugs (134). It is formed *in vivo* from the sulfur-containing amino acid L-cysteine (131). L-cysteine is oxidised to L-cysteine sulfinate by cysteine dioxygenase (CDO, EC 1.13.11.20) then L-cysteine sulfinate to β-sulfinylpyruvate by glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1) which decomposes to pyruvate and SO_2. (134-136). GOT is reported to be abundant in endothelial cells and additionally shown to be located in vascular smooth muscle cells adjacent to the endothelial layer (137).

SO_2 is reported to be endogenously produced in cardiovascular tissues in mammals and is present in plasma (138). Porcine coronary artery rings produce basal levels of SO_2, and interestingly the production of these gases is increased 4 fold by the presence of acetylcholine (1μM) and 1.5 fold by stimulation with the endothelium-dependent calcium ionophore, A23187 (1μM) (2). There is evidence that SO_2 enhances prostacyclin production as it increases 6-keto-$PGF_{1\alpha}$ levels and cAMP production and that vasorelaxation is reduced by the presence of the cyclo-oxygenase inhibitor indomethacin (139). Such data suggest that SO_2 may act as an endothelium-derived relaxing factor.

Production of SO_2 is reported in a range of cardiovascular tissues including heart, blood vessels and plasma (138) and specifically from rat pulmonary artery (140) and porcine coronary artery (2). Interestingly, SO_2 production was increased *in vitro* in porcine coronary artery by treatment with either acetylcholine or the calcium ionophore (A213187), suggesting a role for the endothelium in SO_2 production (2). It is less clear which of the three pathways (see Figure 2) are important in SO_2 generation in vascular tissue. GOT is reported to be abundant in endothelial cells and adjacent vascular smooth muscle cells (137), however the pathway still requires further clarification. The other two pathways (via NADPH oxidase and via thiosulfate reductase) require H_2S or thiosulfate as a substrate and their physiological role in endothelial function or vasorelaxation to SO_2 remain unknown.

Reports of the vasodilator effects of SO_2 are inconsistent. In rat aorta SO_2 is reported to cause vasorelaxation that is K_{ATP}-dependent (141), Ca^{2+} channel-dependent (141) or independent of the endothelium, but mediated via prostacyclin (139, 142). Others found that SO_2 induced a biphasic vasorelaxation response in rat aorta, the 1st phase being endothelium-dependent and the 2nd endothelium-independent and mediated via cGMP as well as being synergistic with NO (143) or dependent on Ca^{2+} and K^+ channels. (144-145). Further studies showed that blocking endogenous SO_2 production with L-aspartate-β-hydroxamate caused a contraction and a shift to the right in

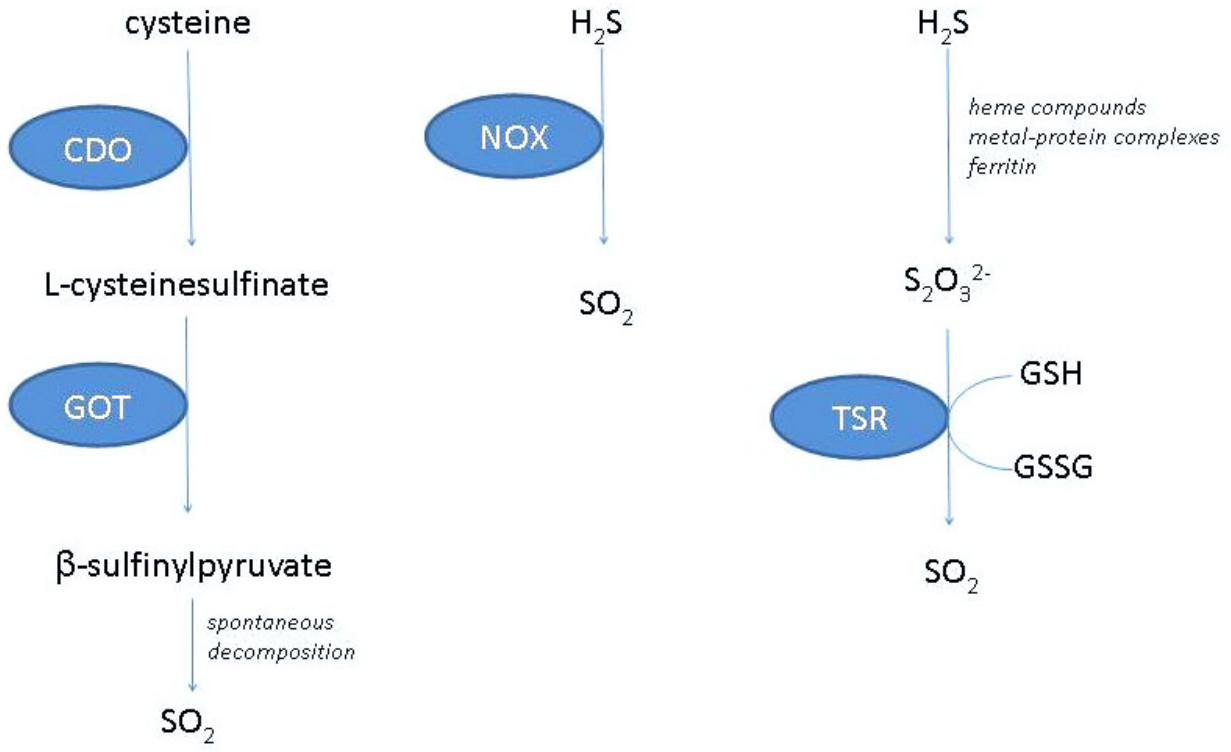

Figure 2. Pathways for endogenous production of SO_2**.** SO_2 can be produced via the oxidation of cysteine and cysteinesulfinate to β-sulfinylpyruvate, which decomposes to SO_2 (131). Additionally, H_2S is the substrate for SO_2 production via NADPH oxidase (133) and via reduction of thiosulphate (132). CDO: cysteine dioxygenase, GOT: glutamate oxaloacetate transaminase, NOX: NADPH oxidase, TSR: thiosulfate reductase.

the noradrenaline response (141), suggesting a role for SO_2 in the basal maintenance of blood vessel tone.

The role of SO_2 in the aetiology of cardiovascular disease as not been extensively studied. SO_2 causes a decrease in blood pressure in rats (146). Exogenous SO_2 is reported to have negative inotropic effects (147) and modulates cardiac Ca^{2+} (148-149), Na^+ channels (150) and K^+ channels (151). Infusion of SO_2 at reperfusion aggravates the effects of experimental ischemia-reperfusion injury in isolated rat hearts (152). Sulfite content and GOT activity were significantly higher in hearts subjected to ischaemia-reperfusion and correlated negatively with cardiac function. Conversely, inhibiting endogenous SO_2 with hydroxamate reduced the deficit in cardiac function and myocardial damage. The mechanism of this effect was associated with increased lipid peroxidation and this is supported by previous work which showed that SO_2 causes oxidative damage of a variety of tissues (153), however SO_2 did not alter formation of reactive oxygen species, suggesting that sulfur radicals may be involved (152). An additional observation was that SO_2 treatment resulted in decreased glutathione (152) which suggests SO_2 induced an increased utilisation or decreased production of this important anti-oxidant. Finally, SO_2 treatment inhibited pulmonary arterial smooth muscle cell proliferation induced by hypoxia (140) and SO_2 exposure has been shown to increase expression of apoptotic processes (154-156) which may have important implications for a variety of disease states. Thus there is preliminary evidence that SO_2 is a vasodilator, the mechanism of this action is yet to be confirmed. SO_2 appears to be detrimental in ischaemia-reperfusion injury and may have a role in apoptosis and inhibition of vascular cell proliferation. However there is much yet to be determined about the physiological and pathophysiological roles of SO_2 in the cardiovascular system.

6. SUMMARY AND PERSPECTIVE

The endothelium is an important source of gasotransmitters, producing NO, CO, H_2S and probably SO_2. These mediators are unique in their ability to traverse cellular membranes, but they also have distinct protein targets. There is no doubt that NO and CO are important endothelium-derived mediators. The role of the endothelium in H_2S production is still being established, however the evidence for involvement of this gas in the cardiovascular system now exists and the mechanisms involved in these effects are becoming clearer. In particular anti-inflammatory and antioxidant effects of H_2S may be useful and potentially exploited for therapeutic benefit in the future. Finally, data is accumulating in support of SO_2 as the next new gasotransmitter with important physiological and pathophysiological roles in the cardiovascular system.

7. REFERENCES

1. M. A. Gillman: Nitrous oxide as neurotransmitter. *Lancet*, 339(8788), 307 (1992)

2. M. Balazy, I. A. Abu-Yousef, D. N. Harpp and J. Park: Identification of carbonyl sulfide and sulfur dioxide in porcine coronary artery by gas chromatography/mass spectrometry, possible relevance to EDHF. *Biochem Biophys Res Commun*, 311(3), 728-34 (2003)

3. M. A. Gillman, L. Kok and F. J. Lichtigfeld: Paradoxical effect of naloxone on nitrous oxide analgesia in man. *Eur J Pharmacol*, 61(2), 175-7 (1980)

4. R. Acin-Perez, E. Salazar, M. Kamenetsky, J. Buck, L. R. Levin and G. Manfredi: Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. *Cell Metab*, 9(3), 265-76 (2009)

5. R. Wang: Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter? *FASEB J*, 16(13), 1792-8 (2002)

6. B. Locey: Hydrogen sulfide. In: *Encyclopaedia of Toxicology*. Elsevier Inc, (2005)

7. K. Abe and H. Kimura: The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci*, 16(3), 1066-71 (1996)

8. R. Patacchini, P. Santicioli, S. Giuliani and C. A. Maggi: Hydrogen sulfide (H2S) stimulates capsaicin-sensitive primary afferent neurons in the rat urinary bladder. *Br J Pharmacol*, 142(1), 31-4 (2004)

9. E. Distrutti, L. Sediari, A. Mencarelli, B. Renga, S. Orlandi, E. Antonelli, F. Roviezzo, A. Morelli, G. Cirino, J. L. Wallace and S. Fiorucci: Evidence that hydrogen sulfide exerts antinociceptive effects in the gastrointestinal tract by activating KATP channels. *J Pharmacol Exp Ther*, 316(1), 325-35 (2006)

10. L. Wu, W. Yang, X. Jia, G. Yang, D. Duridanova, K. Cao and R. Wang: Pancreatic islet overproduction of H_2S and suppressed insulin release in Zucker diabetic rats. *Lab Invest*, 89(1), 59-67 (2009)

11. X. Feng, Y. Chen, J. Zhao, C. Tang, Z. Jiang and B. Geng: Hydrogen sulfide from adipose tissue is a novel insulin resistance regulator. *Biochem Biophys Res Commun*, 380(1), 153-9 (2009)

12. S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah and A. Morelli: The third gas: H2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. *Hepatology*, 42(3), 539-48 (2005)

13. M. Xia, L. Chen, R. W. Muh, P. L. Li and N. Li: Production and actions of hydrogen sulfide, a novel gaseous bioactive substance, in the kidneys. *J Pharmacol Exp Ther*, 329(3), 1056-62 (2009)

14. B. Teague, S. Asiedu and P. K. Moore: The smooth muscle relaxant effect of hydrogen sulphide *in vitro*: evidence for a physiological role to control intestinal contractility. *Br J Pharmacol*, 137(2), 139-45 (2002)

15. D. J. Elsey, R. C. Fowkes and G. F. Baxter: Regulation of cardiovascular cell function by hydrogen sulfide (H_2S). *Cell Biochem Funct*, 28(2), 95-106 (2010)

16. E. Blackstone, M. Morrison and M. B. Roth: H2S induces a suspended animation-like state in mice. *Science*, 308(5721), 518 (2005)

17. M. B. Roth and T. Nystul: Buying time in suspended animation. *Sci Am*, 292(6), 48-55 (2005)

18. J. W. Calvert, W. A. Coetzee and D. J. Lefer: Novel insights into hydrogen sulfide--mediated cytoprotection. *Antioxid Redox Signal*, 12(10), 1203-17

19. H. Zhang and M. Bhatia: Hydrogen sulfide: a novel mediator of leukocyte activation. *Immunopharmacol Immunotoxicol*, 30(4), 631-45 (2008)

20. J. C. Mathai, A. Missner, P. Kugler, S. M. Saparov, M. L. Zeidel, J. K. Lee and P. Pohl: No facilitator required for membrane transport of hydrogen sulfide. *Proc Natl Acad Sci U S A*, 106(39), 16633-8 (2009)

21. O. Kabil and R. Banerjee: The redox biochemistry of hydrogen sulfide. *J Biol Chem* (2010)

22. S. P. Alexander, A. Mathie and J. A. Peters: Guide to Receptors and Channels (GRAC), 3rd edition. *Br J Pharmacol*, 153 Suppl 2, S1-209 (2008)

23. K. Eto, M. Ogasawara, K. Umemura, Y. Nagai and H. Kimura: Hydrogen sulfide is produced in response to neuronal excitation. *J Neurosci*, 22(9), 3386-91 (2002)

24. I. Ishii, N. Akahoshi, X. N. Yu, Y. Kobayashi, K. Namekata, G. Komaki and H. Kimura: Murine cystathionine gamma-lyase: complete cDNA and genomic sequences, promoter activity, tissue distribution and developmental expression. *Biochem J*, 381(Pt 1), 113-23 (2004)

25. L. Diwakar and V. Ravindranath: Inhibition of cystathionine-gamma-lyase leads to loss of glutathione and aggravation of mitochondrial dysfunction mediated by excitatory amino acid in the CNS. *Neurochem Int*, 50(2), 418-26 (2007)

26. V. Vitvitsky, M. Thomas, A. Ghorpade, H. E. Gendelman and R. Banerjee: A functional transsulfuration pathway in the brain links to glutathione homeostasis. *J Biol Chem*, 281(47), 35785-93 (2006)

27. O. W. Griffith: Mammalian sulfur amino acid metabolism: an overview. *Methods Enzymol*, 143, 366-76 (1987)

28. N. Shibuya, M. Tanaka, M. Yoshida, Y. Ogasawara, T. Togawa, K. Ishii and H. Kimura: 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal*, 11(4), 703-14 (2009)

29. N. Shibuya, Y. Mikami, Y. Kimura, N. Nagahara and H. Kimura: Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J Biochem*, 146(5), 623-6 (2009)

30. J. Furne, J. Springfield, T. Koenig, E. DeMaster and M. D. Levitt: Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized function of the colonic mucosa. *Biochem Pharmacol*, 62(2), 255-9 (2001)

31. D. Searcy and S. Lee: Sulfur reduction by human erythrocytes. *Journal of Experimental Zoology*, 282, 310-322 (1998)

32. M. Ishigami, K. Hiraki, K. Umemura, Y. Ogasawara, K. Ishii and H. Kimura: A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid Redox Signal*, 11(2), 205-14 (2009)

33. R. O. Beauchamp, Jr., J. S. Bus, J. A. Popp, C. J. Boreiko and D. A. Andjelkovich: A critical review of the literature on hydrogen sulfide toxicity. *Crit Rev Toxicol*, 13(1), 25-97 (1984)

34. K. R. Olson and J. A. Donald: Nervous control of circulation--the role of gasotransmitters, NO, CO, and H2S. *Acta Histochem*, 111(3), 244-56 (2009)

35. J. Furne, A. Saeed and M. D. Levitt: Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am J Physiol Regul Integr Comp Physiol*, 295(5), R1479-85 (2008)

36. G. Tang, L. Wu, W. Liang and R. Wang: Direct stimulation of K(ATP) channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. *Mol Pharmacol*, 68(6), 1757-64 (2005)

37. A. K. Mustafa, M. M. Gadalla, N. Sen, S. Kim, W. Mu, S. K. Gazi, R. K. Barrow, G. Yang, R. Wang and S. H. Snyder: H2S signals through protein S-sulfhydration. *Sci Signal*, 2(96), ra72 (2009)

38. B. Jiang, G. Tang, K. Cao, L. Wu and R. Wang: Molecular mechanism for H(2)S-induced activation of K(ATP) channels. *Antioxid Redox Signal*, 12(10), 1167-78 (2010)

39. W. Zhao, J. Zhang, Y. Lu and R. Wang: The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J*, 20(21), 6008-16 (2001)

40. Y. Cheng, J. Ndisang, G. Tang, K. Cao and R. Wang: Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. *Am J Physiol Heart Circ Physiol*, 287, 2316-2323 (2004)

41. V. Telezhkin, S. P. Brazier, S. Cayzac, C. T. Muller, D. Riccardi and P. J. Kemp: Hydrogen sulfide inhibits human BK(Ca) channels. *Adv Exp Med Biol*, 648, 65-72 (2009)

42. W. S. Cheang, W. T. Wong, B. Shen, C. W. Lau, X. Y. Tian, S. Y. Tsang, X. Yao, Z. Y. Chen and Y. Huang: 4-Aminopyridine-sensitive K^+ channels contributes to NaHS-induced membrane hyperpolarization and relaxation in the rat coronary artery. *Vascul Pharmacol* (2010)

43. Y. G. Sun, Y. X. Cao, W. W. Wang, S. F. Ma, T. Yao and Y. C. Zhu: Hydrogen sulphide is an inhibitor of L-type calcium channels and mechanical contraction in rat cardiomyocytes. *Cardiovasc Res*, 79(4), 632-41 (2008)

44. W. Zhao and R. Wang: H(2)S-induced vasorelaxation and underlying cellular and molecular mechanisms. *Am J Physiol Heart Circ Physiol*, 283(2), H474-80 (2002)

45. C. C. Bauer, J. P. Boyle, K. E. Porter and C. Peers: Modulation of Ca(2+) signaling in human vascular endothelial cells by hydrogen sulfide. *Atherosclerosis*, 209(2), 374-80 (2010)

46. S. W. Lee, Y. Cheng, P. K. Moore and J. S. Bian: Hydrogen sulphide regulates intracellular pH in vascular smooth muscle cells. *Biochem Biophys Res Commun*, 358(4), 1142-7 (2007)

47. L. Kiss, E. A. Deitch and C. Szabo: Hydrogen sulfide decreases adenosine triphosphate levels in aortic rings and leads to vasorelaxation via metabolic inhibition. *Life Sci*, 83(17-18), 589-94 (2008)

48. H. Yan, J. Du and C. Tang: The possible role of hydrogen sulfide on the pathogenesis of spontaneous hypertension in rats. *Biochem Biophys Res Commun*, 313(1), 22-7 (2004)

49. G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, Q. Meng, A. K. Mustafa, W. Mu, S. Zhang, S. H. Snyder and R. Wang: H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science*, 322(5901), 587-90 (2008)

50. M. Lu, Y. H. Liu, H. S. Goh, J. J. Wang, Q. C. Yong, R. Wang and J. S. Bian: Hydrogen sulfide inhibits plasma Renin activity. *J Am Soc Nephrol*, 21(6), 993-1002 (2010)

51. H. Laggner, M. Hermann, H. Esterbauer, M. K. Muellner, M. Exner, B. M. Gmeiner and S. Kapiotis: The novel gaseous vasorelaxant hydrogen sulfide inhibits angiotensin-converting enzyme activity of endothelial cells. *J Hypertens*, 25(10), 2100-4 (2007)

52. W. Zhao, J. Ndisang and R. Wang: Modulation of endogenous production of H_2S in rat tissues. *Can J Physiol Pharmacol*, 81(9), 848-853 (2003)

53. G. Zhong, F. Chen, Y. Cheng, C. Tang and J. Du: The role of hydrogen sulphide generation in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase *Journal of Hypertension* 21(10), 1879-85 (2003)

54. B. Geng, Y. Cui, J. Zhao, F. Yu, Y. Zhu, G. Xu, Z. Zhang, C. Tang and J. Du: Hydrogen sulfide downregulates the aortic L-arginine/nitric oxide pathway in rats. *Am J Physiol Regul Integr Comp Physiol*, 293(4), R1608-18 (2007)

55. S. Kubo, I. Doe, Y. Kurokawa, H. Nishikawa and A. Kawabata: Direct inhibition of endothelial nitric oxide synthase by hydrogen sulfide: contribution to dual modulation of vascular tension. *Toxicology*, 232(1-2), 138-46 (2007)

56. M. Whiteman, L. Li, I. Kostetski, S. H. Chu, J. L. Siau, M. Bhatia and P. K. Moore: Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. *Biochem Biophys Res Commun*, 343(1), 303-10 (2006)

57. M. Whiteman, J. Armstong, S. Chu, S. Jia-Ling, B. Wong, N. Cheung, B. Halliwell and P. Moore: The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger'? *J Neurochem*, 90, 765-768 (2004)

58. C. C. Dahm, K. Moore and M. P. Murphy: Persistent S-nitrosation of complex I and other mitochondrial membrane proteins by S-nitrosothiols but not nitric oxide or peroxynitrite: implications for the interaction of nitric oxide with mitochondria. *J Biol Chem*, 281(15), 10056-65 (2006)

59. B. Geng, L. Chang, C. Pan, Y. Qi, J. Zhao, Y. Pang, J. Du and C. Tang: Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. *Biochemical and Biophysical Research Communications*, 318, 756-763 (2004)

60. L. Chang, B. Geng, F. Yu, J. Zhao, H. Jiang, J. Du and C. Tang: Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats. *Amino Acids*, 34(4), 573-85 (2008)

61. M. Whiteman, P. Rose, J. L. Siau, N. S. Cheung, G. S. Tan, B. Halliwell and J. S. Armstrong: Hypochlorous acid-mediated mitochondrial dysfunction and apoptosis in human hepatoma HepG2 and human fetal liver cells: role of mitochondrial permeability transition. *Free Radic Biol Med*, 38(12), 1571-84 (2005)

62. H. Laggner, M. K. Muellner, S. Schreier, B. Sturm, M. Hermann, M. Exner, B. M. Gmeiner and S. Kapiotis: Hydrogen sulphide: a novel physiological inhibitor of LDL atherogenic modification by HOCl. *Free Radic Res*, 41(7), 741-7 (2007)

63. M. Y. Ali, C. Y. Ping, Y. Y. Mok, L. Ling, M. Whiteman, M. Bhatia and P. K. Moore: Regulation of vascular nitric oxide *in vitro* and *in vivo*; a new role for endogenous hydrogen sulphide? *Br J Pharmacol*, 149(6), 625-34 (2006)

64. A. Stasko, V. Brezova, M. Zalibera, S. Biskupic and K. Ondrias: Electron transfer: a primary step in the reactions of sodium hydrosulphide, an H_2S/HS(-) donor. *Free Radic Res*, 43(6), 581-93 (2009)

65. S. Muzaffar, N. Shukla, M. Bond, A. C. Newby, G. D. Angelini, A. Sparatore, P. Del Soldato and J. Y. Jeremy: Exogenous hydrogen sulfide inhibits superoxide formation, NOX-1 expression and Rac1 activity in human vascular smooth muscle cells. *J Vasc Res*, 45(6), 521-8 (2008)

66. A. K. Samhan-Arias, M. A. Garcia-Bereguiain and C. Gutierrez-Merino: Hydrogen sulfide is a reversible inhibitor of the NADH oxidase activity of synaptic plasma membranes. *Biochem Biophys Res Commun*, 388(4), 718-22 (2009)

67. H. Kimura: Hydrogen sulfide as a neuromodulator. *Mol Neurobiol*, 26(1), 13-9 (2002)

68. Y. Kimura and H. Kimura: Hydrogen sulfide protects neurons from oxidative stress. *FASEB J.* , 18(10), 1165-7 (2004)

69. Y. Kimura, R. Dargusch, D. Schubert and H. Kimura: Hydrogen sulfide protects HT22 neuronal cells from oxidative stress. *Antioxid Redox Signal*, 8(3-4), 661-70 (2006)

70. H. L. Wei, C. Y. Zhang, H. F. Jin, C. S. Tang and J. B. Du: Hydrogen sulfide regulates lung tissue-oxidized glutathione and total antioxidant capacity in hypoxic pulmonary hypertensive rats. *Acta Pharmacol Sin*, 29(6), 670-9 (2008)

71. G. D. Webb, L. H. Lim, V. M. Oh, S. B. Yeo, Y. P. Cheong, M. Y. Ali, R. El Oakley, C. N. Lee, P. S. Wong, M. G. Caleb, M. Salto-Tellez, M. Bhatia, E. S. Chan, E. A. Taylor and P. K. Moore: Contractile and vasorelaxant effects of hydrogen sulfide and its biosynthesis in the human internal mammary artery. *J Pharmacol Exp Ther*, 324(2), 876-82 (2008)

72. R. Wang: Hydrogen sulfide: a new EDRF. *Kidney Int*, 76(7), 700-4 (2009)

73. V. Brancaleone, F. Roviezzo, V. Vellecco, L. De Gruttola, M. Bucci and G. Cirino: Biosynthesis of H2S is impaired in non-obese diabetic (NOD) mice. *Br J Pharmacol*, 155(5), 673-80 (2008)

74. Y. Chen, W. Yao, B. Geng, Y. Ding, M. Lu, M. Zhao and C. Tang: Endogenous hydrogen sulfide in patients with COPD. *Chest*, 128(5), 3205-3211 (2005)

75. H. Jiang, H. Wu, Z. Li, B. Geng and C. Tang: Changes of the new gaseous transmitter H_2S in patients with coronary heart disease. *Di Yi Junyi Daxue Xuebao*, 25(8), 951-4 (2005)

76. L. Chen, S. Ingrid, Y. G. Ding, Y. Liu, J. G. Qi, C. S. Tang and J. B. Du: Imbalance of endogenous homocysteine and hydrogen sulfide metabolic pathway in essential hypertensive children. *Chin Med J (Engl)*, 120(5), 389-93 (2007)

77. W. Yanfei, S. Lin, D. Junbao and T. Chaoshu: Impact of L-arginine on hydrogen sulfide/cystathionine-gamma-lyase pathway in rats with high blood flow-induced pulmonary hypertension. *Biochem Biophys Res Commun*, 345(2), 851-7 (2006)

78. M. Yusuf, B. Huat, A. Hsu, M. Whiteman, M. Bhatia and P. Moore: Streptozotocin-induced diabetes in the rat is associated with enhanced tissue hydrogen sulfide biosynthesis. *Biochem Biophys Res Commun*, 333, 1146-1152 (2005)

79. S. K. Jain, R. Bull, J. L. Rains, P. F. Bass, S. N. Levine, S. Reddy, R. McVie and J. A. Bocchini: Low levels of hydrogen sulfide in the blood of diabetes patients and streptozotocin-treated rats causes vascular inflammation? *Antioxid Redox Signal*, 12(11), 1333-7 (2010)

80. M. Whiteman, K. M. Gooding, J. L. Whatmore, C. I. Ball, D. Mawson, K. Skinner, J. E. Tooke and A. C. Shore: Adiposity is a major determinant of plasma levels of the novel vasodilator hydrogen sulphide. *Diabetologia*

81. J. W. Calvert, S. Jha, S. Gundewar, J. W. Elrod, A. Ramachandran, C. B. Pattillo, C. G. Kevil and D. J. Lefer: Hydrogen sulfide mediates cardioprotection through Nrf2 signaling. *Circ Res*, 105(4), 365-74 (2009)

82. Z. Zhang, H. Huang, P. Liu, C. Tang and J. Wang: Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening K ATP channels. *Can J Physiol Pharmacol*, 85(12), 1248-53 (2007)

83. M. Bliksoen, M. L. Kaljusto, J. Vaage and K. O. Stenslokken: Effects of hydrogen sulphide on ischaemia-reperfusion injury and ischaemic preconditioning in the isolated, perfused rat heart. *Eur J Cardiothorac Surg*, 34(2), 344-9 (2008)

84. X. Y. Zhu, X. H. Yan and S. J. Chen: H_2S protects myocardium against ischemia/reperfusion injury and its effect on c-Fos protein expression in rats. *Sheng Li Xue Bao*, 60(2), 221-7 (2008)

85. J. W. Elrod, J. W. Calvert, J. Morrison, J. E. Doeller, D. W. Kraus, L. Tao, X. Jiao, R. Scalia, L. Kiss, C. Szabo, H. Kimura, C. W. Chow and D. J. Lefer: Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci U S A*, 104(39), 15560-5 (2007)

86. Y. Hu, X. Chen, T. T. Pan, K. L. Neo, S. W. Lee, E. S. Khin, P. K. Moore and J. S. Bian: Cardioprotection induced by hydrogen sulfide preconditioning involves activation of ERK and PI3K/Akt pathways. *Pflugers Arch*, 455(4), 607-16 (2008)

87. N. R. Sodha, R. T. Clements, J. Feng, Y. Liu, C. Bianchi, E. M. Horvath, C. Szabo, G. L. Stahl and F. W. Sellke: Hydrogen sulfide therapy attenuates the inflammatory response in a porcine model of myocardial ischemia/reperfusion injury. *J Thorac Cardiovasc Surg*, 138(4), 977-84 (2009)

88. A. Sivarajah, M. C. McDonald and C. Thiemermann: The production of hydrogen sulphide limits myocardial ischemia and reperfusion injury and contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia in rats. *Shock*, 26, 154-161 (2006)

89. D. Johansen, K. Ytrehus and G. Baxter: Exogenous hydrogen sulfide (H_2S) protects against regional myocardial ischemia-reperfusion injury - Evidence for a role for K_{ATP} channels. *Basic Res Cardiol*, 101(1), 53-60 (2006)

90. D. J. Elsey, R. C. Fowkes and G. F. Baxter: L-cysteine stimulates hydrogen sulfide synthesis in myocardium associated with attenuation of ischemia-reperfusion injury. *J Cardiovasc Pharmacol Ther*, 15(1), 53-9

91. J. S. Bian, Q. C. Yong, T. T. Pan, Z. N. Feng, M. Y. Ali, S. Zhou and P. K. Moore: Role of hydrogen sulfide in the cardioprotection caused by ischemic preconditioning in the rat heart and cardiac myocytes. *J Pharmacol Exp Ther*, 316(2), 670-8 (2006)

92. G. Zagli, R. Patacchini, M. Trevisani, R. Abbate, S. Cinotti, G. F. Gensini, G. Masotti and P. Geppetti: Hydrogen sulfide inhibits human platelet aggregation. *Eur J Pharmacol*, 559(1), 65-8 (2007)

93. A. Sivarajah, M. Collino, M. Yasin, E. Benetti, M. Gallicchio, E. Mazzon, S. Cuzzocrea, R. Fantozzi and C. Thiemermann: Anti-apoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial I/R. *Shock*, 31(3), 267-74 (2009)

94. G. Rossoni, A. Sparatore, V. Tazzari, B. Manfredi, P. Del Soldato and F. Berti: The hydrogen sulphide-releasing derivative of diclofenac protects against ischaemia-reperfusion injury in the isolated rabbit heart. *Brit J Pharmacol*, 153, 100-109 (2008)

95. L. L. Yao, X. W. Huang, Y. G. Wang, Y. X. Cao, C. C. Zhang and Y. C. Zhu: Hydrogen sulfide protects cardiomyocytes from hypoxia/reoxygenation-induced apoptosis by preventing GSK-3beta-dependent opening of mPTP. *Am J Physiol Heart Circ Physiol*, 298(5), H1310-9 (2010)

96. S. Shi, Q. S. Li, H. Li, L. Zhang, M. Xu, J. L. Cheng, C. H. Peng, C. Q. Xu and Y. Tian: Anti-apoptotic action of hydrogen sulfide is associated with early JNK inhibition. *Cell Biol Int*, 33(10), 1095-101 (2009)

97. N. R. Sodha, R. T. Clements, J. Feng, Y. Liu, C. Bianchi, E. M. Horvath, C. Szabo and F. W. Sellke: The effects of therapeutic sulfide on myocardial apoptosis in

response to ischemia-reperfusion injury. *Eur J Cardiothorac Surg*, 33(5), 906-13 (2008)

98. T. Pan, K. Neo, L. Hu, Q. Yong and J. Bian: H_2S preconditioning-induced PKC activation regulates intracellular calcium handling in rat cardiomyocytes. *American Journal of Physiology - Cell Physiology*, 294(1), C169-77 (2008)

99. Q. C. Yong, S. W. Lee, C. S. Foo, K. L. Neo, X. Chen and J. S. Bian: Endogenous hydrogen sulphide mediates the cardioprotection induced by ischemic postconditioning. *Am J Physiol Heart Circ Physiol*, 295(3), H1330-H1340 (2008)

100. Y. Ji, Q. F. Pang, G. Xu, L. Wang, J. K. Wang and Y. M. Zeng: Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. *Eur J Pharmacol*, 587(1-3), 1-7 (2008)

101. J. Du, Y. Hui, Y. Cheung, G. Bin, H. Jiang, X. Chen and C. Tang: The possible role of hydrogen sulfide as a smooth muscle cell proliferation inhibitor in rat cultured cells. *Heart Vessels*, 19(2), 75-80 (2004)

102. R. Baskar, A. Sparatore, P. Del Soldato and P. K. Moore: Effect of S-diclofenac, a novel hydrogen sulfide releasing derivative inhibit rat vascular smooth muscle cell proliferation. *Eur J Pharmacol*, 594(1-3), 1-8 (2008)

103. G. Yang, K. Cao, L. Wu and R. Wang: Cystathionine gamma-lyase overexpression inhibits cell proliferation via a H2S-dependent modulation of ERK1/2 phosphorylation and p21Cip/WAK-1. *J Biol Chem*, 279(47), 49199-205 (2004)

104. G. Yang, L. Wu and R. Wang: Pro-apoptotic effect of endogenous H2S on human aorta smooth muscle cells. *FASEB J*, 20(3), 553-5 (2006)

105. Q. H. Meng, G. Yang, W. Yang, B. Jiang, L. Wu and R. Wang: Protective effect of hydrogen sulfide on balloon injury-induced neointima hyperplasia in rat carotid arteries. *Am J Pathol*, 170(4), 1406-14 (2007)

106. X. Zhao, L. K. Zhang, C. Y. Zhang, X. J. Zeng, H. Yan, H. F. Jin, C. S. Tang and J. B. Du: Regulatory effect of hydrogen sulfide on vascular collagen content in spontaneously hypertensive rats. *Hypertens Res*, 31(8), 1619-30 (2008)

107. W. J. Cai, M. J. Wang, P. K. Moore, H. M. Jin, T. Yao and Y. C. Zhu: The novel proangiogenic effect of hydrogen sulfide is dependent on Akt phosphorylation. *Cardiovasc Res*, 76(1), 29-40 (2007)

108. R. Wang: Is H2S a stinky remedy for atherosclerosis? *Arterioscler Thromb Vasc Biol*, 29(2), 156-7 (2009)

109. W. Qiao, T. Chaoshu, J. Hongfang and D. Junbao: Endogenous hydrogen sulfide is involved in the pathogenesis of atherosclerosis. *Biochem Biophys Res Commun*, 396(2), 182-186 (2010)

110. R. Clarke: Homocysteine and cardiovascular disease. Overview. *J Cardiovasc Risk*, 5(4), 213-5 (1998)

111. S. K. Yan, T. Chang, H. Wang, L. Wu, R. Wang and Q. H. Meng: Effects of hydrogen sulfide on homocysteine-induced oxidative stress in vascular smooth muscle cells. *Biochem Biophys Res Commun*, 351(2), 485-91 (2006)

112. C. P. Judkins, H. Diep, B. R. Broughton, A. E. Mast, E. U. Hooker, A. A. Miller, S. Selemidis, G. J. Dusting, C. G. Sobey and G. R. Drummond: Direct evidence of a role for Nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in ApoE-/- mice. *Am J Physiol Heart Circ Physiol*, 298(1), H24-32

113. S. Muzaffar, J. Y. Jeremy, A. Sparatore, P. Del Soldato, G. D. Angelini and N. Shukla: H2S-donating sildenafil (ACS6) inhibits superoxide formation and gp91phox expression in arterial endothelial cells: role of protein kinases A and G. *Br J Pharmacol*, 155(7), 984-94 (2008)

114. S. Selemidis, C. G. Sobey, K. Wingler, H. H. Schmidt and G. R. Drummond: NADPH oxidases in the vasculature: molecular features, roles in disease and pharmacological inhibition. *Pharmacol Ther*, 120(3), 254-91 (2008)

115. S. Y. Wu, C. S. Pan, B. Geng, J. Zhao, F. Yu, Y. Z. Pang, C. S. Tang and Y. F. Qi: Hydrogen sulfide ameliorates vascular calcification induced by vitamin D3 plus nicotine in rats. *Acta Pharmacol Sin*, 27(3), 299-306 (2006)

116. Y. Wang, X. Zhao, H. Jin, H. Wei, W. Li, D. Bu, X. Tang, Y. Ren, C. Tang and J. Du: Role of hydrogen sulfide in the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Arterioscler Thromb Vasc Biol*, 29(2), 173-9 (2009)

117. R. C. Zanardo, V. Brancaleone, E. Distrutti, S. Fiorucci, G. Cirino and J. L. Wallace: Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEB J*, 20(12), 2118-20 (2006)

118. W. Yang, G. Yang, J. X, L. Wu and R. Wang: Activation of K_{ATP} channels by H_2S in rat insulin-secreting cells and the underlying mechanisms. *J Physiol*, 569(2), 519-531 (2005)

119. M. Y. Ali, M. Whiteman, C. M. Low and P. K. Moore: Hydrogen sulphide reduces insulin secretion from HIT-T15 cells by a K_{ATP} channel-dependent pathway. *J Endocrinol*, 195(1), 105-12 (2007)

120. G. D. Yang and R. Wang: H_2S and cellular proliferation and apoptosis. *Sheng Li Xue Bao*, 59(2), 133-40 (2007)

121. S. Fiorucci, L. Santucci and E. Distrutti: NSAIDs, coxibs, CINOD and H_2S-releasing NSAIDs: what lies beyond the horizon. *Dig Liver Dis*, 39(12), 1043-51 (2007)

122. K. Baumgart, F. Wagner, M. Groger, S. Weber, E. Barth, J. A. Vogt, U. Wachter, M. Huber-Lang, M. W. Knoferl, G. Albuszies, M. Georgieff, P. Asfar, C. Szabo, E. Calzia, P. Radermacher and V. Simkova: Cardiac and metabolic effects of hypothermia and inhaled hydrogen sulfide in anesthetized and ventilated mice. *Crit Care Med*, 38(2), 588-95 (2010)

123. M. O. Amdur: Toxicologic appraisal of particulate matter, oxides of sulfur, and sulfuric acid. *J Air Pollut Control Assoc*, 19(9), 638-44 (1969)

124. V. Lenters, C. S. Uiterwaal, R. Beelen, M. L. Bots, P. Fischer, B. Brunekreef and G. Hoek: Long-Term Exposure to Air Pollution and Vascular Damage in Young Adults. *Epidemiology* (2010)

125. H. Kan, C. M. Wong, N. Vichit-Vadakan and Z. Qian: Short-term association between sulfur dioxide and daily mortality: the Public Health and Air Pollution in Asia (PAPA) study. *Environ Res*, 110(3), 258-64

126. K. Bhaskaran, S. Hajat, A. Haines, E. Herrett, P. Wilkinson and L. Smeeth: Effects of air pollution on the incidence of myocardial infarction. *Heart*, 95(21), 1746-59 (2009)

127. M. Briet, C. Collin, S. Laurent, A. Tan, M. Azizi, M. Agharazii, X. Jeunemaitre, F. Alhenc-Gelas and P. Boutouyrie: Endothelial function and chronic exposure to air pollution in normal male subjects. *Hypertension*, 50(5), 970-6 (2007)

128. A. Bener, M. Dogan and N. J. Shanks: The impact of air pollution on hospital admission for respiratory and cardiovascular diseases in an oil-rich country. *East Afr J Public Health*, 6(2), 124-7 (2009)

129. A. F. Gunnison and A. W. Benton: Sulfur dioxide: Sulfite. Interaction with mammalian serum and plasma. *Arch Environ Health*, 22(3), 381-8 (1971)

130. J. Xie, R. Fan and Z. Meng: Protein oxidation and DNA-protein crosslink induced by sulfur dioxide in lungs, livers, and hearts from mice. *Inhal Toxicol*, 19(9), 759-65 (2007)

131. M. H. Stipanuk, J. E. Dominy, Jr., J. I. Lee and R. M. Coloso: Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. *J Nutr*, 136(6 Suppl), 1652S-1659S (2006)

132. R. Shapiro: Genetic effects of bisulfite (sulfur dioxide). *Mutat Res*, 39(2), 149-75 (1977)

133. H. Mitsuhashi, S. Yamashita, H. Ikeuchi, T. Kuroiwa, Y. Kaneko, K. Hiromura, K. Ueki and Y. Nojima: Oxidative stress-dependent conversion of hydrogen sulfide to sulfite by activated neutrophils. *Shock*, 24(6), 529-34 (2005)

134. T. Ubuka, S. Yuasa, J. Ohta, N. Masuoka, K. Yao and M. Kinuta: Formation of sulfate from L-cysteine in rat liver mitochondria. *Acta Med Okayama*, 44(2), 55-64 (1990)

135. O. W. Griffith: Cysteinesulfinate metabolism. altered partitioning between transamination and decarboxylation following administration of beta-methyleneaspartate. *J Biol Chem*, 258(3), 1591-8 (1983)

136. M. H. Stipanuk and J. E. Dominy, Jr.: Surprising insights that aren't so surprising in the modeling of sulfur amino acid metabolism. *Amino Acids*, 30(3), 251-6 (2006)

137. S. X. Du, H. F. Jin, D. F. Bu, X. Zhao, B. Geng, C. S. Tang and J. B. Du: Endogenously generated sulfur dioxide and its vasorelaxant effect in rats. *Acta Pharmacol Sin*, 29(8), 923-30 (2008)

138. H. F. Jin, S. X. Du, X. Zhao, S. Q. Zhang, Y. Tian, D. F. Bu, C. S. Tang and J. B. Du: Significance of endogenous sulfur dioxide in the regulation of cardiovascular system. *Beijing Da Xue Xue Bao*, 39(4), 423-5 (2007)

139. Z. Meng and H. Zhang: The vasodilator effect and its mechanism of sulfur dioxide-derivatives on isolated aortic rings of rats. *Inhal Toxicol*, 19(11), 979-86 (2007)

140. Y. Sun, Y. Tian, M. Prabha, D. Liu, S. Chen, R. Zhang, X. Liu, C. Tang, X. Tang, H. Jin and J. Du: Effects of sulfur dioxide on hypoxic pulmonary vascular structural remodeling. *Lab Invest*, 90(1), 68-82 (2010)

141. S. X. Du, J. B. Du and C. S. Tang: Advances in study of physiologic effects of endogenous sulfur dioxide and its derivatives. *Beijing Da Xue Xue Bao*, 38(3), 331-4 (2006)

142. Z. Meng, Y. Li and J. Li: Vasodilatation of sulfur dioxide derivatives and signal transduction. *Arch Biochem Biophys*, 467(2), 291-6 (2007)

143. J. Li and Z. Meng: The role of sulfur dioxide as an endogenous gaseous vasoactive factor in synergy with nitric oxide. *Nitric Oxide*, 20(3), 166-74 (2009)

144. Q. Zhang and Z. Meng: The vasodilator mechanism of sulfur dioxide on isolated aortic rings of rats: Involvement of the K^+ and Ca^{2+} channels. *Eur J Pharmacol*, 602(1), 117-23 (2009)

145. Y. K. Wang, A. J. Ren, X. Q. Yang, L. G. Wang, W. F. Rong, C. S. Tang, W. J. Yuan and L. Lin: Sulfur dioxide relaxes rat aorta by endothelium-dependent and -independent mechanisms. *Physiol Res*, 58(4), 521-7 (2009)

146. Z. Meng, H. Geng, J. Bai and G. Yan: Blood pressure of rats lowered by sulfur dioxide and its derivatives. *Inhal Toxicol*, 15(9), 951-9 (2003)

147. S. Q. Zhang, J. B. Du, Y. Tian, B. Geng, C. S. Tang and X. Y. Tang: [Effects of sulfur dioxide on cardiac function of isolated perfusion heart of rat]. *Zhonghua Yi Xue Za Zhi*, 88(12), 830-4 (2008)

148. A. Nie and Z. Meng: Modulation of L-type calcium current in rat cardiac myocytes by sulfur dioxide derivatives. *Food Chem Toxicol*, 44(3), 355-63 (2006)

149. A. Nie and Z. Meng: Sulfur dioxide derivatives modulate Na/Ca exchange currents and cytosolic [Ca2+]i in rat myocytes. *Biochem Biophys Res Commun*, 358(3), 879-84 (2007)

150. A. Nie and Z. Meng: Study of the interaction of sulfur dioxide derivative with cardiac sodium channel. *Biochim Biophys Acta*, 1718(1-2), 67-73 (2005)

151. A. Nie and Z. Meng: Sulfur dioxide derivative modulation of potassium channels in rat ventricular myocytes. *Arch Biochem Biophys*, 442(2), 187-95 (2005)

152. S. Zhang, J. Du, H. Jin, W. Li, Y. Liang, B. Geng, S. Li, C. Zhang and C. Tang: Endogenous sulfur dioxide aggravates myocardial injury in isolated rat heart with ischemia and reperfusion. *Transplantation*, 87(4), 517-24 (2009)

153. Z. Meng: Oxidative damage of sulfur dioxide on various organs of mice: sulfur dioxide is a systemic oxidative damage agent. *Inhal Toxicol*, 15(2), 181-95 (2003)

154. J. Bai and Z. Meng: Expression of caspase and apoptotic signal pathway induced by sulfur dioxide. *Environ Mol Mutagen*, 51(2), 112-22

155. J. Bai and Z. Meng: Effects of sulfur dioxide on apoptosis-related gene expressions in lungs from rats. *Regul Toxicol Pharmacol*, 43(3), 272-9 (2005)

156. J. Bai and Z. Meng: Expression of apoptosis-related genes in livers from rats exposed to sulfur dioxide. *Toxicology*, 216(2-3), 253-60 (2005)

Abbreviations: CBS: cystathione-β-synthase, CSE: cystathionine-γ-lyase, CAT: cysteine aminotransferase, 3MST: 3-mecaptosulfertransferase, SUR: sulphonylurea receptor, ICAM : intra-cellular adhesion molecule, CDO: cysteine dioxygenase, GOT: glutamate oxaloacetate transaminase

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