Review

Mitochondrial mechanisms by which gasotransmitters (H_2S, NO and CO) protect cardiovascular system against hypoxia

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1. Abstract

Over past few years, there has been a dramatic increase in studying physiological mechanisms of the activity of various signaling low-molecular molecules that directly or indirectly initiate adaptive changes in the cardiovascular system cells (CVSC) to hypoxia. These molecules include biologically active endogenous gases or gasotransmitters (H_2S, NO and CO) that influence on many cellular processes, including mitochondrial biogenesis, oxidative phosphorylation, K^+/Ca^{2+} exchange, contractility of cardiomyocytes (CM) and vascular smooth muscle cells (VSMC) under conditions of oxygen deficiency. The present review focuses on the mechanistic role of the gasotransmitters (NO, H_2S, CO) in cardioprotection. The structural components of these mechanisms involve mitochondrial enzyme complexes and redox signal proteins, K^+ and Ca^{2+} channels, and mitochondrial permeability transition pore (MPTP) that have been considered as the final molecular targets of mechanisms underlying antioxidant and mild mitochondrial uncoupling effects, preconditioning, vasodilatation and adaptation to hypoxia. In this article, we have reviewed recent findings on the gasotransmitters and proposed a unifying model of mitochondrial mechanisms of cardioprotection.

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2. Introduction

Historically mitochondria have been considered as molecular “power stations” that produce and store energy in the form of the high-energy bonds of ATP. This energy is used by cells to sustain their functions, including signaling and adaptation to the effects of negative environmental factors. In the cardiovascular system (CVS), most ATP molecules are generated by oxidative phosphorylation that occurs in mitochondria and supports energy-consuming and long-running biochemical processes underlying myocardial automatism and muscle contraction. About two thirds of the energy consumption by smooth muscle and endothelial cells of blood vessels are covered by anaerobic glycolysis, which makes these cells less vulnerable to oxygen deficiency [1]. Nevertheless, mitochondria both of cardiomyocytes (CM) and blood vessel cells (BVC) remain highly sensitive to the impact of the negative environmental factors and undergo metabolic adaptation in response to changes in environmental conditions. When this adaptation is impaired, a progressive decline in the mitochondrial function contributes to the development of CVS diseases [2]. On the other hand, mitochondria house proteins of signal transduction pathways that regulate activity and adaptation of the CVS cells during hypoxia and development of hypoxia-related chronic diseases.

Over past few years, there has been a dramatic increase in studying physiological mechanisms of the activity of various signaling molecules or messengers that directly or indirectly initiate adaptive changes in the CVS cells to hypoxia and ischemia/reperfusion. These messengers include biologically active endogenous gas molecules (NO, H_2S, CO) that affect many cellular processes, including contractility of cardiomyocytes and vascular smooth muscle cells (VSMCs) under conditions of oxygen deficiency during ischemia and reperfusion [3].

The gas messengers are able to modulate mitochondrial function by targeting mainly the ATP-dependent mitochondrial transport, electron transport chain (ETC), and functions of ATP synthase, mitoK$_{ATP}$, and BK$_{Ca}$ channels since these mitochondrial structures are terminal molecular targets for these gases during hypoxic preconditioning and long-term adaptation (Fig. 1). Many aspects of the protective effect of the gas molecules still remain poorly understood.

An increased sensitivity to the opening of MPTP has been known to result in mitochondrial dysfunction and apoptosis in CVS diseases. Hence, there is an urgent need for clarification of the gasotransmitters role in the mitochondrial mechanisms related to the regulation and formation of MPTP.

Understanding the role of gas transmitters in regulation of the mitochondrial functions and cell signaling that initiate protective mechanisms of the CVS cells may contribute to the development of new antihypoxic drugs aimed at preventing and treating a broad range of pathologies, including ischemic cardiomyopathy and cardiac ischemia-reperfusion injury.

3. Energy-producing function of mitochondria and H_2S

The main function of mitochondria is to generate energy in the form of ATP. The ATP is produced by substrate phosphorylation (glycolysis) and mitochondrial aerobic respiration (oxidative phosphorylation). The oxidative phosphorylation occurs in the inner mitochondrial membrane (IMM) and uses the electrochemical proton gradient to generate ATP. The CVS cells rely on both glycolysis and oxidative phosphorylation to sustain their function. Herewith, the uniqueness of the mitochondria as energy-producing organelles precisely defines the second pathway of the ATP generation. In fact, this is a sequential transformation of the chemical energy of the reducing NADH equivalents into the electrochemical proton gradient across the IMM that activates the membrane-bound ATP synthase and results in the formation of the high-energy bonds of ATP [1].

In light of contemporary insights about energy producing mechanisms in cellular systems, the whole energy production process in mitochondria can be divided into four main stages. The first two, conversion of substrates to acetyl-CoA and its’ oxidation to NADH in the Krebs cycle, occur in the mitochondrial matrix. The last two, electron transfer from NADH to oxygen through the respiratory chain and formation of ATP by ATP synthase complex, occur in the internal membranes of mitochondrial cristae [1]. The electron transfers and ATP synthase activities are membrane potential-dependent processes. Therefore, maintaining a stable mitochondrial membrane potential (MMP) is one of the vital conditions to support healthy mitochondrial function and oxidative phosphorylation. A decrease or, opposite, excessive increase in the MMP that happen during the development of CVS pathology serve as a pharmacological target for treatment of a various CVS diseases associated with the mitochondrial dysfunction and circulatory hypoxia.

Because of the high importance of generation and maintenance of the MMP, the CVS cells (CM and VSMCs) developed special mechanisms intended to support a proper ETC functioning in hypoxic conditions. One of these mechanisms include a rearrangement of the substrate region of the respiratory chain by prioritizing FAD-dependent over NAD-dependent substrates and transferring electrons to the ETC complexes II–IV bypassing complex I. Activation of the alternative metabolic pathways allows maintaining the electron flow to the cytochrome *c* region without disrupting the electron transport function of complexes III, IV, and V. This process is called succinate-oxidase pathway, which is more effective energetically in hypoxic conditions. Thereby, a decrease in the rate of oxidative transformations

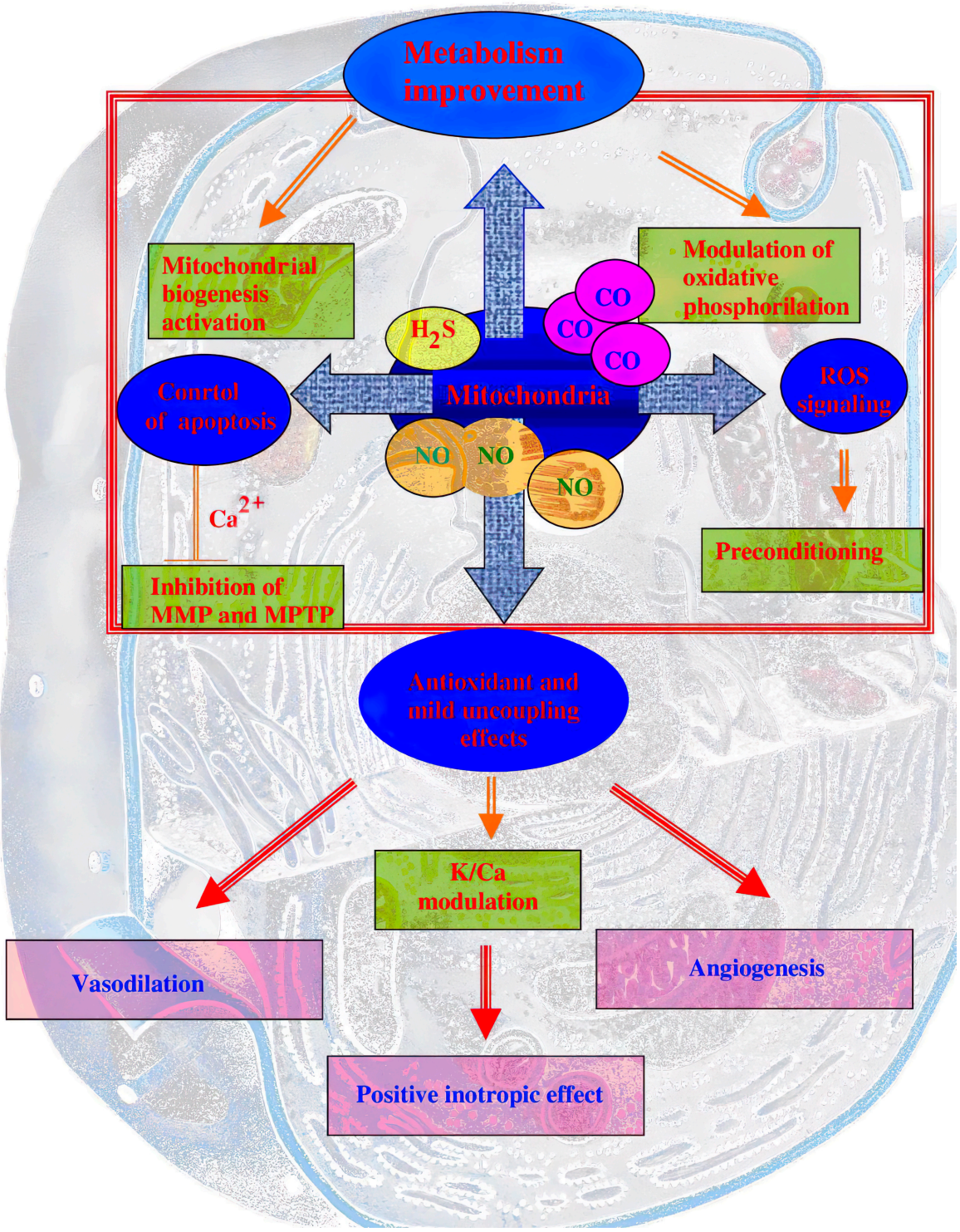

Fig. 1. Influence of gasotransmitters (H_2S, CO, NO) on mitochondrial mechanisms of cardioprotection.

is compensated by this process, "also, a metabolic acidosis as a consequence of hypoxia is eliminated and, as a result, the resistance of the heart muscle to oxygen deficiency is increased" [4]. Since the activation of complex II determines Ca^{2+} influx into mitochondria, the intramitochondrial Ca^{2+} pool increases during hypoxia when myocardial contractility is reduced [5].

Over the past years, there has been a significant increase in attention to the metabolic regulation of the ETC complexes activity and finding endogenous substances that can increase the activity of the complexes in hypoxia. Surprisingly, hydrogen sulfide (H_2S), a highly toxic gas (LD100—1 mg/L), pertains to such regulators. The hydrogen sulfide acts directly on the central nervous system and

can cause instant death at high concentrations with a single inhalation. At the same time, H_2S, an endogenous gas molecule, is continuously produced in animals and humans as a product of cysteine-containing amino acids breakdown indicating that certain mechanisms of H_2S intoxication, its intracellular use and utilization have been developed during evolution [6]. Many studies have been focused on mechanisms underlying the effect of H_2S at the organism and cellular levels etc. [6–10]. In this context, we are interested in studies related directly to the effect of H_2S on mitochondrial targets and mechanisms of its' physiological effect on the CVS cells (CM and VSMCs).

It was originally demonstrated that hydrogen sulfide at high doses causes irreversible inhibition of cytochrome oxidase that prompts the ETC dysfunction, uncoupling oxidative phosphorylation, and subsequent cell de-energization [7]. However, further studies revealed that H_2S at low concentrations vis-à-vis activates mitochondria because it serves as an energetic mitochondrial substrate [8]. In the mitochondria, H_2S oxidation involves several enzymes including sulfide-quinone oxidoreductase, persulfide dioxygenase and sulfite oxidase. During the oxidation, protons released from H_2S enter the ETC, and the remaining oxidized forms, including free sulfur, become part of the mitochondrial signaling system [11, 12].

Analysis of findings on the cytoprotective effect of H_2S on CM and VSMCs has shown that H_2S protective effect is based on its ability to "quench" free radical processes in the mitochondria, reduce production of ROS and intracellular injury caused by oxidative stress. This notion has been supported by an antagonism of the emerging interactions between H_2S and the mitochondrial ROS inducer — homocysteine, which is also a product of metabolism and conversion of S-containing amino acids [13]. Excessive blood homocysteine content [more than 16 microM/L] can lead to the development of a severe CVS pathology associated with membranes of CM and VSMCs impairment due to oxidative stress [14].

In the experimental studies, the intraperitoneal administration of the saturated H_2S aqueous solution led to a decrease in the total concentration of blood plasma homocysteine and lipid peroxidation processes both in blood plasma and myocardium. Moreover, administration of H_2S reduced production of superoxide anion and H_2O_2 and recovered activities of the mitochondrial enzymes, including succinate dehydrogenase, cytochrome oxidase and mitochondrial superoxide dismutase, whose functions are impaired during homocysteinemia [13].

Interestingly, both *in vivo* and *vitro* experiments have revealed that H_2S targets a marker of endoplasmic stress, glucose-regulated protein p78, which is expressed during homocysteinemia and other diseases characterized by impaired energy metabolism [13]. Thus, the protective effect of H_2S on CVS occurs via regulation of the ETC enzyme activity as well as metabolic and redox-dependent pathways which components are signaling and regulatory thiol-containing proteins [8, 15–17].

Other targets of the H_2S protective effect on CVS cells include ATP-dependent K^+ channels that mediate the cardioprotective effects of preconditioning (Fig. 1) [18]. Previously, we described structure and regulatory mechanisms of these channels in MCM with the participation of the hypoxia-inducible factor-1alpha (HIF-1alpha) [19]. The current review elaborates mechanisms that are involved into activation of K_{ATP} channels with the participation of H_2S generated during hypoxia [20] (Table 1, Ref. [14–17, 20–60]).

Previous physiological studies have demonstrated that H_2S donors, 4-carboxyphenyl isothiocyanate (4CPI) and sodium hydrosulfide (NaHS), significantly improve a number of functional and biochemical characteristics of cardiac muscle contractility after ischemia [21, 22]. Pretreatment of rats with 5-hydroxydecanoate (5-HD), a selective blocker of mitoK_{ATP} channels, abolished the 4CPI cytoprotective effects [22]. These findings laid in the basis for further biochemical and molecular studies aimed at understanding cellular mechanisms underlying protective effect of H_2S on the CVS. It has been found that endogenous hydrogen sulfide affects the K_{ATP} channels activity in smooth muscle cells of blood vessels. The intensity of K^+ currents of incoming rectification increased after exposure to H_2S, and decreased after exposure to inhibitors, cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS) [22]. Molecular studies have demonstrated that the S-sulfhydration of cysteine residue 43 (Cys43) of regulatory Kir6.1 subunit (Kir is the output rectification channel) plays a key role in the H_2S-mediated activation of mitochondrial K_{ATP} channels [61]. Herewith, the enhancement in the activity of K_{ATP} channels was accompanied with an increase of the conjugation of PIP_2 with the corresponding Kir6.1 sites that stabilized the channel in the open state and led to an increase in the amplitude of the K^+ current [61]. It has been recently shown that H_2S changes activity of other isoforms of Kir subunits (Kir2 and Kir3) of K_{ATP} channels [62] that may also contribute to cardioprotection [63, 64].

Experiments with isolated mitochondria from rat hearts revealed that the 4CPI hydrogen sulfide donor caused a decrease in MMP and weakened the activity of caspase-9. This effect was canceled by 5-hydroxydecanoate, a selective blocker of mitoK_{ATP} channels [16]. These findings served as the forerunner for the biochemical and molecular studies aimed at unraveling the cellular mechanisms underlying the H_2S effect on cardioprotection and CVS. The endogenous H_2S has been found to have impact on the activity of K_{ATP} channels in the smooth muscle and endothelial cells of blood vessels, where the intensity of the K^+ currents of incoming rectification enhanced by H_2S and decreased by cystathionine gamma-lyase and cystathionine beta-synthase [22]. Unfortunately, there are no electrophysiological data confirming involvement of H_2S into

Table 1. Protective role of hydrogen sulfide, nitric oxide and carbon monoxide in cardiovascular system.

Main molecular targets in CVS	Effects on cells of CVS	Clinical significance and prospects for pharmacological use	References
Hydrogen sulfide (H_2S)			
ETC enzymes	Activation and increase of ATP synthesis	Adaptive response in vessels during hypoxia	[23, 26]
Homocysteine	Restoring the level of CSE activities	Protection the myocardium from oxidative and ER stress induced by HHcy	[14, 15, 17]
Nrf2	Decreasing in generation of ROS	Reducing the risk of hypertension and myocardial infarction	[21–23]
NLRP3 inflammasome	Inhibiting both nuclear translocation of NF-kappaB and NLRP3 inflammasome activation	Inhibition the vicious cycle of oxidative stress and inflammation in hypertension	[45, 46]
KATP	Increasing of MMP,decreasing of mitochondrial Ca^{2+} overload and opening of the MPTP; decreasing in caspase 9 activity	Protection of cells from ischemic and reperfusion damages. Control the ventilatory responses to hypoxia	[16, 20, 24, 25, 203]
Glu-receptors	Activation of NTS neurons	Ventilatory and cardiovascular control	[20]
BKCa	Transient receptor potential vanilloid 4 (TRPV4) channel-mediated Ca^{2+} influx	Promoting K^+ influx in VSMC. Vasodilatation	[47]
ER stress-related proteins	The decrease of activity caspase 1/2, expression of glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP)	Suppressing of ER stress. Reducing of cytotoxicity	[48, 49]
Nitric oxide (NO)			
mitoKATP	Attenuation of mitochondrial respiration caused by complex I substrates	The decrease of ROS production. Protection from IRI	[23, 27, 37, 56, 212]
Nitric oxide—releasing molecules (NO-RMs)	Stimulation of NO/cyclic guanosine 5' monophosphate (cGMP) pathway	Regulation of vascular contractility	[57–59]
HIF-1alpha	Activation of HIF-1alpha and subsequent expressionof glycolysis genes, GLUT family (glucose transporter) genes, EPO and VEGF/R genes. Modulating redox signaling.omega-Alkynyl arachidonic aciddiminished HIF-1alpha binding to the HRE sequence in iNOS promoter	Reducing the inflammatory response in hypertension. Antioxidant effects. Vascular reconstruction and angiogenesis. Reducing infarct size	[27–29]
SIRT1	Suppressing of NF-kappaB signaling via eNOS expression	Amelioration of myocardial ischemia/reperfusion injury	[30]
BH4	Remoting ischemic preconditioning by limiting cardiac eNOS uncoupling	Mitigation of myocardial IR injury. Reducing infarct size	[31]
PKC	iNOS mediated activation of PKC and mitoKATP channel opening	Increasing of cardiac tolerance to ischemia and reperfusion	[32, 60]
CaM	CaM facilitates a conformational shift in NOS allowing for efficient electron transfer	Mitigation of myocardial IR injury	[33]
ETC enzymes	Inhibition of mitochondrial respiration	Protection of cells from ischemic and reperfusion damages	[34–36]

Table 1. Continued.

Main molecular targets in CVS	Effects on cells of CVS	Clinical significance and prospects for pharmacological use	References
Carbon monoxide (CO)			
mitoKATP	Regulation of mitochondrial respiration and membrane potential	Protective response of cardiac muscle to oxidative stress. Vasodilatation	[23, 212]
mitoBKCa	The increase in the oxygen consumption rate in endothelial cells. Inhibition of glycolysis (extracellular acidification rate, and a decrease in ATP-turnoverenhanced non-mitochondrial respiration).	Mild uncoupling of mitochondrial respiration in endothelial cells induces adaptive response in vessels during hypoxia	[50]
TASK-3	Regulation of mitochondrial respiration and membrane potential	Vasodilatation andreducing of cardiac hypertrophy	[23, 51]
Carbon monoxide-releasing molecules (CO-RMs)	Stimulation of cGMP andNa/H exchange. Activation of BKCa through NO via the NOS and through the PKG, PKA, and S-nitrosylation pathways.	Regulation of vascular contractility; attenuation of coronary vasoconstriction and significantly reducing of acute hypertension	[38, 40, 43, 44, 52, 211]
Ntf2	Stimulation of HO-1 and subsequent expression of HSP32, sGC, p38MAP; the decrease of NFkappaB expression	Heme oxygenase suppresses markers of heart failure and ameliorates cardiomyopathy. Facilitating tissue regeneration/repair and the formation of new blood vessels	[39, 41, 42, 53, 212]
T-type Cav	Inhibition of T-type Cav via induction of HO-1	Control of cellproliferation (for example in hypertrophic cardiomyopathy and atherosclerosis)	[54, 210]
L-type CaV	Inhibition of pore-forming subunit CaV cardiac L-type Ca^{2+} channels	Protection of cells from ischemic and reperfusion damages	[55, 210]

the direct regulation of mitoK$_{ATP}$ channels. However, there are studies that support indirect participation of H_2S, when using hydrogen sulfide donors, in the regulation of mitoK$_{ATP}$ channels and muscle cells of the CVS. Studies conducted by Shimanskaia *et al.* [21] showed that the intraperitoneal injection of sodium hydrosulfide (NaHS, 7.4 mg/kg) slightly reduced heart rate and intensity of the myocardial contractile function without the increase of left ventricle pressure in isolated rat hearts. A small increase in coronary blood flow indicated the vasorelaxation effect of NaHS. At the same time, the hearts that had previously been injected with sodium hydrosulfide were more resistant to the additional volume load compared to control animals. When the left ventricle was stretched, the development of a more powerful contraction force and easier relaxation during the diastole was observed that supported an improvement of the heart functional reserves [21].

Results of physiological experiments supporting the cardioprotective effect of H_2S at small doses were confirmed by biochemical studies that demonstrated an increase in the mitochondrial resistance in the presence of this gas transmitter [16]. Moreover, H_2S decreased "mitochondrial factors" and metabolites released from mitochondria during Ca^{2+}-induced MPTP opening in the coronary system, thus proving a high degree of mitochondrial membranes' integrity during reperfusion and the protective effect of H_2S against MPTP [21]. To confirm the protective effect of the hydrogen sulfide donor on MCM, the authors conducted an experiment to evaluate Ca^{2+}-induced swelling of cardiac mitochondria resulting from the MPTP opening. They demonstrated a dose-dependent reduction in the mitochondrial swelling by 31–77% when the mitochondria were pre-treated with physiological concentrations (1–10 microM) of NaHS. This confirmed the regulatory role of endogenous H_2S in the processes of mitochondrial transport and its protective effect against MPTP. Preincubation of isolated mitochondria with 100 microM 5-hydroxydecanoate resulted in reduction of the protective effect of the H_2S donor that pointed out the involvement of mitoK$_{ATP}$ channels in the H_2S-dependent regulation of the MCM membranes permeability and inhibition of MPTP opening in cardiac mitochondria [21]. The authors suggested that, under these circumstances, the protective effect of H_2S molecules could be associated with the protection of thiol groups of mitochondrial proteins, particularly, adenine nucleotide translocase.

In addition, the protective effect of H_2S on the CVS cells could be explained by its modulatory effect on mitochondrial high conductance Ca^{2+}-dependent K^+ channels (mitoBK$_{Ca}$). However, there is no scientific evidence supporting this assumption [23, 65, 66].

So far, H_2S has been known to regulate channels similar to BK$_{Ca}$ located in the plasma membranes of different types of electrically excitable cells. This supports involvement of H_2S into the electrically controlled transport mechanisms that occur directly in the mitochondrial membranes [24, 25, 66, 67]. Studies of H_2S donors' effect on BK$_{Ca}$ channels have revealed that exposure of pituitary tumor cells GH3 to NaHS increased the opening time average of single BK$_{Ca}$ channels [67]. This effect was dependent of the NaHS concentration and membrane potential but not the intracellular concentration of Ca^{2+} $(Ca^{2+})_i$. In addition, the increase in the activity of BK$_{Ca}$ channels by released H_2S was temporary and reversible due to a decrease in the number of oxidized sulfhydryl groups on the cytoplasmic side of the channel-forming protein subunit and its' phosphorylation degree [24].

Thus, the recent studies have demonstrated the protective effect of small (physiological) doses of H_2S on the CVS cells that accounts for its' action on mitochondrial ETC enzymes, thiol groups of signaling and regulatory proteins as well as on K$_{ATP}$ and BK$_{Ca}$ channels [23, 26]. Based on this, H_2S might be considered as an important signaling and regulatory molecule that has a protective effect on the CVS cells under physiological and pathological conditions such as hypoxia, ischemic heart disease (IHD) and ischemia/reperfusion.

4. The role of nitric oxide and mitochondrial nitric oxide synthase in cardioprotection during hypoxia and ischemia/reperfusion

More recently, considerable research has been devoted to the role of nitric oxide (NO) in the development of adaptation to various pathological conditions, including ischemic heart disease and ischemia/reperfusion [68–72]. It has been shown that NO causes relaxation of vasculature, participates in protection of the myocardium against reperfusion injuries, and regulates apoptosis and proliferation of vascular smooth muscle cells [27, 71, 73]. Under physiological conditions, NO reacts with oxygen molecules and forms intermediate compounds, known as reactive nitrogen species (RNS). Formation of NO and RNS in cells is controlled by hormones, neurotransmitters, cytokines, and growth factors. With regard to the latter, NO and its derivatives act as secondary paracrine factors that transmit a signal from NO-producing cells to neighboring cells [28]. Intracellular NO and RNS receptors, which include Src protein-tyrosine kinases, Ras family proteins, cytochrome oxidase, and soluble guanylate cyclase (sGC), are mainly proteins containing heme, active SH- and iron-sulfur groups. They are localized both on the surface of the plasma membrane and in the internal compartments of cells. Most of the NO receptors are key components of intracellular signaling systems that regulate transcription factors AP-1, HIF-1, NF-kappaB, FoxO and the expression of their subordinate genes [28, 29, 74]. A feature that distinguishes NO from other high molecular weight signaling molecules is that the change in the redox potential of the cells switches the redox-dependent NO receptor and modifies the action of NO. De-

pending on the ROS level in cells, NO activates different redox-dependent signaling systems (Fig. 1). This is important in induction and suppression of the cellular protective responses to hypoxia [27] (Table 1).

In the CVS, NO is derived from a spectrum of molecular structures integrated into a nitroxidergic system (NOES) that includes neuronal (neurons) and extraneuronal (endothelium, cardiomyocytes, macrophages, platelets, SMC, glia, etc.) cells [75]. NO in the NOES is produced from L-arginine by three isoforms of NO synthases: neuronal (nNOS), endothelial (eNOS) and macrophage (mNOS) also known as NO synthase I, II, and III, respectively. The endothelial and neuronal NOS belong to a group of constitutive NOS (cNOS). The macrophagal NO synthase belongs to a group of inducible NOS (iNOS). The constitutive isoforms of NO synthase can generate NO in response to background receptor stimulation of the mechanical, neuronal, or humoral nature. The inducible enzyme isoforms are usually formed in response to excessive activation of cells by cytokines. To date, there is evidence of the existence of the inducible isoforms of NOS-I and NOS-II [71]. These isoforms are widely represented in various types of cells including blood vessel epithelial and smooth muscle cells, CM, and skeletal muscle myocytes. They are activated under stress condition, hypoxia, and various pathologies [30, 31, 76]. A subcellular localization and activity of NOS is determined by myristoylation and palmitoylation of N-terminal sequence, while acetylation of N-terminal glycine residues to amide bonds defines a membrane fixation of the enzyme. Therefore, the neuronal and endothelial enzyme forms are usually associated with cell membranes, and the inducible macrophage NOS exists mainly in the dissolved state in the cytosol [71].

NOS phosphorylation by a number of protein kinases is an important mechanism regulating NO production [32, 77]. The phosphorylation of the constitutive NOS leads to a decrease in the enzymatic activity, whereas dephosphorylation with the participation of phosphatases, in particular, calcineurin can increase the catalytic activity of the enzyme [70]. The actual mechanism of regulation of NOS activity is far more diverse and complex than we reported here. The association of constitutive NOS isoforms with the cell membrane directly or indirectly through calmodulin or other specific membrane-associated proteins involves a coordinated modulation of the NOS activity through phosphorylation/dephosphorylation at Ser-1177 and Thr-495 (Find out more about this in seminal work of S. Dimmeler, I. Fleming and R Busse groups [77]).

It has been known that cNOS begin to synthesize NO in response to increase in cytosolic calcium concentration. This makes calmodulin (CaM) to bind a 30-amino acid peptide connecting oxygenase and reductase domains of the NOS subunits. A mechanism of the calmodulin activation for the Ca^{2+}-dependent cNOS has been considered to be due to reductase domain conformation change in CaM binding which leads in turn to an increase in the electron transfer rate to both flavins and terminal electron acceptors of the ETC. A high intracellular calcium level has been revealed to stimulate the constitutive forms of the NOS and long-term NO synthesis, while the NO production by the inducible form does not depend on $(Ca^{2+})_i$ and, at the Ca^{2+} normal level, this form is limited only by the enzyme level, substrate amount and presence of cofactors [33].

The mechanism of the eNOS and nNOS action is similar in the CVS system. Vasodilator agents (acetylcholine, adenosine, 5-hydroxytryptamine, glutamate, bradykinin, histamine, etc.) increase cytosolic Ca^{2+} level in the endothelial cells. As a consequence, Ca^{2+} in combination with the CaM activates Ca^{2+}-dependent NOS isoenzymes in accordance with the mechanism described above. The activity of eNOS and nNOS lasts for minutes after the induction. In addition, eNOS is characterized by a lower maximum rate of catalysis (V_{max}) compared to other isoforms.

The endothelial and neuronal NO synthases are involved in such processes as conductance of nerve impulses, peristalsis provision and practically instantaneous regulation of blood pressure. For example, factors like acetylcholine and bradykinin activate the phosphoinositide signaling pathway in endothelial cells, resulting in $(Ca^{2+})_i$ increase. This leads to the activation of eNOS to produce NO that diffuses into the VSMC and causes their contraction. In neurons, Ca^{2+} level rises during electrical activity that activates nNOS. The resulting NO initiates vasodilation independent of the endothelium due to innervation of the smooth muscles [78, 79].

In the CVS cells, the NOS level frequently increases under conditions of oxygen deficiency, since NO formed by NO synthases is a trigger of mobilization processes aimed at maintaining cell viability during hypoxia. Herewith, the mitochondrial synthase of nitric oxide (mtNOS) plays the most important role. Its function is closely interrelated with other regulatory mitochondrial factors and signaling pathways and involved in implementing adaptive cellular responses to hypoxia [70]. mtNOS has been recognized as a constitutive form of the nNOS and it was first discovered in the mitochondria of the excitable brain and heart cells [80, 81]. The mtNOS is involved in cytochrome oxidase reversible inhibition and functionally associated with complex I of the ETC [70, 81]. At the same time, mtNOS reminds more inducible isoform rather than constitutive enzyme isoform by its main characteristics. Unlike the constructive enzyme isoform that is compartmentalized in the cytosol, mtNOS is localized in the inner mitochondrial membrane (IMM) [81].

The question whether mtNOS is a separate isoform of the enzyme or its post-translational modification remains open and is interpreted in a different manner [34, 82–84]. Regardless, discovering NOS in mitochondria has opened up new possible ways to study the roles of mtNOS and mito-

chondrial NO in mechanisms of the cellular adaptation and cardioprotection.

The mtNOS is directly related to cell functioning in hypoxic and ischemic conditions [35, 85]. On the one hand, tissue hypoxia significantly slows down the NOS-dependent synthesis of NO due to the lack of O_2 needed for the enzymatic reaction. On the other hand, it activates mtNOS via the Ca^{2+}-CaM mechanism [86]. There are studies supporting the stimulating effect of hypoxia on the mtNOS activity [87]. It has been shown that changes in the activity of mtNOS depend on the severity of the hypoxic state. Thus, this phenomenon has importance for practical medicine: the moderate hypoxia can lead to the activation of mtNOS and arginine pathway of NO synthesis since the launch of NO production underlies numerous compensatory-adaptive reactions in response to hypoxia.

NO synthesized in mitochondria during hypoxia modulates the mitoK_{ATP} channels opening that promotes cell energization and underlies the protective effect of preconditioning [88]. Activation of mitoK_{ATP} channels is one of the first steps in the cell adaptation process to hypoxia and ischemia since the mitoK_{ATP} channels opening reduces Ca^{2+} overload of mitochondria, normalizes the cell redox balance thus ensuring their functional activity, and viability in oxygen deficiency conditions [88–90].

A transcription rate of genes regulating mtNOS, content of mtNOS substrates (NADPH, L-arginine) and its cofactors (FAD, FMN, tetrahydrobiopterin (BH_4)) are among the factors that affect the dynamics of the NOS-dependent production of NO in mitochondria. In the organism, NO is produced by arginine conversion and nitrite reductase reactions. The input of nitrite reductase reactions into NO production enhances under hypoxic condition. These reduction reactions are catalyzed by electron donor systems involving NADH, NADPH, flavoproteins, and cytochrome oxidase in mitochondria [91]. It has been demonstrated that mitochondrial NOS switches to the formation of reactive oxygen species instead of NO in L-arginine deficiency that leads to the oxidative stress and the MPTP opening. The Ca^{2+}-induced MPTP opening is then prevented by ROS neutralization with superoxide dismutase mimetics or mtNOS substrates or cofactors such as L-arginine or tetrahydrobiopterin [81]. Therefore, maintaining the physiological level of agonists and key components of the arginine pathway of the NO synthesis during the oxidative stress following hypoxia can have a cytoprotective effect [81, 82].

During hypoxia, when a significant Ca^{2+} overload is observed, NO produced in mitochondria delays MPTP opening. However, it is not clear whether this effect results from a direct action of NO (for example, direct S-nitrosylation of thiol groups) on the pore or ROS neutralization [87].

As discussed earlier, ROS overproduction during many pathological conditions, including hypoxia and ischemia, leads to the interaction of oxygen free radicals with NO and production of the highly reactive peroxynitrite oxidant ($ONOO^-$). Ultimately, peroxynitrite (PN) triggers inhibition of aconitase and iron-containing centers of I–III MTC complexes, suppression of ATP and creatine phosphate synthesis, nitrosylation of membrane thiols that affects permeability of the mitochondrial membranes and results in the MPTP opening and mitoptosis. In turn, mitochondrial dysfunction impairs a reuptake of neurotransmitters, ion transport, generation and conduction of the electrical impulses [36, 92–94].

The effect of NO is multifunctional due to its multidirectional action on cell function that results in different cell responses to the same stimulus [95]. Such effect of NO is determined by the ratio of various NOS isoforms and location of NO production in cell compartments. In this regard, it is important to note that increase in the activity of the NOS and NO production does not always impair cells and lead to the programmed cell death (see below) but also has a positive role [81, 96, 97]. Signaling pathways that involve both pro- and anti-apoptotic proteins are activated in the cells accordingly [97, 98]. The effect of mtNOS and mitochondrial NO on the cells is determined by the ratio of stress factors and cell survival factors that direct NO in one way or another [82, 99].

At low concentrations, NO reversibly binds the electron transport chain cytochrome oxidase and blocks MPTP, therefore, contributing to the cell survival in hypoxia. At high concentrations, it causes S-nitrosylation of the thiol groups of the mitochondrial proteins and inhibits ATP synthesis that results vice versa in MPTP opening, release of apoptogenic factors and cytochrome *c* into the cytosol, and triggering the mitochondrial apoptotic pathway [100, 101]. The toxic NO influence is associated both with the direct effect on cellular iron-containing enzymes and formation of the highly reactive and permeable to membranes peroxynitrite. This results in not only mitochondrial dysfunction and reduction of ATP production but also in damage of cell nuclear apparatus as a consecquence of DNA deamination and ribonucleotide reductase inhibition [94].

It is obvious that the pharmacological regulation of the mtNOS activity is of great scientific and practical importance and involves development of selective drugs blocking mtNOS that could be used specifically for myocardial ischemia-reperfusion treatment. Increased mtNOS activity has been demonstrated in experiments with IHD (the severe hypoxia case especially) and right ventricular hypertrophy in hypoxia-induced pulmonary hypertension. The inhibition of mtNOS has been shown to lead to myocardial contractility increase in cardiomyopathy [70].

In addition to inhibitors, there is a search for new inductors of NOS. Currently, cNOS inductors are of great importance in modern medicine. They can be effective

cardioprotectors in hypoxic conditions since the cNOS-produced NO initiates the activity of hypoxia-induced factor HIF-1 [71]. This factor is transcriptional and regulates the expression of redox-dependent genes that allow cells to adapt to oxygen deficiency. These include genes of glycolysis (aldolase, lactate dehydrogenase, phosphofructokinase genes), glucose transport (GLUT family glucose transporter genes), angiogenesis (erythropoietin (EPO) genes), vascular reconstruction (vascular endothelial growth factor (*VEGF*) gene, and VEGF receptor 1 (VEGF1)) [19]. In addition, HIF-1 activates vasomotor genes that are important for vascular response to hypoxia [102].

The expression of HIF-1 gene itself and the level of its' protein product depend on the concentration and partial pressure of oxygen (pO2) in the blood. The HIF-1 activity is increased during hypoxia. In this regard, a special attention is paid to the search for new substances that mediate the induction of HIF-1 in the CVS cells.

Currently, some of the NO donors (S-nitroso-N-acetyl-D, L-penicillamine; S-nitrosoglutathione) have been demonstrated to induce an increase in the HIF-1 activity [103]. This process is independent of cGMP, however, associated with activation of the redox-sensitive PI3K/AKT/mTOR signaling pathway that controls the key cell functions [19]. NO can bind iron in the HIF hydroxylases and block their binding with oxygen, thereby, inhibiting the hydroxylation reaction of the adaptation factor to hypoxia [104]. This makes relevant the search of the constitutive NOS inducers as well as compounds that enable to prolong the effect of NO and support its transport to various organs and tissues.

Findings from experiments with application of activators and inhibitors of mito- and sarcolemmal K_{ATP} channels explain the protective effect of low NO concentrations (<1 microM) on the CVS system by its stimulating action on mitoK_{ATP} channels [37, 105, 106]. It has been shown that the NO donor, S-nitroso-N-acetyl-DL-penicillamine, activates only mitoK_{ATP} channels and does not alter sarcolemmal K_{ATP} channels since its effect on the mitoK_{ATP} channels is inhibited by 5-hydroxydecanoate (5-HD), a specific blocker of the mitoK_{ATP} channels, or NO scavengers [106].

Since the majority of the NO effects are mediated via cGMP-activated signaling pathways, a study has been conducted to determine the direct effect of 8Br-cGMP, a non-hydrolyzable cGMP analogue, on the activation of the mitoK_{ATP} channels in the excitable cells. Negative results of this study have suggested that the mitoK_{ATP} channels are directly activated by NO [106]. Interestingly, the channels activated by diazoxide were more susceptible to the potentiating effects of NO than those being inactive and closed [106]. The activity of the single mitoK_{ATP} channels embedded into the lipid bilayer was inhibited by specific mitoK_{ATP}-5-HD blockers or glibenclamide [107]. At the same time, activity of mitoK_{ATP} channels was suppressed by NO in non-excitable Jukart cells [108]. Such differences might be related to discrepancies in the molecular mechanisms underlying the NO effect on excitable and non-excitable cells. Unfortunately, no direct studies have been performed on mitochondrial channels to clarify this difference. However, some answers have been provided by studies conducted on plasmalemmal K_{ATP} channels. For example, registration of cellular K_{ATP} currents in the large rat DRG neurons has showed that the K_{ATP} channels are stimulated by NO by reducing their sensitivity to ATP, which inhibited the opening of the K_{ATP} channels [109]. The stimulating effect of NO on K_{ATP} channels remained after the use of inhibitors of cytosolic guanylate cyclase and PKG. This indicated that the sGC/cGMP/PKG pathway was not invoved in the transmission of NO-mediated signals to K_{ATP} channels. The activating effect of NO was abrogated by dithiothreitol and NEM, a thiol-alkylating agent. These results demonstrated that NO activated K_{ATP} channels through direct S-nitrosylation of cysteine residues. Measurement of inward rectifier K^+ currents revealed that the current through recombinant wild-type SUR1/Kir6. 2 channels expressed in COS7 cells was activated by NO, but channels formed only from truncated isoform Kir6.2 subunits without SUR1 subunits were insensitive to NO. Further, mutagenesis of SUR1 indicated that NO-induced K_{ATP} channel activation involves interaction of NO with cysteine residues in the nucleotide binding domain1 (NBD1) of the SUR1 subunit [109].

On the other hand, NO can regulate mitoK_{ATP} channels via the sGC/cGMP/PKG signaling pathway in some studied cell types [110–112]. For example, mitoK_{ATP} channels opening index and activity of the Kir6.2/SUR2A complex has been shown to significantly increase in the presence of NOC-18 (exogenous NO donor) in transfected HEK293 cells and cardiomyocytes isolated from rabbit hearts or genetically modified mice [37]. The activity of mitoK_{ATP} channels was significantly reduced in the presence of compound KT5823, a selective PKG inhibitor [113]. Other studies prove that NO can activate mitoK_{ATP} channels in cardiomyocytes through its participation in regulation of sGC/cGMP/PKG signaling pathway [37, 110, 113]. It has been proven that the sGC-dependent signaling pathway activated by NO can also be involved into the regulation of Ca^{2+}-dependent MPTP and mitoBK_{Ca} channels in MCM [110, 111, 114].

In summary, we affirm that NO is an important signaling molecule in the CVS and modulator of mitochondrial respiration, ATP synthesis, activity of mitoK_{ATP} channels, HIF-1, and an important MPTP regulator. Currently, an assumption has been made regarding a functional dualism of NO in cell injury processes and regulation of metabolism. Analysis of a numerous experimental findings demonstrated that multifunctionality and multidirectional effect of NO on the CVS cells depends of not only

the concentration and location of the NO synthesis, but also its interaction with other signaling molecules, impact force of a pathogenetic factor on cell, and functional metabolic state of the cell [30, 32, 68–71]. The mtNOS is one of the most regulated NO metabolism enzymes in the CVS that opens up the prospect for use of this enzyme as a specific target for pharmacological impact. Such approach would allow looking for effective drugs designed to regulate the processes of adaptation of the CVS and organism altogether to hypoxia and ischemia. This is supported using by mtNOS inhibitors that demonstrated high efficacy in experimental cardiomyopathy, thus, these compounds can find practical applications in minimizing adverse effects of coronary heart disease and ischemia/reperfusion injury. Using such drugs, it becomes possible to selectively modulate the individual isoforms of the NOS, including mtNOS, and change their activity in a targeted way. Thus, cell homeostasis and, more importantly, resistance to hypoxia can be improved by modulating the mtNOS activity and mitochondrial NO synthesis [70, 81, 82].

5. The role of carbon monoxide and heme oxygenase system in vasodilation. Antiarrhythmic effects of endogenic CO

Carbon monoxide (CO), also known as a “silent killer”, is one of the most toxic substances that have harmful effect on all eukaryotic organisms. It is a frequent cause of morbidity and mortality as a result of poisoning [115–118]. Symptoms and signs of CO poisoning and death result from tissue hypoxia due to its high affinity for hemoglobin. Carbon monoxide has approximately 210–250 times higher affinity for hemoglobin than oxygen at normal atmospheric pressure [119]. Binding of CO with heme of hemoglobin molecule causes allosteric conformational change of hemoglobin, resulting in the formation of carboxyhemoglobin (COHb), a strong compound where hemoglobin bound to CO is unable to transport oxygen to tissues of the body [120–122]. Hypoxia induced by oxygen displacement from hemoglobin, known as a carbon monoxide hypoxia, leads to fatal inhibition of ATP synthase, mitochondrial dysfunction, intracellular accumulation of superoxide and cell death [123–125]. However, recent studies have shown that CO at micromolar concentrations can participate in the regulation of physiological functions and even act as a cytoprotector during development of a number of pathological conditions [118, 126, 127].

Endogenous CO is a product of heme catabolism to carbon monoxide, biliverdin, iron, and controlled heme oxygenases. Degradation of heme occurs in the presence of certain enzymatic systems among which heme oxygenase (HO) and biliverdin reductase are directly involved into the oxidative conversion of heme. Heme oxygenase cleaves the tetrapyrrole ring in the heme to form CO and biliverdin [128].

The key enzyme in the heme oxygenase reaction is heme oxygenase. Until recently, HO was assumed to be expressed mainly in brain, liver and spleen cells. However, it has been now established that HO is widely distributed in the cells of the CVS [38, 127, 129]. Three isoforms of heme oxygenases are known. Among them, HO-1 is a stress-induced form and known as a heat shock protein 32 (HSP32), and HO-2/3 are constitutive forms [115]. The HO-1 plays an important role in the mechanisms of cell adaptation to various pathological processes, including hypoxia [130]. Initially, HO-1 was considered as a microsomal protein, mainly localized in the endoplasmic reticulum, however, later the enzyme was found in the cytoplasm, nuclear matrix, peroxisomes, and mitochondria of the spleen and liver [128].

In this context, it is important to note that HO-1 is also expressed in the CVS cells, including CM, endothelial and vascular smooth muscle cells, thereby controlling the formation of CO [131–135]. The HO-1 is activated by various oxidant species, including endogenous prooxidants, such as heme and its derivatives [39, 136]. It is known that “free” heme at high concentrations is a prooxidant and direct participant in the processes of free radical oxidation. In this regard, induction of HO-1 is primarily aimed at preventing from the development of oxidative stress and cytotoxic effects of byproducts of heme protein degradation on the CVS cells [136, 137]. Inhibition of HO-1 has been demonstrated to increase oxidative stress and reperfusion injury of the cells [40–42, 127].

Accumulation of the ROS, in turn, induces transcriptional activity of HO-1 gene that plays a significant role during hypoxia and oxidative stress. The HO-1 knockout mice developed hypertrophy of pulmonary artery and hypertension during hypoxia, while overexpression of *HO-1* was accompanied by a decrease in proinflammatory cytokine production and vasoconstriction under the same condition [43]. These protective mechanisms are caused mainly by products of the heme oxygenase reaction, such as ferritin that binds Fe^{2+} and bilirubin characterized by antioxidant properties, as well as the relaxing effect of CO.

The induction of HO-1 often occurs when the NOS is stimulated by donors of NO and its derivatives, and during S-nitrosotiols and S-nitrosoglutathione formation. Along with the redox-dependent regulation of HO-1 expression, Ca^{2+} ions, transcription factor Nrf2, MAP kinase, soluble guanylate cyclase and other signaling molecules are also involved into the regulation of the HO-1 expression [131, 138].

The constitutive isoform *HO-2* (36 kDa) found in many tissues determines the degradation rate of heme in physiological conditions. It is abundantly expressed in the cardiovascular and nervous systems [40, 136]. The *HO-2* is a Ca^{2+}-dependent enzyme that activated by the Ca^{2+}-calmodulin complex and inhibited by calmidazolium, a CaM-specific inhibitor [40]. Presence of a region highly-

sensitive to O_2 in the structure of HO-2 allows considering it as a heme/oxygen sensor activated by hypoxia. The HO-3 isoform is a constitutive homologue of HO-2. It is abundantly expressed in different types of cells, however, characterized by low catalytic activity and functions only in the presence of oxygen [137]. In addition to the heme oxygenase system that promotes the formation of CO, other alternative sources of the CO formation in the organism have been described. These sources include some products of lipid peroxidation and biotransformation of pharmacological drugs (phenobarbital, diphenin) [43, 137]. Endogenous CO production is limited by substrate availability. Thus, the mechanisms that oversee heme production in cells regulate CO synthesis. In the human body, CO production does not exceed 20 microM/h under physiological conditions and may increase in various pathological conditions, including those that are accompanied by hypoxia [132, 134].

Numerous studies indicate that CO and its donors are involved in regulation of myogenic vascular tone by causing SMC relaxation [38, 44, 139, 140] (Table 1), and also cause anti-inflammatory and aniapoptotic effects [38]. In this regard, it seems to be rational applying the CO positive effects for correcting hypoxia-induced pathological conditions and reducing a course of chronic cardiovascular diseases. Cardiovascular diseases have the utmost potential for therapeutic application of the CO. However, many mechanisms underlying the CO effects on CVS cells are still not well known. Considering the importance of the perspectives of CO use as an endogenous regulator and cytoprotector in the CVS, we will focus on common mechanisms underlying its vasodilating and anti-apoptotic effects (Fig. 1).

To date, it has been known that the vasorelaxing effect of CO is mainly related to its ability to regulate the ion permeability of cell membranes through an increase in the activity of soluble guanylate cyclase and modulation of various types of ion channels [141]. Moreover, activation of BK_{Ca} channels by CO has been considered as the main mechanism of CO action in the CVS cells [142, 143].

Under physiological conditions, BK_{Ca} channels can be activated by electrical stimuli or increased $[Ca^{2+}]_i$. Their function is to repolarize the membrane potential and remove K^+ from the cell [143, 144]. The BK_{Ca} channels contain a pore-forming alpha-subunit and an auxiliary beta 1-subunit, which increases the channel sensitivity to Ca^{2+} ions. The CO sensitizes BK_{Ca} channels and regulates their activity to maintain intracellular Ca^{2+} level within micromolar concentrations [145, 146]. Local Ca^{2+} transients (Ca^{2+} sparks) are required to activate BK_{Ca} channels in SMC. They help to maintain the required concentration of Ca^{2+} in the micromolar range by activating the ryanodine receptors (RyR) localized in the sarcoplasmic reticulum [147, 148]. Some of the BK_{Ca} channels are highly sensitive to Ca^{2+} and can be activated by a single Ca^{2+} spark that result in transient K^+currents. The transient K^+ currents of the arterial wall hyperpolarize the membrane potential and decrease the activity of voltage-dependent Ca^{2+} channels located in the plasma membrane. This leads to a decrease in global intracellular Ca^{2+} concentration and vasorelaxation.

The vasorelaxing activity of CO is mediated by its binding to the alpha-subunit of the BK_{Ca} channel and its subsequent activation by cell heme. The heme, being in the cell in a reduced state, binds to the heme-binding domain (Cys-Lys-Ala-Cys-His) of the alpha-subunit located between amino acids 612 and 616, and this binding inhibits the BK_{Ca} channels [149, 150]. At the same time, the binding of CO to the BK_{Ca} channel and reduced heme iron changes the heme's association with the channel that leads to increased channel capacity [139]. Thus, the heme associated with the BK_{Ca} channel is a CO receptor, and CO binding increases the sensitivity of the BK_{Ca} channel to Ca^{2+}, in turn [139, 151]. The CO increasing sensitivity of BK_{Ca} channels to Ca^{2+} enhances coupling of Ca^{2+} with activated Ca^{2+} sparks of BK_{Ca} channels [139, 151–153]. The CO also raises the conjugation of Ca^{2+} with BK_{Ca} channels by increasing the Ca^{2+} sparks frequency as a result of RyR activation [154, 155].

Since the suppression of Ca^{2+} oscillations or blocking the BK_{Ca} channels eliminates the relaxation of vascular smooth muscles induced by CO, it is believed that the coupling of Ca^{2+} sparks with the BK_{Ca} channel is a key element in ensuring the CO relaxing effect [156]. In addition to a direct effect on these channels, CO can regulate their activity indirectly through interaction with other molecules involved in the regulation of these channels, and in particular PKG, which phosphorylates serine residues (Ser855, Ser869 and Ser1072) localized in the cytoplasmic domain of BK_{Ca}, increasing the probability of opening the gate of the channel. The CO is able to stimulate soluble guanylate cyclase and PKG activation, as a result [157].

In turn, activation of the BK_{Ca} channels leads to hyperpolarization of the vascular SMC membrane, closing of the voltage-dependent Ca^{2+} channels, and decrease in Ca^{2+} entry into the cells [158]. Thus, despite of some differences in the SMC response to CO, the CO vasodilating effect can be explained by an increase in the sensitivity of the BK_{Ca} channels to Ca^{2+} as well as an enhance in transient K^+ current, which causes PM hyperpolarization and closure of the voltage-dependent Ca^{2+} channels.

On the other hand, the relaxing effect of CO on SMC is conditioned by activation of the soluble guanylate cyclase and an increase in intracellular cGMP concentration [159–161]. The cGMP-dependent protein kinase G (PKG) is a key participant in the mechanism of cGMP-mediated CO effect on vascular SMC. The kinase induces re-uptake of Ca^{2+} by sarcoplasmic reticulum through phosphorylation of a number of signaling proteins and lead to reduction of $[Ca^{2+}]_i$ followed by smooth muscle relaxation [162]. In addition, activated PKG phosphorylates RyR in SR, which

contributes to an increase in the intensity of Ca^{2+}-sparks associated with vasorelaxation [163].

Although further studies are needed to determine the more precise effect of CO on the molecular structures of the cells during vasorelaxation, CO donors can already be used in practical medicine to reduce blood pressure in hypertensive patients. In addition, the endogenous CO induction might be one of the ways to reduce a stage of ischemic injury caused by circulatory disorders associated with pathological vasoconstriction during acute coronary syndrome and angina pectoris.

The K_{ATP} channels are considered as targets of carbon dioxide in SMC along with the proteins that have been mentioned previously. Their participation in the CO-mediated vasorelaxation was established by using selective K_{ATP} channel blockers. For example, a decrease in the CO relaxing effect on vascular SMCs was observed in experiments with 10 microM glibenclamide, a selective blocker of ATP-dependent K^+ channels [164]. However, the interaction of CO and K_{ATP} channels remains poorly understood.

To date, the CO activation of the K_{ATP} channels has been revealed to depend on presence of the heme. Also, it has been demonstrated that CO tightly binds the iron heme-SUR2A615–933 complex similar way to the CO bindings found in other studied heme-dependent regulatory systems. This supports the fact that CO regulates heme binding by the SUR2A subunit of the K_{ATP} channel. The data obtained for the heme-SUR2A615-933 complex are consistent with ideas about the activity of the 6-coordinate low-spin heme forms with histidine and cysteine as axial ligands. In the presence of CO, the cysteine ligand becomes displaced for the interaction of the CO-bound porphyrin complex with proximal histidine, which significantly increases the functional activity of the channel. Bonds in Fe-Cys are weak, therefore, iron-protein complexes are expected to easily dissociate in the presence of CO, a strong pi-acceptor ligand [161]. The interaction of the heme with the SUR2A subunit of the K_{ATP} channel is flexible and reversible that implies conformational changes in the heme molecule and the heme pocket opening for interaction with signaling molecules [165]. These molecules primarily include ROS, which modify the cysteine residues of channel proteins [164] and soluble guanylate cyclase [166]. The CO binding to the heme iron is accompanied by a change in sGC conformation underlying the enzyme activation [166]. The sGC modification leads in turn to increase in the formation of cGMP, an inducer of signaling processes, which lead to vasodilation [39, 159, 166].

Most of the cGMP effects are mediated via cGMP-dependent PKG, which phosphorylates a wide range of regulatory target proteins in the CVS cells, and thereby modulates the functional activity of these cells. Inhibition of the cGMP synthesis or the kinase itself causes a weakening of the contractile effects of CO on various SMC types [39, 167]. In the organism, vessels are often influenced by two gas transmitters (CO and NO), and the CO effect is enhanced in the presence of NO [159]. These effects are associated with sGC stimulation. *In vitro* experiments have shown that NO is 30–100 times more potent sGC stimulator than CO [44], and this explains why the NO-induced vasorelaxation is significantly more pronounced than the CO-modulated one.

Along with existing information of CO as a vasodilator, during the oxidative stress CO can exhibit a constrictive effect and promote ROS formation in mitochondria [140, 168]. In turn, the CO-induced ROS production [169] is a prerequisite for activation of antioxidant enzymes and redox-dependent expression of corresponding genes. Modulating various signaling cascades, including PI3K/Akt [38], NF-kappaB, HIF-1alpha [132], p38 MAPK [169], JNK1/2 [128], sGC/cGMP [170–174], CO is able to exert a protective anti-apoptotic, anti-inflammatory, and anti-proliferative effect.

6. Antiapoptotic properties of CO, H_2S and NO

This chapter will focus on the protective mechanisms that underlie the CO, H_2S and NO effects predominantly in the CVS cells (CM and CVS smooth muscle cells). Apoptosis in the CVS cells can be initiated by endogenous and exogenous factors that are intracellular signals generated during cell stress. In this case, the apoptosis induction depends on release of proapoptotic proteins from the mitochondrial intermembrane space. The exogenous factors are considered as extracellular ligands that bind “death receptors” on the cell surface that leads to the death-inducing signaling complex (DISC) formation [175]. The cytoprotective CO effect is associated with the induction of protective mechanisms that weaken effects of both internal and death-dependent external apoptotic signaling pathways [176]. The endogenous factor-induced apoptosis is associated with mitochondrial signaling pathways and increased permeability of the mitochondrial membranes [177]. Permeabilization of the outer and inner mitochondrial membranes leads to the irreversible programmed cell death. It is related, first to a loss of mitochondrial membrane potential and cytochrome *c* release to the cytosol, second to uncoupling of oxidative phosphorylation, third to ROS hyperproduction, fourth to ATP synthesis cessation, and fifth to release of pro-apoptotic proteins [178].

6.1 Antiapoptotic properties of CO

The main mechanism by which CO mediates antiapoptotic effect in the internal mitochondrial pathway is preventing association of Bid and Bax proteins, which are pro-apoptotic members of the Bcl-2 family, on the surface of the external mitochondrial membrane. The CO inhibits caspase-8, whose function is to activate the pro-apoptotic protein Bax by cleaving it to the tBid active fragment [176,

179]. The activated tBid is translocated into the mitochondria, where it binds Bax protein, whose oligomeric form causes permeabilization of the outer mitochondrial membrane, release of cytochrome *c* and other pro-apoptotic proteins from the mitochondrial intramembrane space, apoptosome formation and ultimately cell death [180–185].

As for the external receptor-dependent apoptotic pathway, CO inhibits formation and movement of the death-inducing signaling complex DISC from the Golgi apparatus to the plasma membrane. This signaling pathway is initiated by the FasL (Fas cell death ligand) that interacts with its receptor (Fas-R) localized to the cell membrane [186]. The FAS activation induces oligomerization and rapid recruitment of an adapter protein (FADD) that interacts with the death domain of the Fas receptor (Fas-associated death domain FADD) and caspase-8 that form DISC. Inside the signal complex, auto-proteolytic generation of caspase-8 occurs from procaspase-8 [187]. Although exact mechanisms underlying the DISC formation and translocation of Fas, FADD, and caspase-8 have not been fully characterized, the DISC assembly has been demonstrated to occur in the Golgi apparatus and its activation happens in the plasma membrane [176, 188, 189]. Activated in the apoptosome, the caspase-8 cleaves Bid to the active tBid fragment, which transfers from the cytosol into the mitochondrial membrane, where it promotes activation of Bax, a main molecule of the internal mitochondrial apoptotic pathway [190].

It is assumed that CO is also involved in other cytoprotective mechanisms during activation of the external apoptotic pathway, particularly through activation of the p38 MAP kinase signaling pathway and regulation of the transcription factor NF-kappaB activity. The interaction of the signaling proteins with CO results in activation of the FADD-like ICE-inhibitory protein, which inhibits the TNF-alpha/Act-D-induced caspase-8 cleavage [191, 192].

The anti-apoptotic CO effects can be useful for practical medical applications in cases when improving cell survival is essential to protect against acute stress or chronic destructive changes. For example, ischemic stroke and acute coronary syndrome are representative diseases when ischemic injury is caused by failure of circulation. Treatment of these diseases is associated with repeated vascularization (blood flow restoration in the damaged area), which causes additional ischemic-reperfusion injury (IRI). In such conditions, CO by exerting an anti-apoptotic effect on cells can reduce tissue damage caused both by the IRI and initial ischemia.

In addition, favorable CO effects on the CVS can include its antiproliferative effects on VSMC and mitochondrial respiration modulation associated with mild uncoupling of the oxidative phosphorylation and preconditioning [193, 194]. The CO directly regulates the expression both of cyclin D1, a key regulator of cell cycle progression in the G_1 phase, and $p21^{cip1}$ gene, a potent inhibitor of cell cycle progression, which leads to the G_0/G_1 cell cycle arrest [195]. Moreover, CO abrogates transition of the SMC from proliferative dormancy to the growth phase by inhibiting growth factors or cytokines inducing cell proliferation [196–198]. Thus, with the participation of these mechanisms, CO can exert an antiproliferative effect on the CVS smooth muscle cells.

In addition, CO contributes to the uncoupling of the mitochondrial respiration and modulates the production of ROS. During the mitochondrial oxidative phosphorylation, 1–3% of consumed oxygen is not completely reduced to the superoxide produced by the ETC and form primary moderately reactive oxygen derivatives that contribute to the formation of more reactive or secondary oxygen derivatives even under physiological conditions [177, 199]. In pathological conditions, reverse of the electron flow can lead to a persistent and enhanced ROS generation. Thus, the mild mitochondrial uncoupling is an integral cellular mechanism for limiting ROS overproduction and oxidative stress [193]. Uncoupling of the mitochondrial respiration by CO via stimulation of mitochondrial uncouplers and/or the ATP/ADP translocase plays an important role in the uncoupling of the oxidative phosphorylation at low CO levels [194].

At the same time, CO partially can inhibit electron transfer along the ETC, which results in the preconditioning at the cellular level (ATP production increase and mitochondrial respiration stimulation) [177, 193]. These CO-mediated preconditioning effects have a positive effect on the survival of the CVS cells during ischemia/reperfusion [177].

6.2 Antiapoptotic properties of H_2S

Recent studies have shown that some pharmacological drugs, which increase the endogenous synthesis of H_2S, can protect the heart from IR injury by reducing apoptosis of CM [200, 201]. In addition, H_2S improves the contractile function of the myocardium by inhibiting apoptosis of ventricular myocytes and reducing the infarction zone (preconditioning effect) [202]. At the same time, the infarction zone has been demonstrated to decrease after use of exogenous and endogenous H_2S and to increase because of pharmacological inhibition of cystathionine γ-lyase (CGL) [203].

Molecular studies have shown that the protective preconditioning effect of H_2S is associated with an increase in microRNAs (miRs) levels [204]. MicroRNAs are a recently discovered class of small noncoding RNAs that regulate gene expression at post transcriptional levels. A previous study by Kang *et al*. [205] showed that level of miR-1 was upregulated by 2.21-fold in the IR group compared to the group preconditioned with H_2S. Also, preconditioning with H_2S is protective in IR-exposed CM by regulating the expression of miR-1 and apoptosis-related genes. Histone deacetylase 4 (HDAC4) is one of the downstream target

genes of miR-1. Histone deacetylation alters the chromosome structure and affects access of the transcription factors to DNA. HDAC4 does not bind to DNA directly but indirectly via transcription factors, MEF2C/D that play a critical role in transcriptional regulation. Thus, HDAC4 is involved in the protective effect of H_2S against IR-induced apoptosis of CM.

6.3 Antiapoptotic properties of NO

As noted earlier, the effects of NO on CVS cells depend on its concentration. Higher NO concentrations depress CM function, mediate inflammatory processes following IR, impair MMP, mitochondrial respiration, IMM permeability, and finally inducing apoptosis or necrosis in CM.

Lower concentration of NO or its donor SNAP (2 μM) increase the MMP via activation of mitoK$_{ATP}$ channels [206]. Any increase in MMP will reduce the uptake of Ca^{2+} by mitochondria, restore Ca^{2+} homeostasis in CM and prevent the formation of MPTP and the initiation of the caspase cascade leading to CM apoptosis.

In addition, there is information that β3-AR adrenoreceptors and associated eNOS and nNOS pathways may be involved in the protection of cardiomyocytes from apoptosis [207]. The authors demonstrated that the number of apoptotic CM in mice with induced myocardial infarction (MI) was lower if animals were administrated with β3-AR agonist BRL37344 (BRL) at 0.1 mg/kg/hour one day after MI operation. The apoptosis index in mice with MI pretreated with BRL was by 12% lower compared to the MI group [207].

In addition, the authors evaluated the expressions of NOS isoforms after MI, as well as the role they played in the cardioprotective effects of β3-AR [207]. It is known that the eNOS expression and activation which is generally modulated by 4 phosphorylation sites, eNOS^{Ser1177}, eNOS^{Ser114}, eNOS^{Ser633} and eNOS^{Thr495} [77]. Representative blotting results and semiquantitative analyses showed that total eNOS, phosphorylated eNOS^{Ser114} and phosphorylated eNOS^{Ser633} were unchanged in all groups. However, phosphorylation of eNOS^{Ser1177}, which indicates eNOS activation, significantly decreased in MI group, whereas the expressions of phospho-eNOS^{Thr495} increased in MI group. At the same time, BRL-37344 treatment increased the expression of phosphorylated eNOS^{Ser1177} and decreased the level of phosphorylated eNOS^{Thr495}.

It was also found that mRNA expression of nNOS was significantly increased in the MI+BRL group compared to MI group. These results showed that the modulation of β3-AR on nNOS is carried out by a transcriptional pathway. Moreover, the protein expression of nNOS was increased in MI group compared to the sham group. BRL-37344 treatment resulted in a 2-fold increase in total nNOS protein expression, increase in expression of phospho-nNOS^{Ser1417} and decrease in phospho-nNOS^{Ser847} expression compared to the protein expression in MI group. In contrary, there were no differences in the expression of iNOS and phospho-iNOS in the experimental and control groups.

7. Interactions of H_2S, NO and CO in the cardiovascular system during hypoxia

Various studies have shown that the effect of the studied gas transmitters H_2S, NO and CO on the CVS cells depends on their concentration. High concentrations of these gas transmitters are toxic to cells, and low, physiological concentrations, induce vasorelaxation, angiogenesis, promote cardioprotection and inhibition of apoptosis. The transmitters share common features due to the interaction and intersection of their common cardioprotective signal pathways. For example, BK$_{Ca}$ channels play an important role in the mechanism of the cardioprotective effect of H_2S on cardiomyocytes during hypoxia, but they are also activated in response to the stimulation of CM by endogenous CO. Jaggar *et al.* [208, 209] found that CO regulates these channels by binding to reduced heme. Activators of BK$_{Ca}$ channels may have a protective effect on the CVS cells and vascular resistance during hypoxia and I/R [210]. NO is also a trigger molecule for the activation of BK$_{Ca}$ channels but increases their activity indirectly through PKG and PKA-related pathways [211]. Similar effects of the gas transmitters on molecular targets in other cardioprotective signaling pathways during hypoxia and I/R are known too [212]. These findings may be useful for the search for new therapeutic agents that modulate the metabolism and interaction of gas transmitters with each other in the CVS cells in I/R or others pathological condition accompanied by hypoxia [212, 213].

8. Prospects for the use of donors and inducers of gasotransmitters (NO, H_2S, CO) synthesis in clinical practice

8.1 Clinical significance of endogenous NO

Nitric oxide refers to compounds that have a polyfunctional effect and can have both physiological and toxic effects. The toxic effect of NO is primarily manifested in the inhibition of mitochondrial respiratory chain enzymes, which leads to a decrease in the production of ATP, as well as enzymes involved in DNA replication. In addition, excessive NO production leads to hyperactivation of the NMDA subtype of Glu receptors and increase of $[Ca^{2+}]_i$, contributing to neuropathology [214]. High concentrations of Ca^{2+} in the cytoplasm of neurons trigger neurotoxic processes, including uncoupling of the electron transport chain, activation of enzymes that may impair neurons [215]. Examples of the consequences of NO toxic effect include neurodegenerative diseases such as ischemic stroke, epilepsy, Parkinson's and Alzheimer's diseases, etc.

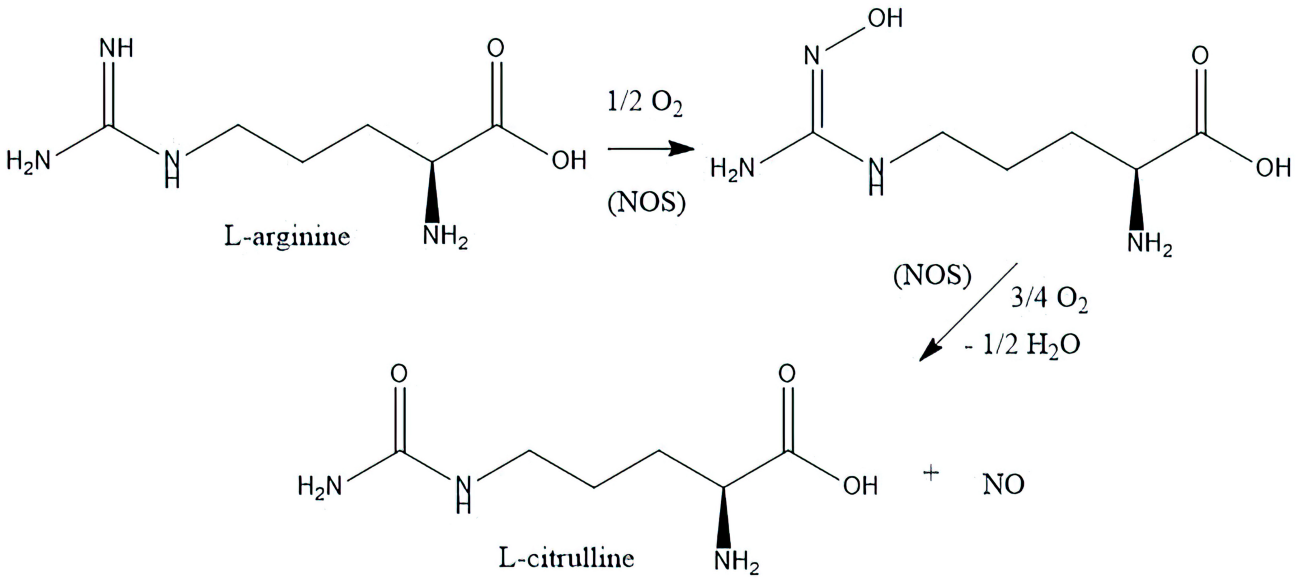

Fig. 2. Schematic representation of NO synthesis from L-arginine.

The participation of NO has been also demonstrated in the development of insulin-dependent diabetes although the direct target of the action of NO and other free radicals is the DNA of the pancreatic beta-cells in the islets of Langerhans [216]. Furthermore, excessive production of NO by the iNOS is an important link in the pathogenesis of acute circulatory failure in thermal, cardiogenic, septic and other types of shock [217].

Multiple factors such as low-density lipoproteins, high glucose concentrations, and ischemia can cause a decrease in NO production, both by inhibiting of NOS and by reducing their expression. Low levels of NO may lead to increased vascular tone, blood clotting and reduced immunity, thereby contributing to the development of hypertension, atherosclerosis, thrombosis, coronary heart disease, infectious diseases, and tumor growth [217–219].

Nitric oxide is synthesized via the oxidative reaction catalyzed by the NOS from L-arginine (Fig. 2) [220].

The formation of excessive amounts of NO is mainly caused by the activation of iNOS, localized in the cytosol of cells (mainly macrophages) and expressed under the influence of cytokines and bacterial polysaccharides. The inducible NOS produces NO hundreds and thousands of times more than the constitutive isoforms of the enzyme. It has been recently shown that iNOS is synthesized not only by macrophages, but many other cells under certain external stimuli, mainly during pathological conditions. Interaction of NO with the oxygen radical O_2^- results in formation of peroxynitrite ($ONOO^-$), which in combination with NO damages DNA and causes apoptosis in cardiomyocytes and other cells [221].

Nitric oxide is involved in various functional processes via interaction with regulatory molecules. One of the most studied functions is the relaxation of SMC. Multiple molecules such as acetylcholine, histamine, bradykinin, serotonin, adenine nucleotides, and some others are called “endothelium-dependent vasodilators”. Under physiological conditions, stimulation of the endothelium by these molecules leads to the NO synthesis. In turn, NO diffuses to SMC and stimulates GC, resulting in formation of cGMP. In the SMC of the internal organs, cGMP reduces the $[Ca^{2+}]_i$ and activates the myosin light chain kinase, causing relaxation of the SMC of digestive tract and respiratory system.

One of the most important and well-studied target organs for NO is heart. In myocardium, NO becomes one of the cardioprotective regulatory factors. Nitric oxide is synthesized in the coronary endothelium, endocardium, and cardiomyocytes. It enhances ventricular relaxation and contributes to diastolic heart function by increasing the intracellular concentration of cGMP. Under experimental conditions, NO has been demonstrated to have a pronounced effect on heart and hemodynamics by causing a decrease in heart rate, stroke volume, an increase in the duration of the PQ interval and period of expulsion.

In some cases, an increase in the level of NO can be protective. For example, it reduces mortality in patients with moderate hypercholesterolemia and atherogenic stenosis of the internal carotid artery [222].

In extreme conditions, changes in NO level might be considered as an indicator that reflects the ability of the organism to provide an adequate regional perfusion. A decrease in the level of NO metabolites in patients with ischemic heart disease is a poor prognostic sign for a long-term ischemia. The results of numerous studies justify the need for use of nitrates in the treatment of various forms of IHD in patients with reduced levels of NO metabolites [222]. Thus, NO donor drugs or drugs that stimulate the release of NO from endothelium have a therapeutic inter-

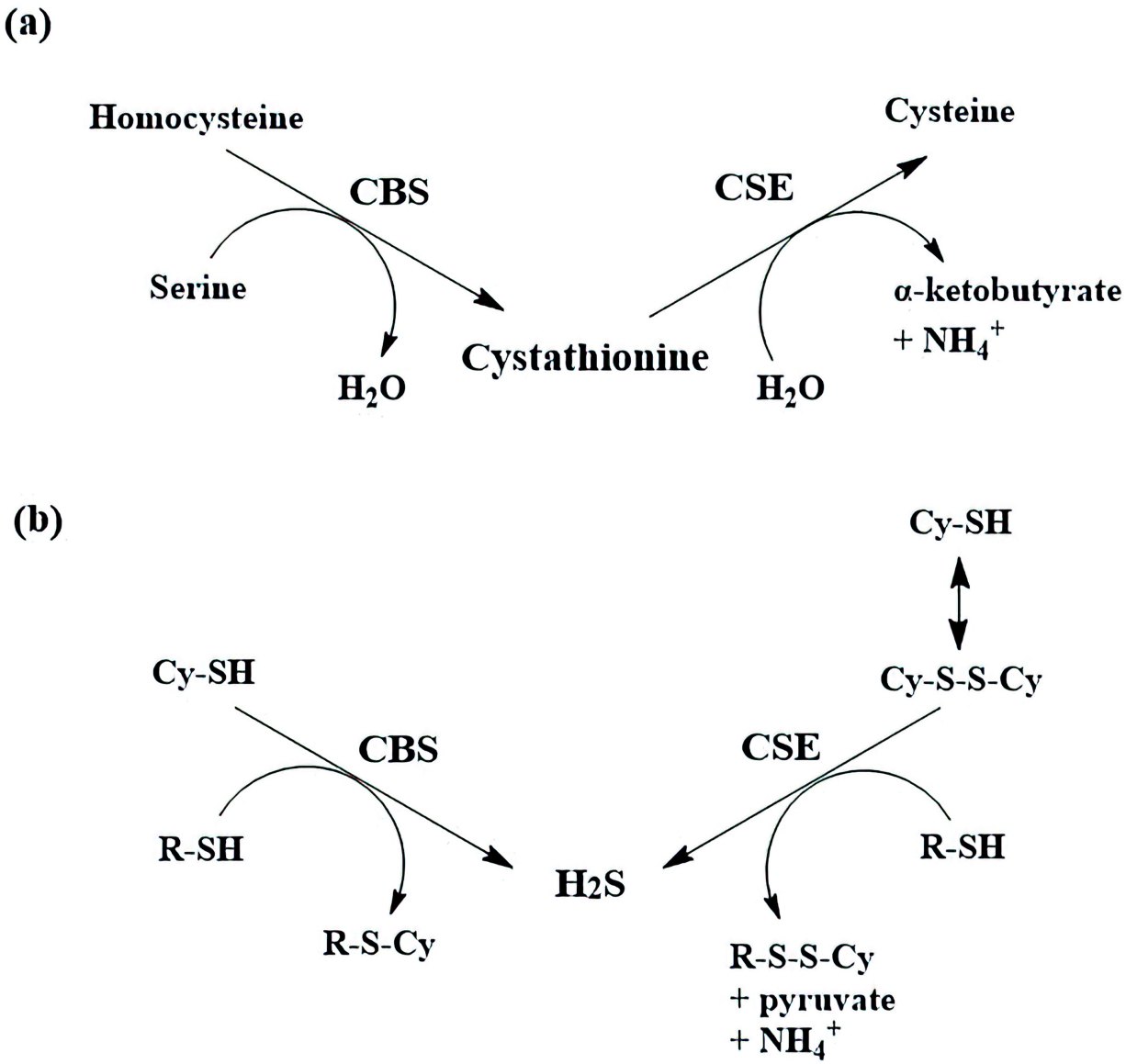

Fig. 3. Sulfur metabolism and H_2S producing reactions. (a) The classically described roles of CBS and CSE in sulfur metabolism. CBS condenses homocysteine with serine to generate the thiol ether cystathionine. CSE hydrolyzes cystathionine into cysteine, α-ketobutyrate and ammonia. (b) H_2S producing reactions catalyzed by CBS and CSE. CBS catalyzes the β-replacement reaction of cysteine (Cy–SH) with a variety of thiols (R–SH) to generate H_2S and the corresponding thiol ether (R–S–Cy). CSE catalyzes the β-disulfide elimination reaction of cystine (Cy–S–S–Cy), this is followed by a reaction with a variety of thiols, to generate H_2S and the corresponding disulfide (R–S–S–Cy) [226].

est. The donors of NO include traditional heart drugs such as nitroglycerin and other organic nitrates, which serve as exogenous sources of NO. They have a strong side effect caused by a sudden drop in blood pressure due to NO hyperproduction. In this regard, more attention has been currently given to the development of new drugs for clinical use that have modulating properties without significant side effects. Such modulators include drugs of nitrate-like action molsidomine, sodium nitroprusside that stimulate the activity of guanylate cyclase and NOS. Nebivolol is another potent option to patients with newly diagnosed or poorly controlled hypertension. This drug, a representative of the latest group of β_1-blockers, is characterized by a very high degree of cardioselectivity (the index of blocking β_1/β_2-receptors is 293, 10–20 times higher than the similar index of the vast majority of other β_1-blockers) [223] and modulates NO release by endotheliocytes. The results of different studies proved efficacy and safety profile of nebivolol in patients with heart failure, arterial hypertension and CHD [224].

At low concentrations of NO, other endogenous gasotransmitters (H_2S and CO) can act as NO mimetics causing similar physiological changes as vasodilation.

8.2 Clinical significance of donors and inducers of H_2S

Hydrogen sulfide is involved in the regulation of many physiological processes, including homeostasis, immunity, and transmission of nerve impulses in the cells of the central and peripheral nervous system. It also plays a vital role in vasodilation and reducing blood pressure. The discovery of these properties of H_2S marked the beginning of a new direction in pharmacology associated with the development of a fundamentally new group of antihypertensive drugs whose actions is based on release of H_2S molecules from endogenic or exogenic sources.

The endogenous source of H_2S in the cells is cysteine (8. Hydrogen sulfide synthesis is carried out by three enzymes, namely cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST), depending on the cell type [225]. Cystathionine-β-synthase synthesizes H_2S primarily in neurons. In CM and VSMC, the synthesis of H_2S is carried out by CSE. The third H2S – producing enzyme, 3-MST, was found in endothelial cells) (Fig. 3, Ref. [226]).

Clinical studies have shown that the level of H_2S in the blood plasma of individuals with normal blood pressure was 34 microM, while it was reduced to 20 microM in patients with arterial hypertension. Inhalation of H_2S gas contributed to a decrease in blood pressure parameters in hypertensive patients [227]. In experiments on rat models, it was found that intravenous administration of a H_2S solution caused a dose-dependent decrease in blood pressure [226].

In vitro, H_2S donor sodium hydrosulfide (NaHS), which is actively used in experimental practice, also caused relaxation of thoracic aorta, mesenteric, renal arteries and portal vein. The relaxing effect of H_2S on smooth muscle cells is associated with effects on cGC, and, as we noted earlier, on K_{ATP} channels [23, 228, 229].

Hydrogen sulfide reduces myocardial contractility both *in vitro* and *in vivo* [66, 228, 229]. This effect is partially related to the activation of K_{ATP} channels in CM [230]. It has been demonstrated that myocardial infarction in rats is associated with reduction in concentration of H_2S by 60% compared to the control group. In addition, the intraperitoneal injection of NaHS (14 microM/kg) significantly reduced mortality among rats with myocardial infarction [231].

Given the importance of H_2S in the regulation of vascular tone, new drugs that are exogenous H_2S donors, inducers, and inhibitors of endogenous H_2S synthesis are currently being developed [232].

Right now, sodium hydrosulfide (NaHS) and sodium sulfide Na_2S are the most often used H_2S donors in experimental practice. However, when these molecules are dissolved, H_2S is released too quickly that causes a sharp drop in blood pressure *in vivo*, resulting in vascular collapse [231]. In this case, difficulties related to handling H_2S release make NaHS and Na_2S unsuitable for therapeutic use.

Li Ling and co-authors [233] obtained a new H_2S donor designated as GYY4137. Unlike NaHS, GYY4137 releases H_2S gradually, which makes this molecule more promising for further pharmacological applications. In experiments on rat models and *in vitro*, GYY4137 has demonstrated vasodilatory properties and antihypertensive effect [234].

Another direction in the development of H_2S-based drugs is based on incorporation of H_2S-releasing groups into already existing and widely used drug molecules. Alternative H_2S donors can be obtained by attaching sulfide groups to nonsteroidal anti-inflammatory drugs [235, 236].

Furthermore, inhibitors of enzymes H_2S synthesis can be used in order to reduce pathologically high concentrations of H_2S. These inhibitors include DL-propargylglycine with high lipophilic properties that allow the inhibitor to pass easily through cell membrane without causing noticeable damage to it [237]. However, DL-propargylglycine is characterized by low selectivity and inhibits not only CSE, which is an enzyme of H_2S synthesis in the cardiovascular system, but also CBS in neurons, affecting the central nervous system function [238].

8.3 Clinical significance of endogenous CO donors

Carbon monoxide (CO) is formed during the oxidative cleavage of protoheme IX by heme oxygenase-1 (HO-1) [128]. In turn, protoheme IX is formed in the process of heme catabolism from hemoglobin and myoglobin as well as other hemeproteins (Fig. 4).

During the reaction, heme is converted to biliverdin by the enzyme heme oxygenase, CO is produced, and the iron is released from the heme as the ferrous ion. Biliverdin then is converted to bilirubin by the biliverdin reductase. All three products of the heme oxygenase reaction are biologically active.

Studies on the role of endogenous CO as an anti-inflammatory agent and cytoprotector have been conducted in numerous laboratories around the world [239]. These properties of endogenous CO make it an interesting therapeutic target for the treatment of such pathological conditions as tissue injury caused by ischemia and subsequent reperfusion (for example, myocardial infarction, ischemic stroke), graft rejection, vascular atherosclerosis, severe sepsis, severe malaria, and autoimmune diseases. Human clinical trials have also been conducted, but the results have not been published yet [240].

Experimental approaches of cancer therapies include the use of free CO donors ($[Ru(CO)_3Cl_2]_2$, $Mn_2(CO)_{10}$, etc.) and inhalation of CO gas. Despite the certain effectiveness of these approaches in mouse models, their clinical trials are stalled due to doubts about the therapeutic index [241].

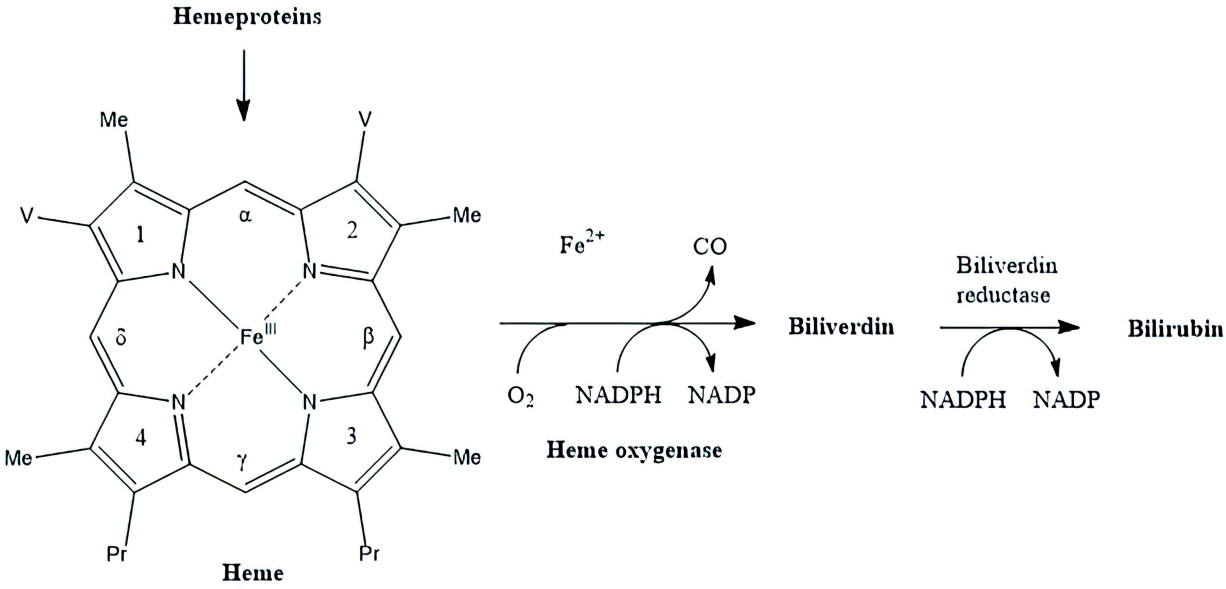

Fig. 4. Schematic representation of CO formation as a result of heme catabolism.

9. Conclusions

In summary, the adaptive changes in the CVS cells in chronic diseases and response to hypoxia are closely associated with the participation of Ca^{2+} ions and gas transmitters (NO, H_2S, CO). These molecules affect blood vessels tone, angiogenesis, and cell survival under conditions of hypoxia and oxidative stress initiated by hypoxia. Effects of these gas molecules and Ca^{2+} are mediated through activation of signaling mechanisms affecting the mitochondrial function and activity of such important regulators of intracellular processes as PKG, PI3K, p38 MAPK, JNK1/2, sGC, cGMP, NF-kappaB, HIF-1alpha, and ion channels (mitoK_{ATP} and BK_{Ca} channels). The energy synthesizing and Ca^{2+}-depositing function of the cells depend on throughput capacity of these channels. Development of new drugs which molecular targets are mitochondrial channels will offer new ways for prevention and treatment of the CVS diseases.

10. Author contributions

IS generated the idea of this review, collected materials and wrote the main text; VN participated in the formulation of the research problem; LE participated in the writing an article; SK collected literary materials and participated in data analysis. All authors participated in the drafting, writing and approval of the final version of this review.

11. Ethics approval and consent to participate

Not applicable.

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14. Conflict of interest

The authors declare no conflict of interest.

15. References

[1] Zorov DB, Isaev NK, Plotnikov EY, Zorova LD, Stelmashook EV, Vasileva AK, *et al*. The mitochondrion as Janus Bifrons. Biochemistry. 2007; 72: 1115–1126.

[2] Okamura K, Nakagama Y, Takeda N, Soma K, Sato T, Isagawa T, *et al*. Therapeutic targeting of mitochondrial ROS ameliorates murine model of volume overload cardiomyopathy. Journal of Pharmacological Sciences. 2019; 141: 56–63.

[3] Lukyanova LD, Germanova EL, Tsybina TA, Chernobaeva GN. Energotropic Effect of Succinate-Containing Derivatives of 3-Hydroxypyridine. Bulletin of Experimental Biology and Medicine. 2009; 148: 587–591.

[4] Lukyanova LD, Kirova YI, Germanova EL. The Role of Succinate in Regulation of Immediate HIF-1alpha Expression in Hypoxia. Bulletin of Experimental Biology and Medicine. 2018; 164; 298–303.

[5] Levrat C, Louisot P. Study of the succinate dehydrogenase activation in permeabilized mitochondria through the Ca(2+)-stimulated phospholipase a2. Biochemistry and Molecular Biology International. 1994; 34: 569–578.

[6] Tabibzadeh S. Nature creates, adapts, protects and sustains life using hydrogen sulfide. Frontiers in Bioscience. 2016; 21: 528–560.

[7] Khan AA, Schuler MM, Prior MG, Yong S, Coppock RW, Florence LZ, *et al*. Effects of hydrogen sulfide exposure on lung mitochondrial respiratory chain enzymes in rats. Toxicology and Applied Pharmacology. 1990; 103: 482–490.

[8] Goubern M, Andriamihaja M, Nübel T, Blachier F, Bouillaud F. Sulfide, the first inorganic substrate for human cells. FASEB Journal. 2007; 21: 1699–1706.

[9] Semenykhina OM, Strutyns'ka NA, Bud'ko AI, Vavilova HL, Sahach VF. Effect of hydrogen sulfide donor NaHs on the functional state of the respiratory chain of the rat heart mitochondria. Fiziolohichnyi Zhurnal. 2013; 59: 9–17. (In Ukrainian)

[10] Chang L, Geng B, Yu F, Zhao J, Jiang H, Du J, *et al*. Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats. Amino Acids. 2008; 34: 573–585.

[11] Jackson MR, Loll PJ, Jorns MS. X-Ray Structure of Human Sulfide: Quinone Oxidoreductase: Insights into the Mechanism of Mitochondrial Hydrogen Sulfide Oxidation. Structure. 2019; 27: 794–805.e4.

[12] Bełtowski J. Hydrogen sulfide in pharmacology and medicine–an update. Pharmacological Reports. 2015; 67: 647–658.

[13] Djuric D, Jakovljevic V, Zivkovic V, Srejovic I. Homocysteine and homocysteine-related compounds: an overview of the roles in the pathology of the cardiovascular and nervous systems. Canadian Journal of Physiology and Pharmacology. 2018; 96: 991–1003.

[14] Yang Q, He G. Imbalance of Homocysteine and H2S: Significance, Mechanisms, and Therapeutic Promise in Vascular Injury. Oxidative Medicine and Cellular Longevity. 2019; 2019: 1–11.

[15] Nandi SS, Mishra PK. H2S and homocysteine control a novel feedback regulation of cystathionine beta synthase and cystathionine gamma lyase in cardiomyocytes. Scientific Reports. 2017; 7: 3639.

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

[17] Tyagi N, Mishra PK, Tyagi SC. Homocysteine, hydrogen sulfide (H2S) and NMDA-receptor in heart failure. Indian Journal of Biochemistry & Biophysics. 2009; 46: 441–446.

[18] Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiological Reviews. 2003; 83: 1113–1151.

[19] Shemarova IV, Nesterov VP. Molecular Basis of Cardioprotection in Ischemic Heart Disease. Journal of Evolutionary Biochemistry and Physiology. 2019; 55: 163–173.

[20] Sabino JPJ, Traslaviña GAA, Branco LGS. Role of central hydrogen sulfide on ventilatory and cardiovascular responses to hypoxia in spontaneous hypertensive rats. Respiratory Physiology & Neurobiology. 2016; 231: 21–27.

[21] Shimanskaia TV, Strutinskaia NA, Vavilova GL, Goshovskaia IV, Semenikhina EN, Sagach VF. Cyclosporin a-sensitive mitochondrial pore as a target of cardioprotective action of hydrogen sulfide donor. Rossiiskii Fiziologicheskii Zhurnal Imeni i.M. Sechenova. 2013; 99: 261–272. (In Russian)

[22] Testai L, Marino A, Piano I, Brancaleone V, Tomita K, Di Cesare Mannelli L, *et al*. The novel H 2 S-donor 4-carboxyphenyl isothiocyanate promotes cardioprotective effects against ischemia/reperfusion injury through activation of mitoK ATP channels and reduction of oxidative stress. Pharmacological Research. 2016; 113: 290–299.

[23] Walewska A, Szewczyk A, Koprowski P. Gas Signaling Molecules and Mitochondrial Potassium Channels. International Journal of Molecular Sciences. 2018; 19: E3227.

[24] Sitdikova GF, Fuchs R, Kainz V, Weiger TM, Hermann A. Phosphorylation of BK channels modulates the sensitivity to hydrogen sulfide (H2S). Frontiers in Physiology. 2014; 5: 431.

[25] Telezhkin V, Brazier SP, Cayzac S, Müller CT, Riccardi D, Kemp PJ. Hydrogen Sulfide Inhibits Human BKCa Channels. Advances in Experimental Medicine and Biology. 2009; 368: 65–72.

[26] Cordeiro B, Shinn C, Sellke FW, Clements RT. Rottlerin-induced BKCa channel activation impairs specific contractile responses and promotes vasodilation. The Annals of Thoracic Surgery. 2015; 99: 626–634.

[27] Ahmad A, Dempsey SK, Daneva Z, Azam M, Li N, Li PL, *et al*. Role of nitric oxide in the cardiovascular and renal systems. International Journal of Molecular Sciences. 2018; 19: E2605.

[28] Nishikawa Y, Miki T, Awa M, Kuwata K, Tamura T, Hamachi I. Development of a Nitric Oxide-Responsive Labeling Reagent for Proteome Analysis of Live Cells. ACS Chemical Biology. 2019; 14: 397–404.

[29] Cheng Y, Feng Y, Xia Z, Li X, Rong J. omega-Alkynyl arachidonic acid promotes anti-inflammatory macrophage M2 polarization against acute myocardial infarction via regulating the cross-talk between PKM2, HIF-1alpha and iNOS. BBA Molecular and Cell Biology of Lipids. 2017; 1862: 1595–1605.

[30] Li D, Wang X, Huang Q, Li S, Zhou Y, Li Z. Cardioprotection of CAPE-oNO(2) against myocardial ischemia/reperfusion induced ROS generation via regulating the SIRT1/eNOS/NF-kappaB pathway in vivo and in vitro. Redox Biology. 2018; 15: 62–73.

[31] Santana MNS, Souza DS, Miguel-dos-Santos R, Rabelo TK, Vasconcelos CMLD, Navia-Pelaez JM, *et al*. Resistance exercise mediates remote ischemic preconditioning by limiting cardiac eNOS uncoupling. Journal of Molecular and Cellular Cardiology. 2018; 125: 61–72.

[32] Tsibulnikov SY, Maslov LN, Naryzhnaya NV, Ma H, Lishmanov YB, Oeltgen PR, *et al*. Role of protein kinase C, PI3 kinase, tyrosine kinases, no-synthase, KATP channels and MPT pore in the signaling pathway of the cardioprotective effect of chronic continuous hypoxia. General Physiology and Biophysics. 2018; 37: 537–547.

[33] Piazza M, Dieckmann T, Guillemette JG. Investigation of the structure and dynamic of calmodulin-nitric oxide synthase complexes using NMR spectroscopy. Frontiers in Bioscience. 2018; 23: 1902–1922.

[34] Taylor CT, Moncada S. Nitric Oxide, Cytochrome C Oxidase, and the Cellular Response to Hypoxia. Arteriosclerosis, Thrombosis, and Vascular Biology. 2010; 30: 643–647.

[35] Giulivi C. Mitochondria as generators and targets of nitric oxide. Novartis Foundation Symposium. 2007; 287: 92–94.

[36] Parihar MS, Parihar A, Villamena FA, Vaccaro PS, Ghafourifar P. Inactivation of mitochondrial respiratory chain complex i leads mitochondrial nitric oxide synthase to become pro-oxidative. Biochemical and Biophysical Research Communications. 2008; 367: 761–767.

[37] Li W, Wu N, Shu W, Jia D, Jia P. Pharmacological preconditioning and postconditioning with nicorandil attenuates ischemia/reperfusion-induced myocardial necrosis and apoptosis in hypercholesterolemic rats. Experimental and Therapeutic Medicine. 2015; 10: 2197–2205.

[38] Kim Y, Pae H, Park JE, Lee YC, Woo JM, Kim N, *et al*. Heme oxygenase in the regulation of vascular biology: from molecular mechanisms to therapeutic opportunities. Antioxidants & Redox Signaling. 2011; 14: 137–167.

[39] Araujo JA, Zhang M, Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. Frontiers in Pharmacology. 2012; 3: 119.

[40] Bilban M, Haschemi A, Wegiel B, Chin BY, Wagner O, Otterbein LE. Heme oxygenase and carbon monoxide initiate homeostatic signaling. Journal of Molecular Medicine. 2008; 86: 267–279.

[41] Dulak J, Deshane J, Jozkowicz A, Agarwal A. Heme oxygenase-1 and carbon monoxide in vascular pathobiology: focus on angiogenesis. Circulation. 2008; 117: 231–241.

[42] Ndisang JF. Heme oxygenase in cardiac repair and regeneration. Frontiers in Bioscience. 2016; 21: 251–277.

[43] Origassa CST, Câmara NOS. Cytoprotective role of heme oxygenase-1 and heme degradation derived end products in liver injury. World Journal of Hepatology. 2013; 5: 541–549.

[44] Motterlini R, Clark JE, Foresti R, Sarathchandra P, Mann BE, Green CJ. Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities. Circulation Research. 2002; 90: E17–E24.

[45] Perry MM, Tildy B, Papi A, Casolari P, Caramori G, Rempel KL, *et al*. The anti-proliferative and anti-inflammatory response of COPD airway smooth muscle cells to hydrogen sulfide. Respiratory Research. 2018; 19: 85.

[46] Li J, Teng X, Jin S, Dong J, Guo Q, Tian D, *et al*. Hydrogen sulfide improves endothelial dysfunction by inhibiting the vicious cycle of NLRP3 inflammasome and oxidative stress in spontaneously hypertensive rats. Journal of Hypertension. 2019; 37: 1633–1643.

[47] Gao D, Xu J, Qin W, Peng L, Qiu Z, Wang L, *et al*. Cellular Mechanism Underlying Hydrogen Sulfide Mediated Epithelial K+ Secretion in Rat Epididymis. Frontiers in Physiology. 2019; 9: 1886.

[48] Li F, Luo J, Wu Z, Xiao T, Zeng O, Li L, *et al*. Hydrogen sulfide exhibits cardioprotective effects by decreasing endoplasmic reticulum stress in a diabetic cardiomyopathy rat model. Molecular Medicine Reports. 2016; 14: 865–873.

[49] Zhang R, Lin Y, Wang W, Wang X. Excessive nNOS/no/AMPK signaling activation mediated by the blockage of the CBS/H2S system contributes to oxygen-glucose deprivation-induced endoplasmic reticulum stress in PC12 cells. International Journal of Molecular Medicine. 2017; 40: 549–557.

[50] Kaczara P, Motterlini R, Kus K, Zakrzewska A, Abramov AY, Chlopicki S. Carbon monoxide shifts energetic metabolism from glycolysis to oxidative phosphorylation in endothelial cells. FEBS Letters. 2016; 590: 3469–3480.

[51] Duan W, Hicks J, Makara MA, Ilkayeva O, Abraham DM. TASK-1 and TASK-3 channels modulate pressure overload-induced cardiac remodeling and dysfunction. American Journal of Physiology-Heart and Circulatory Physiology. 2020; 318: H566–H580.

[52] Musameh MD, Fuller BJ, Mann BE, Green CJ, Motterlini R. Positive inotropic effects of carbon monoxide-releasing molecules (CO-RMs) in the isolated perfused rat heart. British Journal of Pharmacology. 2006; 149: 1104–1112.

[53] Ndisang JF, Chibbar R, Lane N. Heme oxygenase suppresses markers of heart failure and ameliorates cardiomyopathy in L-NAME-induced hypertension. European Journal of Pharmacology. 2014; 734: 23–34.

[54] Duckles H, Boycott HE, Al-Owais MM, Elies J, Johnson E, Dallas ML, *et al*. Heme oxygenase-1 regulates cell proliferation via carbon monoxide-mediated inhibition of T-type Ca2+ channels. Pflugers Archiv: European Journal of Physiology. 2015; 467: 415–427.

[55] Peers C, Dallas ML, Scragg JL. Ion channels as effectors in carbon monoxide signaling. Communicative & Integrative Biology. 2009; 2: 241–242.

[56] Wang J, Sun J, Qiao S, Li H, Che T, Wang C, *et al.* Effects of isoflurane on complex II associated mitochondrial respiration and reactive oxygen species production: Roles of nitric oxide and mitochondrial KATP channels. Molecular Medicine Reports. 2019; 20: 4383–4390.

[57] Jaitovich A, Jourd'heuil D. A Brief Overview of Nitric Oxide and Reactive Oxygen Species Signaling in Hypoxia-Induced Pulmonary Hypertension. Advances in Experimental Medicine and Biology. 2017; 967: 71–81.

[58] Mughal A, Sun C, O'Rourke ST. Activation of Large Conductance, Calcium-Activated Potassium Channels by Nitric Oxide Mediates Apelin-Induced Relaxation of Isolated Rat Coronary Arteries. The Journal of Pharmacology and Experimental Therapeutics. 2019; 366: 265–273.

[59] Zhao Y, Ge J, Li X, Guo Q, Zhu Y, Song J, *et al*. Vasodilatory effect of formaldehyde via the no/cGMP pathway and the regulation of expression of KATP, BKCa and L-type Ca2+ channels. Toxicology Letters. 2019; 312: 55–64.

[60] Rochette L, Lorin J, Zeller M, Guilland J, Lorgis L, Cottin Y, *et al*. Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: possible therapeutic targets? Pharmacology & Therapeutics. 2013; 140: 239–257.

[61] Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, *et al*. Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. Circulation Research. 2011; 109: 1259–1268.

[62] Ha J, Xu Y, Kawano T, Hendon T, Baki L, Garai S, *et al*. Hydrogen sulfide inhibits Kir2 and Kir3 channels by decreasing sensitivity to the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2). The Journal of Biological Chemistry. 2018; 293: 3546–3561.

[63] Xu H, Zhao M, Liang S, Huang Q, Xiao Y, Ye L, *et al*. The Effects of Puerarin on Rat Ventricular Myocytes and the Potential Mechanism. Scientific Reports. 2016; 6: 35475.

[64] Henn MC, Janjua MB, Kanter EM, Makepeace CM, Schuessler RB, Nichols CG, *et al*. Adenosine Triphosphate-Sensitive Potassium Channel Kir Subunits Implicated in Cardioprotection by Diazoxide. Journal of the American Heart Association. 2015; 4: e002016.

[65] Zhao Y, Wei H, Kong G, Shim W, Zhang G. Hydrogen sulfide augments the proliferation and survival of human induced pluripotent stem cell-derived mesenchymal stromal cells through inhibition of BKCa. Cytotherapy. 2013; 15: 1395–1405.

[66] Li Y, Zang Y, Fu S, Zhang H, Gao L, Li J. H2S relaxes vas deferens smooth muscle by modulating the large conductance Ca2+ -activated K+ (BKCa) channels via a redox mechanism. The Journal of Sexual Medicine. 2012; 9: 2806–2813.

[67] Sitdikova GF, Weiger TM, Hermann A. Hydrogen sulfide increases calcium-activated potassium (BK) channel activity of rat pituitary tumor cells. PflüGers Archiv - European Journal of Physiology. 2010; 459: 389–397.

[68] Kanno S, Lee PC, Zhang Y, Ho C, Griffith BP, Shears LL, *et al*. Attenuation of myocardial ischemia/reperfusion injury by superinduction of inducible nitric oxide synthase. Circulation. 2000; 101: 2742–2748.

[69] Alánová P, Kolář F, Ošťádal B, Neckář J. Role of no/cGMP Signaling Pathway in Cardiac Ischemic Tolerance of Chronically Hypoxic Rats. Physiological Research. 2015; 64: 783–787.

[70] Griecsová L, Farkašová V, Gáblovský I, Khandelwal VKM, Bernátová I, Tatarková Z, *et al*. Effect of maturation on the resistance of rat hearts against ischemia. Study of potential molecular mechanisms. Physiological Research. 2015; 64 Suppl 5: S685–S696.

[71] Belge C, Massion PB, Pelat M, Balligand JL. Nitric oxide and the heart: update on new paradigms. Annals of the New York Academy of Sciences. 2005; 1047: 173–182.

[72] Shao J, Miao C, Geng Z, Gu M, Wu Y, Li Q. Effect of eNOS on Ischemic Postconditioning-Induced Autophagy against Ischemia/Reperfusion Injury in Mice. BioMed Research International. 2019; 2019: 1–11.

[73] Farrell K, Simmers P, Mahajan G, Boytard L, Camardo A, Joshi J, *et al*. Alterations in phenotype and gene expression of adult human aneurysmal smooth muscle cells by exogenous nitric oxide. Experimental Cell Research. 2019; 384: 111589.
[74] Li Q, Ye T, Long T, Peng X. Ginkgetin exerts anti-inflammatory effects on cerebral ischemia/reperfusion-induced injury in a rat model via the TLR4/NF-kappaB signaling pathway. Bioscience, Biotechnology, and Biochemistry. 2019; 83: 675–683.
[75] Talman WT, Nitschke Dragon D. Neuronal nitric oxide mediates cerebral vasodilatation during acute hypertension. Brain Research. 2007; 1139: 126–132.
[76] Wang Z, Yan Y, Wang Y, Tong F. The interaction between CSE/H2S and the iNOS/no-mediated resveratrol/poly(ethylene glycol)-poly(phenylalanine) complex alleviates intestinal ischemia/reperfusion injuries in diabetic rats. Biomedicine & Pharmacotherapy. 2019; 112: 108736.
[77] Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature. 1999; 399: 601–605.
[78] Murthy S, Koval OM, Ramiro Diaz JM, Kumar S, Nuno D, Scott JA, *et al*. Endothelial CaMKII as a regulator of eNOS activity and no-mediated vasoreactivity. PLoS ONE. 2017; 12: e0186311.
[79] Wang W, Jiang D, Zhu Y, Liu W, Duan J, Dai S. Relaxing effects of phytoestrogen alpha-zearalanol on rat thoracic aorta rings in vitro. Chinese Journal of Physiology. 2009; 52: 99–105.
[80] Kanai AJ, Pearce LL, Clemens PR, Birder LA, VanBibber MM, Choi SY, *et al*. Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98: 14126–14131.
[81] Dedkova EN, Blatter LA. Characteristics and function of cardiac mitochondrial nitric oxide synthase. The Journal of Physiology. 2009; 587: 851–872.
[82] Zaobornyj T, Ghafourifar P. Strategic localization of heart mitochondrial NOS: a review of the evidence. American Journal of Physiology. Heart and Circulatory Physiology. 2012; 303: H1283–H1293.
[83] Forstermann U, Sessa WS. Nitric oxide syntheses: regulation and function. European Heart Journal. 2012; 33: 829–837.
[84] Tang L, Wang H, Ziolo MT. Targeting NOS as a therapeutic approach for heart failure. Pharmacology & Therapeutics. 2014; 142: 306–315.
[85] Shumaev KB, Sviriaeva IV, Gubkina SA, Krivova TS, Topunov AF, Vanin AF, *et al*. Formation of dinitrosyl iron complexes in cardiac mitochondria. Biofizika. 2010; 55: 460–466. (In Russian)
[86] Zanella B, Giordano E, Muscari C, Zini M, Guarnieri C. Nitric oxide synthase activity in rat cardiac mitochondria. Basic Research in Cardiology. 2004; 99: 159–164.
[87] Nagendran J, Michelakis ED. Mitochondrial NOS is upregulated in the hypoxic heart: implications for the function of the hypertrophied right ventricle. American Journal of Physiology-Heart and Circulatory Physiology. 2009; 296: H1723–H1726.
[88] O'Rourke B. Evidence for mitochondrial K+ channels and their role in cardioprotection. Circulation Research. 2004; 94: 420–432.
[89] Mironova GD, Shigaeva MI, Gritsenko EN, Murzaeva SV, Gorbacheva OS, Germanova EL, *et al*. Functioning of the mitochondrial ATP-dependent potassium channel in rats varying in their resistance to hypoxia. Involvement of the channel in the process of animal's adaptation to hypoxia. Journal of Bioenergetics and Biomembranes. 2010; 42: 473–481.
[90] Zaugg M, Schaub MC. Signaling and cellular mechanisms in cardiac protection by ischemic and pharmacological preconditioning. Journal of Muscle Research and Cell Motility. 2003; 24: 219–249.
[91] Reutov VP, Sorokina EG. No-synthase and nitrite-reductase components of nitric oxide cycle. Biochemistry. Biokhimiia. 1998; 63: 874–884.
[92] Chistiakov DA, Shkurat TP, Melnichenko AA, Grechko AV, Orekhov AN. The role of mitochondrial dysfunction in cardiovascular disease: a brief review. Annals of Medicine. 2018; 50: 121–127.
[93] Gualano B, Artioli GG, Poortmans JR, Lancha Junior AH. Exploring the therapeutic role of creatine supplementation. Amino Acids. 2010; 38: 31–44.
[94] Vico TA, Marchini T, Ginart S, Lorenzetti MA, Adán Areán JS, Calabró V, *et al*. Mitochondrial bioenergetics links inflammation and cardiac contractility in endotoxemia. Basic Research in Cardiology. 2018; 114: 38.
[95] Tsibulnikov SY, Maslov LN, Gorbunov AS, Voronkov NS, Boshchenko AA, Popov SV, *et al*. A Review of Humoral Factors in Remote Preconditioning of the Heart. Journal of Cardiovascular Pharmacology and Therapeutics. 2019; 24: 403–421.
[96] Dedkova EN, Ji X, Lipsius SL, Blatter LA. Mitochondrial calcium uptake stimulates nitric oxide production in mitochondria of bovine vascular endothelial cells. American Journal of Physiology. Cell Physiology. 2004; 286: C406–C415.
[97] Luo R, Chen X, Ma H, Yao C, Liu M, Tao J, *et al*. Myocardial caspase-3 and NF-kappaB activation promotes calpain-induced septic apoptosis: The role of Akt/eNOS/NO pathway. Life Science. 2019; 222: 195–202.
[98] Vaux DL. Apoptogenic factors released from mitochondria. Biochimica Et Biophysica Acta. 2011; 1813: 546–550.
[99] Erusalimsky JD, Moncada S. Nitric oxide and mitochondrial signaling: from physiology to pathophysiology. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007; 27: 2524–2531.
[100] Beltrán B, Mathur A, Duchen MR, Erusalimsky JD, Moncada S. The effect of nitric oxide on cell respiration: a key to understanding its role in cell survival or death. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97: 14602–14607.
[101] Moncada S, Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? Nature Reviews. Molecular Cell Biology. 2002; 3: 214–220.
[102] Si J, Wang N, Wang H, Xie J, Yang J, Yi H, *et al*. HIF-1alpha signaling activation by post-ischemia treatment with astragaloside IV attenuates myocardial ischemia-reperfusion injury. PLoS ONE. 2014; 9: e107832.
[103] Levchenkova OS, Novikov VE, Abramova ES, Feoktistova ZA. Signal Mechanism of the Protective Effect of Combined Preconditioning by Amtizole and Moderate Hypoxia. Bulletin of Experimental Biology and Medicine. 2018; 164: 320–323.
[104] Nagle DG, Zhou Y. Natural product-derived small molecule activators of hypoxia-inducible factor-1 (HIF-1). Current Pharmaceutical Design. 2006; 12: 2673–2688.
[105] Simonovic N, Jakovljevic V, Jeremic J, Finderle Z, Srejovic I, Nikolic Turnic T, *et al*. Comparative effects of calcium and potassium channel modulators on ischemia/reperfusion injury in the isolated rat heart. Molecular and Cellular Biochemistry. 2019; 450: 175–185.
[106] Sasaki N, Sato T, Ohler A, O'Rourke B, Marbán E. Activation of mitochondrial ATP-dependent potassium channels by nitric oxide. Circulation. 2000; 101: 439–445.
[107] Ljubkovic M, Shi Y, Cheng Q, Bosnjak Z, Jiang MT. Cardiac mitochondrial ATP-sensitive potassium channel is activated by nitric oxide in vitro. FEBS Letters. 2007; 581: 4255–4259.
[108] Dahlem YA, Horn TFW, Buntinas L, Gonoi T, Wolf G, Siemen D. The human mitochondrial KATP channel is modulated by calcium and nitric oxide: a patch-clamp approach. Biochimica Et Biophysica Acta. 2004; 1656: 46–56.

[109] Kawano T, Zoga V, Kimura M, Liang M, Wu H, Gemes G, *et al*. Nitric Oxide Activates ATP-Sensitive Potassium Channels in Mammalian Sensory Neurons: Action by Direct S-Nitrosylation. Molecular Pain. 2009; 5: 1744–1712.

[110] Kim J, Ohshima S, Pediaditakis P, Lemasters JJ. Nitric oxide: a signaling molecule against mitochondrial permeability transition- and pH-dependent cell death after reperfusion. Free Radical Biology & Medicine. 2004; 37: 1943–1950.

[111] Costa ADT, Jakob R, Costa CL, Andrukhiv K, West IC, Garlid KD. The mechanism by which the mitochondrial ATP-sensitive K+ channel opening and H2O2 inhibit the mitochondrial permeability transition. The Journal of Biological Chemistry. 2006; 281: 20801–20808.

[112] Sánchez-Duarte E, Trujillo X, Cortés-Rojo C, Saavedra-Molina A, Camargo G, Hernández L, *et al*. Nicorandil improves post-fatigue tension in slow skeletal muscle fibers by modulating glutathione redox state. Journal of Bioenergetics and Biomembranes. 2017; 49: 159–170.

[113] Zhang D, Chai Y, Erickson JR, Brown JH, Bers DM, Lin Y. Intracellular signalling mechanism responsible for modulation of sarcolemmal ATP-sensitive potassium channels by nitric oxide in ventricular cardiomyocytes. The Journal of Physiology. 2014; 592: 971–990.

[114] Frankenreiter S, Bednarczyk P, Kniess A, Bork NI, Straubinger J, Koprowski P, *et al*. cGMP-elevating compounds and ischemic conditioning provide cardioprotection against ischemia and reperfusion injury via cardiomyocyte-specific BKCa channels. Circulation. 2017; 136: 2337–2355.

[115] Piantadosi CA. Biological chemistry of carbon monoxide. Antioxidants & Redox Signaling. 2002; 4: 259–270.

[116] Ernst A, Zibrak JD. Carbon monoxide poisoning. The New England Journal of Medicine. 1998; 339: 1603–1608.

[117] Hampson NB: U.S. Mortality due to carbon monoxide poisoning, 1999–2014. Accidental and intentional deaths. American Thoracic Society. 2016; 13: 1768–1774.

[118] Kim H, Choi S. Therapeutic Aspects of Carbon Monoxide in Cardiovascular Disease. International Journal of Molecular Sciences. 2018; 19: E2381.

[119] Townsend CL, Maynard RL. Effects on health of prolonged exposure to low concentrations of carbon monoxide. Occupational and Environmental Medicine. 2002; 59: 708–711.

[120] Haldane J. The Action of Carbonic Oxide on Man. The Journal of Physiology. 2005; 18: 430–462.

[121] Sawicki CA, Gibson QH. The relation between carbon monoxide binding and the conformational change of hemoglobin. Biophysical Journal. 1979; 24: 21–33.

[122] Rose JJ, Wang L, Xu Q, McTiernan CF, Shiva S, Tejero J, *et al*. Carbon Monoxide Poisoning: Pathogenesis, Management, and Future Directions of Therapy. American Journal of Respiratory and Critical Care Medicine. 2017; 195: 596–606.

[123] Almeida AS, Figueiredo-Pereira C, Vieira HLA. Carbon monoxide and mitochondria-modulation of cell metabolism, redox response and cell death. Frontiers in Physiology. 2015; 6: 33.

[124] Huang Y, Ye Z, Ma T, Li H, Zhao Y, Chen W, *et al*. Carbon monoxide (CO) modulates hydrogen peroxide (H2O2)-mediated cellular dysfunction by targeting mitochondria in rabbit lens epithelial cells. Experimental Eye Research. 2018; 169: 68–78.

[125] Ryter SW. Heme oxygenase-1/carbon monoxide as modulators of autophagy and inflammation. Archives of Biochemistry and Biophysics. 2019; 678:108186.

[126] Morita T, Perrella MA, Lee ME, Kourembanas S. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. Proceedings of the National Academy of Sciences of the United States of America. 1995; 92: 1475–1479.

[127] Ndisang JF, Tabien HEN, Wang R. Carbon monoxide and hypertension. Journal of Hypertension. 2004; 22: 1057–1074.

[128] Wu L, Wang R. Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. Pharmacological Reviews. 2005; 57: 585–630.

[129] Maines MD. The heme oxygenase system: a regulator of second messenger gases. Annual Review of Pharmacology and Toxicology. 1997; 37: 517–554.

[130] Li C, Zhang C, Wang T, Xuan J, Su C, Wang Y. Heme oxygenase 1 induction protects myocardiac cells against hypoxia/reoxygenation-induced apoptosis the role of JNK/c-Jun/Caspase-3 inhibition and Akt signaling enhancement. Herz. 2016; 41: 715–724.

[131] Zhang J, Cai W, Fan Z, Yang C, Wang W, Xiong M, *et al*. MicroRNA-24 inhibits the oxidative stress induced by vascular injury by activating the Nrf2/Ho-1 signaling pathway. Atherosclerosis. 2019; 290: 9–18.

[132] Ryter SW, Alam J, Choi AMK. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiological Reviews. 2006; 86: 583–650.

[133] Stec DE, Drummond HA, Vera T. Role of carbon monoxide in blood pressure regulation. Hypertension. 2008; 51: 597–604.

[134] Untereiner AA, Wu L, Wang R. The role of carbon monoxide as a gasotransmitter in cardiovascular and metabolic regulation. In: Hermann A, Sitdikova GF, Weiger ThM (eds.) Gasotransmitters: Physiology and Pathophysiology (pp. 37–70). Springer, Heidelberg: Berlin. 2012.

[135] Kozakowska M, Dulak J, Józkowicz A. Heme oxygenase-1 - more than the cytoprotection. Postepy Biochemii. 2015; 61: 147–158. (In Polish)

[136] Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. Pharmacological Reviews. 2008; 60: 79–127.

[137] Khan AA, Quigley JG. Control of intracellular heme levels: heme transporters and heme oxygenases. Biochimica Et Biophysica Acta. 2011; 1813: 668–682.

[138] Jin X, Xu Z, Fan R, Wang C, Ji W, Ma Y, *et al*. HO-1 alleviates cholesterol-induced oxidative stress through activation of Nrf2/ERK and inhibition of PI3K/AKT pathways in endothelial cells. Molecular Medicine Reports. 2017; 16: 3519–3527.

[139] Chodorowski Z, Sein Anand J, Nowak-Banasik L, Szydłowska M, Klimek J, Kaletha K. Carbon monoxide-a regulator of vascular tone in hypoxia? Przeglad Lekarski. 2005; 62: 438–440.

[140] Leffler CW, Parfenova H, Jaggar JH. Carbon monoxide as an endogenous vascular modulator. American Journal of Physiology. Heart and Circulatory Physiology. 2011; 301: H1–H11.

[141] Yuan P, Leonetti MD, Pico AR, Hsiung Y, MacKinnon R. Structure of the human BK channel Ca2+-activation apparatus at 3.0 a resolution. Science. 2010; 329: 182–186.

[142] Naik JS, Walker BR. Heme oxygenase-mediated vasodilation involves vascular smooth muscle cell hyperpolarization. American Journal of Physiology. Heart and Circulatory Physiology. 2003; 285: H220–H228.

[143] Yang H, Zhang G, Cui J. BK channels: multiple sensors, one activation gate. Frontiers in Physiology. 2015; 6: 29.

[144] Miller C. An overview of the potassium channel family. Genome Biology. 2000; 1: REVIEWS0004.

[145] Brenner R, Peréz GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, *et al*. Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. Nature. 2000; 407: 870–876.

[146] Wang R, Wu L, Wang Z. The direct effect of carbon monoxide on K Ca channels in vascular smooth muscle cells. PfluGers Archiv: European Journal of Physiology. 1997; 434: 285–291.

[147] Pérez GJ, Bonev AD, Nelson MT. Micromolar Ca(2+) from sparks activates Ca(2+)-sensitive K(+) channels in rat cerebral artery smooth muscle. American Journal of Physiology. Cell Physiology. 2001; 281: C1769–C1775.

[148] Jin H, Du J, Li X, Wang Y, Liang Y, Tang C. Interaction between hydrogen sulfide/cystathionine gamma-lyase and carbon monoxide/heme oxygenase pathways in aortic smooth muscle cells. Acta Pharmacologica Sinica. 2006; 27: 1561–1566.
[149] Tang XD, Xu R, Reynolds MF, Garcia ML, Heinemann SH, Hoshi T. Heme can bind to and inhibit mammalian calcium-dependent slo1 BK channels. Nature. 2003; 425: 531–535.
[150] Horrigan FT, Heinemann SH, Hoshi T. Heme regulates allosteric activation of the Slo1 BK channel. The Journal of General Physiology. 2005; 126: 7–21.
[151] Xi Q, Tcheranova D, Parfenova H, Horowitz B, Leffler CW, Jaggar JH. Carbon monoxide activates KCa channels in newborn arteriole smooth muscle cells by increasing apparent Ca2+ sensitivity of alpha-subunits. American Journal of Physiology. Heart and Circulatory Physiology. 2004; 286: H610–H618.
[152] Wellman GC, Nathan DJ, Saundry CM, Perez G, Bonev AD, Penar PL, *et al*. Ca2+ sparks and their function in human cerebral arteries. Stroke. 2002; 33: 802–808.
[153] Li A, Adebiyi A, Leffler CW, Jaggar JH. KCa channel insensitivity to Ca2+ sparks underlie fractional uncoupling in newborn cerebral artery smooth muscle cells. American Journal of Physiology-Heart and Circulatory Physiology. 2006; 291: 1118–1125.
[154] Li A, Xi Q, Umstot ES, Bellner L, Schwartzman ML, Jaggar JH, *et al*. Astrocyte-derived CO is a diffusible messenger that mediates glutamate-induced cerebral arteriolar dilation by activating smooth muscle Cell KCa channels. Circulation Research. 2008; 102: 234–241.
[155] Jaggar JH, Leffler CW, Cheranov SY, Tcheranova D, E S, Cheng X. Carbon monoxide dilates cerebral arterioles by enhancing the coupling of Ca2+ sparks to Ca2+-activated K+ channels. Circulation Research. 2002; 91: 610–617.
[156] Orlov SN, Tremblay J, Hamet P. CAMP signaling inhibits dihydropyridine-sensitive Ca2+ influx in vascular smooth muscle cells. Hypertension. 1996; 27: 774–780.
[157] Hou S, Heinemann SH, Hoshi T. Modulation of BKCa Channel Gating by Endogenous Signaling Molecules. Physiology. 2009; 24: 26–35.
[158] Anfinogenova YJ, Baskakov MB, Kovalev IV, Kilin AA, Dulin NO, Orlov SN. Cell-volume-dependent vascular smooth muscle contraction: role of Na+, K+, 2Cl- cotransport, intracellular Cl- and L-type Ca2+ channels. Pflugers Archiv: European Journal of Physiology. 2004; 449: 42–55.
[159] Motterlini R, Foresti R. Biological signaling by carbon monoxide and carbon monoxide-releasing molecules. American Journal of Physiology. Cell Physiology. 2017; 312: C302–C313.
[160] Hosein S, Marks GS, Brien JF, McLaughlin BE, Nakatsu K. An extracellular source of heme can induce a significant heme oxygenase mediated relaxation in the rat aorta. Canadian Journal of Physiology and Pharmacology. 2002; 80: 761–765.
[161] Marks GS, Brien JF, Nakatsu K, McLaughlin BE. Does carbon monoxide have a physiological function? Trends in Pharmacological Sciences. 1991; 12: 185–188.
[162] Koneru P, Leffler CW. Role of cGMP in carbon monoxide-induced cerebral vasodilation in piglets. American Journal of Physiology. Heart and Circulatory Physiology. 2004; 286: H304–H309.
[163] Khavandi K, Baylie RA, Sugden SA, Ahmed M, Csato V, Eaton P, *et al*. Pressure-induced oxidative activation of PKG enables vasoregulation by Ca2+ sparks and BK channels. Science Signaling. 2016; 9: ra100.
[164] Wilkinson WJ, Kemp PJ. Carbon monoxide: an emerging regulator of ion channels. The Journal of Physiology. 2011; 589: 3055–3062.
[165] Kapetanaki SM, Burton MJ, Basran J, Uragami C, Moody PCE, Mitcheson JS, *et al*. A mechanism for CO regulation of ion channels. Nature Communications. 2018; 9: 907.
[166] Heinemann SH, Hoshi T, Westerhausen M, Schiller A. Carbon monoxide–physiology, detection and controlled release. Chemical Communications. 2014; 50: 3644–3660.
[167] Gagov H, Kadinov B, Hristov K, Boev K, Itzev D, Bolton T, *et al*. Role of constitutively expressed heme oxygenase-2 in the regulation of guinea pig coronary artery tone. Pflugers Archiv: European Journal of Physiology. 2003; 446: 412–421.
[168] Lamon BD, Zhang FF, Puri N, Brodsky SV, Goligorsky MS, Nasjletti A. Dual Pathways of Carbon Monoxide–Mediated Vasoregulation: modulation by redox mechanisms. Circulation Research. 2009; 105: 775–783.
[169] Choi YK, Por ED, Kwon Y, Kim Y. Regulation of ROS production and vascular function by carbon monoxide. Oxidative Medicine and Cellular Longevity. 2012; 2012: 794237.
[170] Peers C, Lefer DJ. Emerging roles for gasotransmitters. Experimental Physiology. 2011; 96: 831–832.
[171] Morita T, Mitsialis SA, Koike H, Liu Y, Kourembanas S. Carbon monoxide controls the proliferation of hypoxic vascular smooth muscle. Journal of Biological Chemistry. 1997; 272: 32804–32809.
[172] Snijder PM, van den Berg E, Whiteman M, Bakker SJL, Leuvenink HGD, van Goor H. Emerging Role of Gasotransmitters in Renal Transplantation. American Journal of Transplantation. 2013; 13: 3067–3075.
[173] Kourembanas S. Hypoxia and carbon monoxide in the vasculature. Antioxidants & Redox Signaling. 2002; 4: 291–299.
[174] Moody BF, Calvert JW. Emergent role of gasotransmitters in ischemia-reperfusion injury. Medical Gas Research. 2011; 1: 3.
[175] Elmore S. Apoptosis: a review of programmed cell death. Toxicologic Pathology. 2007; 35: 495–516.
[176] Wang X, Wang Y, Kim HP, Nakahira K, Ryter SW, Choi AMK. Carbon monoxide protects against hyperoxia-induced endothelial cell apoptosis by inhibiting reactive oxygen species formation. The Journal of Biological Chemistry. 2007; 282: 1718–1726.
[177] Queiroga CSF, Almeida AS, Vieira HLA. Carbon monoxide targeting mitochondria. Biochemistry Research International. 2012; 2012: 749845.
[178] Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. Physiological Reviews. 2007; 87: 99–163.
[179] Kantari C, Walczak H. Caspase-8 and bid: caught in the act between death receptors and mitochondria. Biochimica Et Biophysica Acta. 2011; 1813: 558–563.
[180] Desagher S, Osen-Sand A, Nichols A, Eskes R, Montessuit S, Lauper S, *et al*. Bid-induced Conformational Change of Bax is Responsible for Mitochondrial Cytochrome c Release during Apoptosis. Journal of Cell Biology. 1999; 144: 891–901.
[181] Eskes R, Desagher S, Antonsson B, Martinou JC. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Molecular and Cellular Biology. 2000; 20: 929–935.
[182] Terrones O, Antonsson B, Yamaguchi H, Wang H, Liu J, Lee RM, *et al*. Lipidic pore formation by the concerted action of proapoptotic BAX and tBID. The Journal of Biological Chemistry. 2004; 279: 30081–30091.
[183] Martinou JC, Green DR. Breaking the mitochondrial barrier. Nature Reviews. Molecular Cell Biology. 2001; 2: 63–67.
[184] Antonsson B, Montessuit S, Lauper S, Eskes R, Martinou JC. Bax oligomerization is required for channel-forming activity in liposomes and to trigger cytochrome c release from mitochondria. The Biochemical Journal. 2000; 345 Pt 2: 271–278.
[185] Huang DC, Strasser A. BH3-only proteins-essential initiators of apoptotic cell death. Cell. 2000; 103: 839–842.
[186] Walsh CM, Luhrs KA, Arechiga AF. The “fuzzy logic” of the death-inducing signaling complex in lymphocytes. Journal of Clinical Immunology. 2003; 23: 333–353.

[187] Salvesen GS, Riedl SJ. Structure of the Fas/FADD complex: a conditional death domain complex mediating signaling by receptor clustering. Cell Cycle. 2009; 8: 2723–2727.
[188] Wang X, Zhang J, Kim HP, Wang Y, Choi AMK, Ryter SW. Bcl-XL disrupts death-inducing signal complex formation in plasma membrane induced by hypoxia/reoxygenation. FASEB Journal. 2004; 18: 1826–1833.
[189] Wang X, Wang Y, Zhang J, Kim HP, Ryter SW, Choi AMK. FLIP protects against hypoxia/reoxygenation-induced endothelial cell apoptosis by inhibiting Bax activation. Molecular and Cellular Biology. 2005; 25: 4742–4751.
[190] Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998; 94: 491–501.
[191] Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, *et al*. Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. The Journal of Experimental Medicine. 2000; 192: 1015–1026.
[192] Kim HS, Loughran PA, Kim PK, Billiar TR, Zuckerbraun BS. Carbon monoxide protects hepatocytes from TNF-alpha/Actinomycin D by inhibition of the caspase-8-mediated apoptotic pathway. Biochemical and Biophysical Research Communications. 2006; 344: 1172–1178.
[193] Long R, Salouage I, Berdeaux A, Motterlini R, Morin D. CORM-3, a water soluble CO-releasing molecule, uncouples mitochondrial respiration via interaction with the phosphate carrier. Biochimica Et Biophysica Acta. 2014; 1837: 201–209.
[194] Lo Iacono L, Boczkowski J, Zini R, Salouage I, Berdeaux A, Motterlini R, *et al*. A carbon monoxide-releasing molecule (CORM-3) uncouples mitochondrial respiration and modulates the production of reactive oxygen species. Free Radical Biology & Medicine. 2011; 50: 1556–1564.
[195] Song R, Mahidhara RS, Liu F, Ning W, Otterbein LE, Choi AMK. Carbon Monoxide Inhibits Human Airway Smooth Muscle Cell Proliferation via Mitogen-Activated Protein Kinase Pathway. American Journal of Respiratory Cell and Molecular Biology. 2002; 27: 603–610.
[196] Schachter M. Vascular smooth muscle cell migration, atherosclerosis, and calcium channel blockers. International Journal of Cardiology. 1997; 62: S85–S90.
[197] Libby P, Sukhova G, Lee RT, Liao JK. Molecular biology of atherosclerosis. International Journal of Cardiology. 1997; 62 Suppl 2: S23–S29.
[198] Schwartz SM. Smooth muscle migration in atherosclerosis and restenosis. The Journal of Clinical Investigation. 1997; 100: S87–S89.
[199] Ribas V, García-Ruiz C, Fernández-Checa JC. Glutathione and mitochondria. Frontiers in Pharmacology. 2014; 5: 151.
[200] Wu H, Ye M, Yang J, Ding J, Yang J, Dong W, *et al*. Nicorandil Protects the Heart from Ischemia/Reperfusion Injury by Attenuating Endoplasmic Reticulum Response-induced Apoptosis through PI3K/Akt Signaling Pathway. Cellular Physiology and Biochemistry. 2015; 35: 2320–2332.
[201] Wang X, Cao Y, Shen M, Wang B, Zhang W, Liu Y, *et al*. DIDS reduces ischemia/reperfusion-induced myocardial injury in rats. Cellular Physiology and Biochemistry. 2015; 35: 676–688.
[202] Zhang Y, Li H, Zhao G, Sun A, Zong NC, Li Z, *et al*. Hydrogen sulfide attenuates the recruitment of CD11b+Gr-1+ myeloid cells and regulates Bax/Bcl-2 signaling in myocardial ischemia injury. Scientific Reports. 2014; 4: 4774.
[203] Sivarajah A, McDonald MC, Thiemermann C. The production of hydrogen sulfide limits myocardial ischemia and reperfusion injury and contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia in the rat. Shock. 2006; 26: 154–161.
[204] Kang B, Li W, Xi W, Yi Y, Ciren Y, Shen H, *et al*. Hydrogen Sulfide Protects Cardiomyocytes against Apoptosis in Ischemia/Reperfusion through MiR-1-Regulated Histone Deacetylase 4 Pathway. Cellular Physiology and Biochemistry. 2017; 41: 10–21.
[205] Kang B, Hong J, Xiao J, Zhu X, Ni X, Zhang Y, *et al*. Involvement of miR-1 in the protective effect of hydrogen sulfide against cardiomyocyte apoptosis induced by ischemia/reperfusion. Molecular Biology Reports. 2014; 41: 6845–6853.
[206] Bell RM, Maddock HL, Yellon DM. The cardioprotective and mitochondrial depolarising properties of exogenous nitric oxide in mouse heart. Cardiovascular Research. 2003; 57: 405–415.
[207] Schulz R, Kelm M, Heusch G. Nitric oxide in myocardial ischemia/reperfusion injury. Cardiovascular Research. 2004; 61: 402–413.
[208] Jaggar JH, Li A, Parfenova H, Liu J, Umstot ES, Dopico AM, *et al*. Heme is a carbon monoxide receptor for large-conductance Ca2+-activated K+ channels. Circulation Research. 2005; 97: 805–812.
[209] Hou S, Xu R, Heinemann SH, Hoshi T. The RCK1 high-affinity Ca2+ sensor confers carbon monoxide sensitivity to Slo1 BK channels. Proceedings of the National Academy of Sciences. 2008; 105: 4039–4043.
[210] Mahan V. Cardiac function dependence on carbon monoxide. Medical Gas Research. 2020; 10: 37.
[211] Bae H, Lim I. Effects of nitric oxide on large-conductance Ca2+ -activated K+ currents in human cardiac fibroblasts through PKA and PKG-related pathways. Clinical and Experimental Pharmacology & Physiology. 2017; 44: 1116–1124.
[212] Andreadou I, Iliodromitis EK, Rassaf T, Schulz R, Papapetropoulos A, Ferdinandy P. The role of gasotransmitters no, H2S and CO in myocardial ischaemia/reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. British Journal of Pharmacology. 2015; 172: 1587–1606.
[213] Wu D, Hu Q, Zhu D. An Update on Hydrogen Sulfide and Nitric Oxide Interactions in the Cardiovascular System. Oxidative Medicine and Cellular Longevity. 2018; 2018: 4579140.
[214] Schröter A, Andrabi SA, Wolf G, Horn TFW. Nitric oxide applications prior and simultaneous to potentially excitotoxic NMDA-evoked calcium transients: cell death or survival. Brain Research. 2005; 1060: 1–15.
[215] Ghasemi M, Mayasi Y, Hannoun A, Eslami SM, Carandang R. Nitric Oxide and Mitochondrial Function in Neurological Diseases. Neuroscience. 2018; 376: 48–71.
[216] Welsh N, Eizirik DL, Sandler S. Nitric oxide and pancreatic beta-cell destruction in insulin dependent diabetes mellitus: don't take no for an answer. Autoimmunity. 1994; 18: 285–290.
[217] Kilbourn RG, Traber DL, Szabó C. Nitric oxide and shock. Disease-a-Month. 1997; 43: 277–348.
[218] Holotiuk* VV, Kryzhanivska AY, Churpiy IK, Tataryn BB, Ivasiutyn DY. Role of nitric oxide in pathogenesis of tumor growth and its possible application in cancer treatment. Experimental Oncology. 2019; 41: 210–215.
[219] Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. Journal of the American College of Cardiology. 2013; 62: 263–271.
[220] Böger RH. The pharmacodynamics of L-arginine. Alternative Therapies in Health and Medicine. 2014; 20: 48–54.
[221] Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiological Reviews. 2007; 87: 315–424.
[222] Naseem KM. The role of nitric oxide in cardiovascular diseases. Molecular Aspects of Medicine. 2005; 26: 33–65.
[223] Sanaee F, Jamali F. Action and disposition of the β3-agonist nebivolol in the presence of inflammation; an alternative to conventional β1-blockers. Current Pharmaceutical Design. 2014; 20: 1311–1317.

[224] DiNicolantonio JJ, Fares H, Niazi AK, Chatterjee S, D'Ascenzo F, Cerrato E, *et al*. B-Blockers in hypertension, diabetes, heart failure and acute myocardial infarction: a review of the literature. Open Heart. 2015; 2: e000230.
[225] Bełtowski J. Synthesis, Metabolism, and Signaling Mechanisms of Hydrogen Sulfide: an Overview. Methods in Molecular Biology. 2019; 16: 1–8.
[226] Gadalla MM, Snyder SH. Hydrogen sulfide as a gasotransmitter. Journal of Neurochemistry. 2010; 113: 14–26.
[227] Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H2S as novel endogenous gaseous KATP channel opener. EMBO Journal. 2001; 20: 6008–6016.
[228] Zhao W, Wang R. H(2)S-induced vasorelaxation and underlying cellular and molecular mechanisms. American Journal of Physiology. Heart and Circulatory Physiology. 2002; 283: H474–H480.
[229] Tang G, Wu L, Liang W, Wang R. Direct stimulation of K(ATP) channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. Molecular Pharmacology. 2005; 68: 1757–1764.
[230] Łowicka E, Bełtowski J. Hydrogen sulfide (H2S) - the third gas of interest for pharmacologists. Pharmacological Reports. 2007; 59: 4–24.
[231] Geng B, Yang J, Qi Y, Zhao J, Pang Y, Du J, *et al*. H2S generated by heart in rat and its effects on cardiac function. Biochemical and Biophysical Research Communications. 2004; 313: 362–368.
[232] Elsey DJ, Fowkes RC, Baxter GF. Regulation of cardiovascular cell function by hydrogen sulfide (H(2)S). Cell Biochemistry and Function. 2010; 28: 95–106.
[233] Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, Lee SW, *et al*. Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. Circulation. 2008; 117: 2351–2360.
[234] Rose P, Dymock BW, Moore PK. GYY4137, a novel water-soluble, H2S-releasing molecule. Methods in Enzymology. 2015; 554: 143–167.
[235] Gemici B, Wallace JL. Anti-inflammatory and cytoprotective properties of hydrogen sulfide. Methods in Enzymology. 2015; 555: 169–193.
[236] Gemici B, Elsheikh W, Feitosa KB, Costa SKP, Muscara MN, Wallace JL. H2S-releasing drugs: anti-inflammatory, cytoprotective and chemopreventative potential. Nitric Oxide: Biology and Chemistry. 2015; 46: 25–31.
[237] Zhou X, Tang S, Hu K, Zhang Z, Liu P, Luo Y, *et al*. DL-Propargylglycine protects against myocardial injury induced by chronic intermittent hypoxia through inhibition of endoplasmic reticulum stress. Sleep & Breathing. 2018; 22: 853–863.
[238] Guo W, Cheng Z, Zhu Y. Hydrogen sulfide and translational medicine. Acta Pharmacologica Sinica. 2013; 34: 1284–1291.
[239] Ryter SW, Choi AMK. Cytoprotective and anti-inflammatory actions of carbon monoxide in organ injury and sepsis models. Novartis Foundation Symposium. 2007; 280: 165–181.
[240] Ryter SW. Therapeutic Potential of Heme Oxygenase-1 and Carbon Monoxide in Acute Organ Injury, Critical Illness, and Inflammatory Disorders. Antioxidants. 2020; 9: 1153.
[241] Ryter SW, Choi AM. Carbon monoxide in exhaled breath testing and therapeutics. Journal of Breath Research. 2013; 7: 017111.

Abbreviations: BH_4, tetrahydrobiopterin; BK_{Ca} channel, Ca^{2+}-activated potassium channel BK; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; CaM, calmodulin; CBS, cystathione beta synthase; cGMP, cyclic guanosine monophosphate; CM, cardiomyocytes; cNOS, constitutive NO-synthase; CO, carbon monoxide; COHb, carboxyhemoglobin; CSE, cystathione gamma lyase; CVS, cardiovascular system; DISC, death-inducing signal complex; DTT, dithiothreitol; eNOS, endothelial NO synthase; EPO, erythropoietin; ETC, electron transport chain; FAD, flavin adenine dinucleotide; FADD, Fas-associated death domain; FMN-P, flavin mononucleotide phosphate; HIF-1, hypoxia-induced factor-1; Hcy, homocysteine; HHcy, hyperhomocysteinemia; tHcy, total Hcy; HO, heme oxygenase; HDAC4, histone deacetylase 4; H_2S, hydrogen sulfide; MI, myocardial infarction; IMM, inner mitochondrial membrane; IHD, ischemic heart disease; iNOS, inducible NO synthase; IRI, ischemic reperfusion injury; KATP channels, ATP-dependent potassium channels; MEC, mitochondrial enzyme complexes; METC, mitochondrial electron transport chain; mitoBK_{Ca}, mitochondrial Ca^{2+}-activated potassium channel BK; mitoK_{ATP}, mitochondrial ATP-dependent potassium channels; MCM, mitochondria of cardiomyocytes; MMP, membrane mitochondrial potential; mNOS, macrophage NO synthase; MPTP, mitochondrial permeability transition pore; mtNOS, mitochondrial NO synthase; NADH, nicotinamide adenine dinucleotide reduced; NADPH, nicotinamide adenine dinucleotide phosphate reduced; NaHS, sodium hydrosulfide; nNOS, neuronal nitric oxide synthase; NODS, nitroxidergic system; NO, nitric oxide; NOES, nitroxidergic system; PIP_2, phosphatidyl inositol bisphosphate ($PI(4;5)P_2$); PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PM, plasma membrane; PN, peroxynitrite; ROS, Reactive Oxygen Species; RNS, Reactive Nitrogen Species; RyR, ryanodine receptor; sGC, soluble guanylate cyclase; SMC, smooth muscle cells; SNAP, S-nitroso-N-acetylpenicillamine; SR, sarcoplasmic reticulum; TASK-3, tandem pore domain acid-sensitive K^+ (TASK)-3 channels; TCA cycle, tricarboxylic acid cycle; VSMC, vascular smooth muscle cells; VEGF, vascular endothelial growth factor; 4-CPI, 4-carboxyphenyl isothiocyanate; 5-HD, 5-hydroxydecanoate.

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