Review

Mitochondrial mechanisms by which gasotransmitters (H$_2$S, 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$_2$S, NO and CO) that influence on many cellular processes, including mitochondrial biogenesis, oxidative phosphorylation, K$^+$/$\text{Ca}^{2+}$ 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$_2$S, 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.
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, H₂S, 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<sub>ATP</sub>, and BK<sub>Ca</sub> 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<sub>2</sub>S

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 (H₂S, 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 Ca²⁺ influx into mitochondria, the intramitochondrial Ca²⁺ 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 (H₂S), 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, H2S, 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 H2S intoxication, its intracellular use and utilization have been developed during evolution [6]. Many studies have been focused on mechanisms underlying the effect of H2S at the organism and cellular levels etc. [6–10]. In this context, we are interested in studies related directly to the effect of H2S 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 H2S at low concentrations vis-à-vis activates mitochondria because it serves as an energetic mitochondrial substrate [8]. In the mitochondria, H2S oxidation involves several enzymes including sulfide-quinone oxidoreductase, persulfide dioxygenase and sulfite oxidase. During the oxidation, protons released from H2S 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 H2S on CM and VSMCs has shown that H2S 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 H2S 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 H2S 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 H2S reduced production of superoxide anion and H2O2 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 H2S 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 H2S 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 H2S 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-1 alpha (HIF-1alpha) [19]. The current review elaborates mechanisms that are involved into activation of KATP channels with the participation of H2S generated during hypoxia [20] (Table 1, Ref. [14–17, 20–60]).

Previous physiological studies have demonstrated that H2S 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 mitoKATP 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 H2S on the CVS. It has been found that endogenous hydrogen sulfide affects the KATP channels activity in smooth muscle cells of blood vessels. The intensity of K+ currents of incoming rectification increased after exposure to H2S, 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 H2S-mediated activation of mitochondrial KATP Channels [61]. Herewith, the enhancement in the activity of KATP channels was accompanied with an increase of the conjugation of PIP2 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 H2S changes activity of other isoforms of Kir subunits (Kir2 and Kir3) of KATP 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 mitoKATP channels [16]. These findings served as the forerunner for the biochemical and molecular studies aimed at unraveling the cellular mechanisms underlying the H2S effect on cardioprotection and CVS. The endogenous H2S has been found to have impact on the activity of KATP channels in the smooth muscle and endothelial cells of blood vessels, where the intensity of the K+ currents of incoming rectification enhanced by H2S and decreased by cystathionine gamma-lyase and cystathionine beta-synthase [22]. Unfortunately, there are no electrophysiological data confirming involvement of H2S 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 (H₂S) 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 inflamma- some activation | Inhibition the vicious cycle of oxidative stress and inflammation in hypertension | [45, 46] |
| KATP | Increasing of MMP, decreasing of mitochondrial Ca²⁺ 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 Ca²⁺ 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 expression of glycolysis genes, GLUT family (glucose transporter) genes, EPO and VEGF/R genes. Modulating redox signaling, omega-Alkynyl arachidonic acid diminished 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] |
| 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 and reducing of cardiac hypertrophy                                                                      | [23, 51]   |
| Carbon monoxide-releasing molecules (CO-RMs) | Stimulation of cGMP and Na/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 cell proliferation (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$\text{ATP}$ channels. However, there are studies that support indirect participation of H$_2$S, when using hydrogen sulfide donors, in the regulation of mitoK$\text{ATP}$ 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$_2$S 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$_2$S 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$_2$S 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$_2$S 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$_2$S donor that pointed out the involvement of mitoK$\text{ATP}$ channels in the H$_2$S-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$_2$S molecules could be associated with the protection of thiol groups of mitochondrial proteins, particularly, adenine nucleotide translocase.

In addition, the protective effect of H$_2$S on the CVS cells could be explained by its modulatory effect on mitochondrial high conductance Ca$^{2+}$-dependent K$^+$ channels (mitoBK$\text{Ca}_n$). However, there is no scientific evidence supporting this assumption [23, 65, 66].

So far, H$_2$S has been known to regulate channels similar to BK$\text{Ca}_n$ located in the plasma membranes of different types of electrically excitable cells. This supports involvement of H$_2$S into the electrically controlled transport mechanisms that occur directly in the mitochondrial membranes [24, 25, 66, 67]. Studies of H$_2$S donors’ effect on BK$\text{Ca}_n$ channels have revealed that exposure of pituitary tumor cells GH3 to NaHS increased the opening time average of single BK$\text{Ca}_n$ 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$\text{Ca}_n$ channels by released H$_2$S 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$_2$S 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$\text{ATP}$ and BK$\text{Ca}_n$ channels [23, 26]. Based on this, H$_2$S 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 nitrooxidergic 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 NO belong to a group of constitutive NOS (cNOS). The macrophage 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 NO 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 NO synthase 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 Ca²⁺-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 (Ca²⁺), and, at the Ca²⁺ 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 Ca²⁺ level in the endothelial cells. As a consequence, Ca²⁺ in combination with the CaM activates Ca²⁺-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 (Vmax) 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 (Ca²⁺), increase. This leads to the activation of eNOS to produce NO that diffuses into the VSMC and causes their contraction. In neurons, Ca²⁺ 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. Hereewith, 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 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$_3$)) 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 thios 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 consequence 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 inducers of NOS. Currently, cNOS inducers 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 sarcosomal 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-D,L-penicillamine, activates only mitoK_{ATP} channels and does not alter sarcosomal 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 involved 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 and wild-type SUR1/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-nitrosothiols 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 O₂ 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, diphenil) [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 anapoptotic 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 BKₐCa channels by CO has been considered as the main mechanism of CO action in the CVS cells [142, 143].

Under physiological conditions, BKₐCa channels can be activated by electrical stimuli or increased [Ca²⁺]. Their function is to repolarize the membrane potential and remove K⁺ from the cell [143, 144]. The BKₐCa channels contain a pore-forming alpha-subunit and an auxiliary beta 1-subunit, which increases the channel sensitivity to Ca²⁺ ions. The CO sensitizes BKₐCa channels and regulates their activity to maintain intracellular Ca²⁺ level within micromolar concentrations [145, 146]. Local Ca²⁺ transients (Ca²⁺ sparks) are required to activate BKₐCa channels in SMC. They help to maintain the required concentration of Ca²⁺ in the micromolar range by activating the ryanodine receptors (RyR) localized in the sarcoplasmic reticulum [147, 148]. Some of the BKₐCa channels are highly sensitive to Ca²⁺ and can be activated by a single Ca²⁺ 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 Ca²⁺ channels located in the plasma membrane. This leads to a decrease in global intracellular Ca²⁺ concentration and vasorelaxation.

The vasorelaxing activity of CO is mediated by its binding to the alpha-subunit of the BKₐCa 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 BKₐCa channels [149, 150]. At the same time, the binding of CO to the BKₐCa 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 BKₐCa channel is a CO receptor, and CO binding increases the sensitivity of the BKₐCa channel to Ca²⁺, in turn [139, 151]. The CO increasing sensitivity of BKₐCa channels to Ca²⁺ enhances coupling of Ca²⁺ with activated Ca²⁺ sparks of BKₐCa channels [139, 151–153]. The CO also raises the conjugation of Ca²⁺ with BKₐCa channels by increasing the Ca²⁺ sparks frequency as a result of RyR activation [154, 155].

Since the suppression of Ca²⁺ oscillations or blocking the BKₐCa channels eliminates the relaxation of vascular smooth muscles induced by CO, it is believed that the coupling of Ca²⁺ sparks with the BKₐCa 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 BKₐCa, 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 BKₐCa channels leads to hyperpolarization of the vascular SMC membrane, closing of the voltage-dependent Ca²⁺ channels, and decrease in Ca²⁺ 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 BKₐCa channels to Ca²⁺ as well as an enhance in transient K⁺ current, which causes PM hyperpolarization and closure of the voltage-dependent Ca²⁺ 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 Ca²⁺ by sarcoplasmic reticulum through phosphorylation of a number of signaling proteins and lead to reduction of [Ca²⁺], 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 constructive 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$_2$S and NO

This chapter will focus on the protective mechanisms that underlie the CO, H$_2$S 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],
FADD-like ICE-inhibitory protein, which inhibits the TNF-signaling proteins with CO results in activation of the transcription factor NF-κB activity. The interaction of p38 MAP kinase signaling pathway and regulation of the protective mechanisms during activation of the external 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. 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. 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. 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.

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.

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. The CO directly regulates the expression of miR-1, a key regulator of cell cycle progression in the G1 phase, and p21^cip1 gene, a potent inhibitor of cell cycle progression, which leads to the G0/G1 cell cycle arrest. Moreover, CO abrogates transition of the SMC from proliferative dormancy to the growth phase by inhibiting growth factors or cytokines inducing cell proliferation. 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. 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. 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.

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). These CO-mediated preconditioning effects have a positive effect on the survival of the CVS cells during ischemia/reperfusion.

### 6.2 Ant apoptotic properties of H₂S

Recent studies have shown that some pharmacological drugs, which increase the endogenous synthesis of H₂S, can protect the heart from IR injury by reducing apoptosis of CM. In addition, H₂S improves the contractile function of the myocardium by inhibiting apoptosis of ventricular myocytes and reducing the infarction zone (preconditioning effect). At the same time, the infarction zone has been demonstrated to decrease after use of exogenous and endogenous H₂S and to increase because of pharmacological inhibition of cystathionine γ-lyase (CGL).

Molecular studies have shown that the protective preconditioning effect of H₂S is associated with an increase in microRNAs (miRs) levels. 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. showed that level of miR-1 was upregulated by 2.21-fold in the IR group compared to the group preconditioned with H₂S. Also, preconditioning with H₂S 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...
genes of miR-1. Histone deacetylation alters the chromo-
some structure and affects access of the transcription fac-
tors 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 H2S 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 de-
press CM function, moderate inflammatory processes fol-
lowing 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 mitoKATP chan-
nels [206]. Any increase in MMP will reduce the uptake
of Ca2+ by mitochondria, restore Ca2+ 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 pre-
treated 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 gener-
ally modulated by 4 phosphorylation sites, eNOSSer1177,
eNOSSer114, eNOSSer633 and eNOSThr495 [77]. Rep-
resentative blotting results and semiquantitative analyses
showed that total eNOS, phosphorylated eNOSSer114 and
phosphorylated eNOSSer633 were unchanged in all groups.
However, phosphorylation of eNOSSer1177, which indica-
es eNOS activation, significantly decreased in MI group,
whereas the expressions of phospho-eNOSThr495 increased
in MI group. At the same time, BRL-37344 treatment in-
creased the expression of phosphorylated eNOSSer1177 and
decreased the level of phosphorylated eNOSThr495.

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 path-
way. Moreover, the protein expression of nNOS was in-
creased 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-
nNOSSer1417 and decrease in phospho-nNOSSer847 ex-
pression 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 H2S, NO and CO in the
cardiovascular system during hypoxia

Various studies have shown that the effect of the
studied gas transmitters H2S, NO and CO on the CVS cells
depends on their concentration. High concentrations of
these gas transmitters are toxic to cells, and low, physio-
logical concentrations, induce vasorelaxation, angiogene-
sis, promote cardioprotection and inhibition of apoptosis.
The transmitters share common features due to the interac-
tion and intersection of their common cardioprotective sig-
nal pathways. For example, BKCa channels play an impor-
tant role in the mechanism of the cardioprotective effect of
H2S on cardiomyocytes during hypoxia, but they are also
activated in response to the stimulation of CM by endoge-
nous CO. Jaggar et al. [208, 209] found that CO regulates
these channels by binding to reduced heme. Activators of
BKCa 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 BKCa
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 inter-
action of gas transmitters with each other in the CVS cells
in I/R or others pathological condition accompanied by hyp-
oxia [212, 213].

8. Prospects for the use of donors and
inducers of gasotransmitters (NO, H2S, CO)
synthesis in clinical practice

8.1 Clinical significance of endogenous NO

Nitric oxide refers to compounds that have a poly-
functional 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 [Ca2+]i,
contributing to neuropathology [214]. High concentrations
of Ca2+ in the cytoplasm of neurons trigger neurotoxic pro-
cesses, including uncoupling of the electron transport chain,
activation of enzymes that may impair neurons [215]. Ex-
amples of the consequences of NO toxic effect include neu-
rodegenerative 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$^{2+}$];, 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 $\text{H}_2\text{S}$ 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, $\alpha$-ketobutyrate and ammonia. (b) $\text{H}_2\text{S}$ producing reactions catalyzed by CBS and CSE. CBS catalyzes the $\beta$-replacement reaction of cysteine (Cy–SH) with a variety of thiols (R–SH) to generate $\text{H}_2\text{S}$ and the corresponding thiol ether (R–S–Cy). CSE catalyzes the $\beta$-disulfide elimination reaction of cystine (Cy–S–S–Cy), this is followed by a reaction with a variety of thiols, to generate $\text{H}_2\text{S}$ and the corresponding disulfide (R–S–S–Cy) [226].

... 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 $\beta_1$-blockers, is characterized by a very high degree of cardioselectivity (the index of blocking $\beta_1/\beta_2$-receptors is 293, 10–20 times higher than the similar index of the vast majority of other $\beta_1$-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 ($\text{H}_2\text{S}$ and CO) can act as NO mimetics causing similar physiological changes as vasodilation.
8.2 Clinical significance of donors and inducers of H₂S

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 vasodilatation and reducing blood pressure. The discovery of these properties of H₂S 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 H₂S molecules from endogenic or exogenic sources.

The endogenous source of H₂S 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-mercaptoppyruvate sulfurtransferase (3-MST), depending on the cell type [225]. Cystathionine-β-synthase synthesizes H₂S primarily in neurons. In CM and VSMC, the synthesis of H₂S 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 H₂S 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 H₂S gas contributed to a decrease in blood pressure parameters in hypertensive patients [227]. In experiments on rat models, it was found that intraventricular administration of a H₂S solution caused a dose-dependent decrease in blood pressure [226].

In vitro, H₂S 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 H₂S on smooth muscle cells is associated with effects on eGC, 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 H₂S 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 H₂S in the regulation of vascular tone, new drugs that are exogenous H₂S donors, inducers, and inhibitors of endogenous H₂S synthesis are currently being developed [232].

Right now, sodium hydrosulfide (NaHS) and sodium sulfide Na₂S are the most often used H₂S donors in experimental practice. However, when these molecules are dissolved, H₂S 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 H₂S release make NaHS and Na₂S unsuitable for therapeutic use.

Li Ling and co-authors [233] obtained a new H₂S donor designated as GYY4137. Unlike NaHS, GYY4137 releases H₂S 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 H₂S-based drugs is based on incorporation of H₂S-releasing groups into already existing and widely used drug molecules. Alternative H₂S donors can be obtained by attaching sulfide groups to nonsteroidal anti-inflammatory drugs [235, 236].

Furthermore, inhibitors of enzymes H₂S synthesis can be used in order to reduce pathologically high concentrations of H₂S. 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 H₂S 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 catalysis 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)₃Cl₂], Mn₄(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\(_2\)S, 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.

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Abbreviations: BH₄, tetrahydrobiopterin; BK⁰Ca channel, Ca²⁺-activated potassium channel BK; [Ca²⁺]ᵢ, intracellular Ca²⁺ concentration; CaM, calmodulin; CBS, cystathionine 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; DTI, 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; HIF, total Hcy; HO, heme oxygenase; HDAC4, histone deacetylase 4; H₂S, hydrogen sulfide; MI, myocardial infarction; IMM, inner mitochondrial membrane; IHD, ischemic heart disease; INOS, inducible NO synthase; IR, ischemic reperfusion injury; KATP channel, ATP-dependent potassium channels; MEC, mitochondrial enzyme complexes; METC, mitochondrial electron transport chain; mitoBK⁰Ca, mitochondrial Ca²⁺-activated potassium channel BK; mitoK⁰ATP, mitochondrial ATP-dependent potassium channels; MCM, mitochondria of cardiomyocytes; MPP, 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; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PM, plasma membrane; PN, peroxynitrite; ROS, Reactive Oxygen Species; RNS, Reactive Nitrogen Species; RyrR, 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.

Keywords: Carbon monoxide; Cardioprotection; Gasotransmitters; Hydrogen sulfide; Hypoxia; Ionic channels; Nitric oxide; Signaling; Review

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