Hydrogen sulfide and PKG in ischemia–reperfusion injury: sources, signaling, accelerators and brakes

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Abstract Over the past decade, hydrogen sulfide has emerged as an important cardioprotective molecule with potential for clinical applications. Although several pathways have been proposed to mediate the beneficial effects of H₂S, the NO/cGMP axis has attracted significant attention. Recent evidence has suggested that cGMP-dependent protein kinase can lie both downstream and upstream of H₂S. The current literature on this topic is reviewed and data from recent studies are integrated to propose a unifying model.

Keywords Hydrogen sulfide · Nitric oxide · PKG · Myocardial infarction · Cardioprotection · Ischemia–reperfusion

Introduction

Hydrogen sulfide (H₂S) was initially of interest solely to toxicologists that have been studying its biohazardous properties for decades. The first study to propose that H₂S

could be an endogenous signaling molecule in mammalian cells serving neuromodulator functions was reported in 1996 [1]. Soon after that, the same group described the role of H₂S as a mediator of vasorelaxation [22]. A steady interest for the role of H₂S in physiology and disease has been noted since. H₂S is produced by three different enzymes, namely cystathionine gamma-lyase (CSE), cystathionine beta-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST) [50], all of which are expressed in the heart [29]. In spite of the lack of detailed studies on the relative level of expression of CBS, CSE and 3MST in different vascular beds and in the myocardium, it is claimed that CSE is the predominant source of enzymatically derived H₂S in the cardiovascular system. In the heart, this is true as H₂S levels are reduced by 80 % in CSE KO mice [28]. To deliver H₂S to cells, a number of donor compounds have been developed that differ in the mode and rate of H₂S release [37]. Most studies have been performed using H₂S salts (NaHS or Na₂S) that are gradually being replaced by agents that more slowly release H₂S, mimicking the endogenous generation of this gasotransmitter.

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H₂S and cardioprotection

Both endogenously produced and exogenously supplied H₂S exhibit cardioprotective actions [3, 26, 39–41, 47, 50]. While no studies on the role of CBS or 3MST in the heart have been published so far, mice with targeted disruption of the CSE gene locus are more susceptible to myocardial damage after left coronary artery ligation [28]. In contrast, mice overexpressing CSE in their cardiomyocytes exhibit greater degree of protection against ischemia–reperfusion injury [17]. Pharmacological treatment with H₂S donors in

ischemia–reperfusion injury models applied either at the time of reperfusion or as a preconditioning agent, preserves mitochondrial respiration, attenuates the expression of inflammatory cytokines, inhibits leukocyte recruitment, reduces oxidative stress levels, improves left ventricular function and reduces myocardial infarct size [4, 5, 11, 17, 45]. Improved myocardial survival and function have also been noted with H₂S donors in heart failure and cardiomyopathy models [12, 29, 39, 50]. Although the role of H₂S as a cardioprotective molecule is well established, the signaling pathways mediating its effects are still under investigation. As efforts are under way to harness the therapeutic potential of H₂S [49], the dosing scheme (chronic vs acute H₂S administration and timing of H₂S application), as well as the choice of donors to be used for cardioprotection, are important issues to consider if drug development efforts are to come to fruition.

H₂S has been demonstrated to activate a number of kinases, ion channels and signaling molecules, including ERK1/2, Akt, PKC and ATP-sensitive potassium channels (K_{ATP}) in cardiomyocytes [3, 47, 50] and to affect the activity of the NO/cGMP pathway, the RISK pathway and downstream effectors of cardioprotective conditioning [21, 47]. Based on the importance of the NO/cGMP pathway in attenuating I/R injury [3, 9, 18, 19, 21] and the contribution of NO to the cardioprotective action of H₂S [5, 28, 29, 41], herein we will focus on the interaction between H₂S, cGMP and its target kinase, cGMP-dependent protein kinase G in animal model of myocardial infarction.

Interplay between NO and H₂S

Ample evidence for a cross-talk between H₂S and the endothelial NO pathway exists with most of the evidence pointing towards a synergistic action of the two gasotransmitters. H₂S enhances Akt activity [36], possibly through PTEN inhibition [20], triggering eNOS phosphorylation on Ser1177 and increased NO production [15, 28]. At the same time, H₂S serves as a potent and selective PDE5 inhibitor, exhibiting a 30-fold selectivity for PDE5 over other PDEs [6, 35]. Moreover, H₂S also prevents nitrosation of eNOS on Cys443 keeping it in the active, dimeric form [2]. H₂S also keeps the soluble guanylyl cyclase, the NO “receptor”, in its ferrous, NO-responsive form [52].

At the functional level, it has been demonstrated that the actions of H₂S and NO are mutually dependent in the vessel wall, as inhibition of the production of one gasotransmitter abolishes or reduces angiogenesis and vascular relaxation triggered by the other [15]. The interaction between H₂S and NO has been confirmed to occur in the heart, where H₂S donor administration resulted in

increased eNOS phosphorylation and enhanced NO availability leading to cardioprotection in myocardial infarction, heart failure and cardiac arrest models; the H₂S protective effects were abolished in eNOS KO mice [5, 28, 29, 32, 38].

H₂S signaling and PKG

A role for cGMP-dependent protein kinase (PKG) in H₂S signaling has been reported in vascular cells. It was shown that exposure of endothelial cells or vessels to NaHS led to increased cGMP accumulation and activation of PKG, as suggested by an increase in VASP phosphorylation, a surrogate marker of its activity [15]. Both vasorelaxation and angiogenic responses to NaHS (proliferation, migration, network formation) were attenuated by the PKG-I α inhibitor DT-2 [7, 15]. Moreover, L-cysteine- and NaHS-induced relaxations in mouse aortas from PKG-I KO mice were reduced compared to wild-type controls, reinforcing the notion that PKG is an integral part of H₂S signaling [7]. Activation of PKG-I was also shown to mediate the effects of NaHS in vivo, since pretreatment of mice with DT-2 abolished the hypotensive response to NaHS [7].

Does PKG lie downstream or upstream of H₂S in the heart?

We have recently shown that bolus administration of NaHS at the end of prolonged ischemia (and prior to reperfusion) leads to increased cardiac cGMP levels and PKG activation. In both rabbits and mice, the cardioprotective effect exhibited by the acute administration of NaHS was reduced by DT-2 [5], thus, placing PKG downstream of H₂S. In the same study, we identified phospholamban (PLN) as a downstream target of PKG; PLN has also been shown to be regulated by phosphodiesterase 2 and protein kinase A in the context of post-conditioning [24]. PLN once phosphorylated reduces free intracellular Ca²⁺ concentration by dissociating from SERCA allowing it to pump Ca²⁺ ions back into the sarcoplasmic reticulum [30], limiting the damage from hypercontracture and mPTP opening. Additional downstream targets for PKG in cardiomyocytes ameliorating I/R injury have been reported and include among others the Na/H exchanger and K_{ATP} channels [9, 10, 19, 21, 23]; K_{ATP} channels are also direct H₂S targets [50]. It should be kept in mind that PKG-independent cardioprotective pathways of NO also exist [13, 14, 31]. Using a mitochondrial targeted NO donor, Chouchani et al. demonstrated that S-nitrosation of a cysteine switch on mitochondrial complex I limits infarct size following I/R injury [13] and this was independent of PKG [31].

Moreover, it should be noted that cGMP-independent activation of PKG by NaHS has also been reported. NaHS will form polysulfides in the presence of oxygen, which in turn catalyze the formation of an activating interprotein disulfide within PKG-I α , triggering vasorelaxation and lowering blood pressure [46]. Transgenic knock-in mice, in which the cysteine 42 redox sensor of PKG has been replaced by serine, exhibit reduced responses to NaHS.

Earlier studies had shown that treatment with the PDE5 inhibitor tadalafil 1 h prior to coronary artery ligation, results in increased H₂S production, reduced infarct size and preserved LV contractility. The reduction in infarct size by tadalafil was abolished in CSE KO mice or following CSE pharmacological inhibition [42], suggesting that PKG lies upstream of H₂S. In a similar experimental set up, administration of the sGC activator, cinaciguat,

increased PKG activity leading to enhanced CSE expression and elevated cardiac H₂S [43]. These biochemical changes translated into improved cardiac function and reduced infarct size [43].

In a recent issue of this journal it was demonstrated that adenovirus-mediated gene transfer of PKG-I α protects against ischemia/reperfusion injury through up regulation of CSE expression in cultured cardiomyocytes, as well as in vivo [16], reinforcing the notion that PKG is upstream of H₂S in cardioprotective pathways. CBS and 3MST levels remained unaltered in response to PKG-I over expression. Inhibition of CSE with PAG augmented LDH release and trypan blue staining in cardiomyocytes infected with PKG-I adenovirus. In vivo, PAG reversed the beneficial effects of PKG-I α over expression [16]. Although the mechanisms responsible for PKG-mediated up regulation of CSE

Fig. 1 Cross-talk and interactions between H₂S and PKG pathways in cardiac myocytes. Exposure of cardiomyocytes to H₂S donors (or endogenously produced H₂S) leads to increased cGMP levels through activation of eNOS and/or PDE inhibition. Increased cGMP-dependent protein kinase activity results in upregulation of CSE levels and H₂S production that further increases cGMP levels and PKG activity. Phosphorylation and inhibition of CSE by PKG serves as a brake to control H₂S output. It should be noted that PKG has actions on effectors of cardioprotection that are not shown in the above schematic and that H₂S also targets additional cardioprotective pathways (see text for details)

expression were not investigated in any of the aforementioned studies, Sp1 has been shown to act as a robust trans-activator of CSE expression in different cell types [25, 44].

Avoiding excessive H₂S production

After combining the observations made in the experiments exposing cardiac tissue to H₂S donors that indicate that PKG is downstream of H₂S, with those made using cGMP-elevating agents and PKG-I over expression that place PKG upstream of CSE and H₂S, one would expect that a feed-forward, self-perpetuating cycle is established (Fig. 1). H₂S would increase PKG activity and this would upregulate CSE, leading to more H₂S production and greater PKG activation. Examples where treatment with H₂S releasing agents leads to increased CSE expression have been reported in the literature [8, 48]. The ability of exogenously supplied H₂S to augment endogenous H₂S production is in stark contrast to most agonists or enzyme activators that cause down regulation of their cognate receptors or targets. If this positive feedback loop indeed existed, H₂S levels would constantly increase over time reaching toxic H₂S levels. It is well known that H₂S in high concentrations is deleterious as it inhibits cytochrome *c* oxidase, acting as a respiratory chain poison [50]. Thus, the need for a brake that would prevent cells from accumulating toxic H₂S concentrations becomes apparent. The brake might come in the form of a posttranslational modification, such as the one reported very recently by Yuan et al. These authors demonstrated that CSE is a PKG substrate; phosphorylation of CSE by PKG on Ser377 inhibits its activity by 75 % [51].

H₂S, CSE and ischemia

Under normoxic conditions, cells oxidize H₂S in the mitochondria to thiosulfate and then sulfate, thereby maintaining a low intracellular H₂S concentration [27, 34]. As oxygen levels fall during ischemia, H₂S oxidation begins to fail and intracellular H₂S increases. At the same time, in spite of the presumably higher tissue levels of H₂S, several studies in different animal models have shown a gradual decrease in myocardial cGMP content during ischemia [19]. A drop in cGMP levels will translate into lower PKG activity, leading to reduced CSE phosphorylation and enhanced H₂S output. Thus, H₂S levels during ischemia would be expected to rise both due to increased production and limited degradation. As long as some oxygen is present to act as end acceptor, H₂S will serve as an electron donor for the respiratory chain [33] becoming

an energy fuel and preserving ATP levels. The above-mentioned observations are entirely speculative and need to be experimentally confirmed, but for the time being provide a rational as to why endogenously produced H₂S during ischemia is beneficial.

Conclusion

The NO/cGMP pathway is an integral part of H₂S signaling in the cardiovascular system. Several levels of interaction between NO and H₂S have been established to occur and more links between H₂S, PKG and CSE are emerging. These interactions are complex and require intense experimental effort to prove their functional consequences and relevance to different cardiac diseases. Understanding the molecular mechanisms of action of H₂S donors will help design better treatment regimens and target appropriate pathological conditions to capitalize on the progress made in H₂S biology for the benefit of patients.

Compliance with ethical standards

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Conflict of interest None.

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