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Review

Hydrogen sulfide as a regulator of calcium channels

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ABSTRACT

An increasing body of evidence suggests the involvement of hydrogen sulfide (H_2S) in different physiological and pathological processes. Similarly to the other gasotransmitters nitric oxide (NO) and carbon monoxide (CO), this bioactive compound is rapidly diffusible through the biological membranes and acts in a paracrine fashion. Despite the large amount of biological actions observed *in vitro* and *in vivo* upon stimulation with H_2S donors, as well as by interfering with its synthesis, the molecular targets and mechanisms through which it exerts its intracellular effects are only partially known. A number of proteins are covalently modified by H_2S through sulfhydration of specific cysteine residues. However, only in few cases their identity has been discovered and the functional role of this post-translational modification needs to be investigated in more detail. Great attention has been devoted to potassium channels, particularly K_{ATP}, as they are considered key mediators of H_2S-induced effects, and their sulfhydration has been clearly demonstrated. Recently, different authors reported the ability of H_2S to interfere with calcium homeostasis in neurons, cardiomyocytes and endothelial cells. Since calcium signaling is involved in all cell processes, these observations attracted increasing attention from basic biology and medicine. Although some effects of H_2S on calcium signals can be ascribed to K_{ATP} modulation, there is growing consensus about the existence of other targets for the gasotransmitter. Some of them are Ca^{2+}-permeable channels. In this review we discuss the state of the art in this specific field, providing an updated report of H_2S interaction with Ca^{2+} channels and its functional outcomes.

1. Introduction

Hydrogen sulfide (H_2S) is a colorless, flammable gas with a characteristic smell of rotten eggs that has long been regarded as a toxic environmental pollutant with minimal, if any, physiological significance [1]. It is, however, now evident that H_2S may be endogenously synthesized in mammalian tissues from L-cysteine by three pyridoxal-50-phosphate (PLP)-dependent enzymes, namely cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and cysteine aminotransferase (CAT) [2]. The latter, in turn, acts in concert with the zinc-dependent enzyme, 3-mercaptopyruvate sulfurtransferase (3-MST), to release H_2S from L-cysteine and keto acids (*e.g.*, α-ketoglutarate) [1,3,4]. The distribution of H_2S-generating enzymes may be tissue specific, whereas CBS is highly expressed in the hippocampus and in the cerebellum within the central nervous system (CNS), and CSE is far more abundant in vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) [1,3,5]. Nevertheless, recent studies have detected CSE in microglial cells, spinal cord and cerebellar granule neurons [6]. An additional source of H_2S is provided by bound sulfur, an intracellular reservoir of sulfur, as reported in rodent neurons and astrocytes in the presence of physiologic levels of endogenous reducing substances, *i.e.* glutathione and cysteine [1]. Interconversion of sulfur-containing amino acids and metabolites is carried out by cysteine CAT, cysteine dioxygenase (CDO), and cysteine lyase (CL) [1]. The assessment of the physiological concentration of free H_2S has engendered a remarkable controversy. It has long been thought that H_2S levels in biological tissues and plasma ranged from 50 μM up to 160 μM [7,8]. However, recent studies have disclosed that H_2S is rapidly catabolized such that (1) whole tissue concentrations of the free gasotransmitter fall within the low nanomolar range and (2) H_2S may be undetectable in peripheral blood [1,9,10]. In order to reconcile this evidence with the notion that H_2S impacts on cellular activities *in vitro* at concentrations that are orders of magnitude larger (100 μM), it has been hypothesized that the equilibrium between H_2S production and consumption results in an

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intracellular microenvironment with enough H_2S to induce a local signaling cascade without affecting systemic levels of the gas [9,11].

A number of signal transduction pathways may be recruited by H_2S to finely tune cardiovascular and CNS functions. For instance, H_2S relaxes VSMCs and contributes to the regulation of blood pressure by activating ATP-sensitive K^+ channels (K_{ATP}); it promotes angiogenesis and vascular remodeling *via* phosphatidylinositol 3-kinase (PI3-K)/Akt/survivin axis in ECs and by augmenting the phosphorylation of extracellular signal-related kinase (ERK) and p38 in VSMCs; moreover, it downregulates a number of pro-inflammatory genes involved in the cardiac ischemic/reperfusion injury by preventing the nuclear translocation of the nuclear factor-κB (NF-κB); it also stimulates long term synaptic potentiation by enhancing the activity of NMDA receptors upon the activation of the cAMP/protein kinase A cascade [1,3,5–8,11].

When considering the impact exerted by intracellular Ca^{2+} concentrations (Ca_i) dynamics on cell physiology, it is not surprising that a growing number of studies are attempting to elucidate the involvement of Ca^{2+}-permeable channels in H_2S-related signaling. The present review aims at providing an updated and concise description of the interaction of H_2S with different types of plasmalemmal Ca^{2+} channels and the associated functional outcomes (Fig. 1 and Table 1).

2. S-sulfhydration is a regulatory mechanism for ion channels

Gasotransmitters, such as nitric oxide (NO), carbon monoxide (CO) and H_2S, selectively interact with different types of ion channels [1,3,5,12–16]. Carbon monoxide modulates (positively or negatively) large-conductance calcium-activated K^+ (BK_{Ca}), voltage-activated K^+ ($K_{V2.1}$) and L-type Ca^{2+} channels, ligand-gated P2X2 and P2X4 receptors, tandem P domain K^+ channels (TREK1) and the epithelial Na^+ channel (ENaC). The detailed mechanisms underlying these effects are not clear. Carbon monoxide activates soluble guanylyl cyclase (sGC), leading to the release of cGMP, but it can also directly modify target proteins such as K_{Ca} α-subunit through interaction with aspartate and histidine residues [17,18]. Nitric oxide covalently modifies free sulfhydryl (—SH) of cysteine residues *via* protein S-nitrosylation. Among ion channels, K_{Ca}, ultra-rapid delayed rectifier K^+ current ($K_{V1.5}$), K_{ATP}, delayed rectifier K^+, L-type Ca^{2+} channels, and transient receptor potential (TRP) channels are (positively or negatively) modulated by S-nitrosylation [19,20].

H_2S donors can also modify cysteine residues of different proteins through S-sulfhydration [1,5]. The —SH from sulfhydryl donor is transferred to free cysteine sulfhydryl and forms covalent persulfide (—SSH). Sulfhydration can be detected by a modified biotin-switch assay used for nitrosylation as well as by mass spectrometry [5]. A number of H_2S-releasing drugs have been utilized to mimic the endogenous effects of H_2S under experimental conditions [1,2]. The most popular H_2S donor is sodium hydrosulfide (NaHS), which presents a fast releasing rate in aqueous solution and liberates one third of H_2S compared to the concentration of the salt [8].

Several proteins are sulfhydrated including actin, tubulin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), strengthening the idea that this signaling pathways is biologically relevant [5]. Accordingly, a recent report showed that H_2S-linked sulfhydration of NF-kB p65 subunit at cysteine-38 mediates its anti-apoptotic action in macrophages and liver cells [21].

In the vascular tissue, hydrogen sulfide is considered an endothelium-derived hyperpolarizing factor (EDHF) that is released by ECs and affects K^+ channels including intermediate calcium-dependent K^+ (IK_{Ca}), small calcium-dependent K^+ (SK_{Ca}) and K_{ATP} in VSMCs [13]. In VSMCs of rat mesenteric arteries H_2S sulfhydrates Kir 6.1 subunit of K_{ATP} in cysteine-43, both constitutively and during cholinergic simulation [13]. Previous observations by Jiang and co-workers showed that H_2S directly interacts with cysteine-6 and cysteine-26 residues of the extracellular NH_2 terminal of rat vascular sulfonylurea receptor (rvSUR1) subunit of rvKir6.1 K_{ATP} channels [13,22]. This study, however, did not assess whether H_2S formed persulfides with the exposed free cysteine residues or disassembled the related disulfide bonds [12].

In addition to K^+ channels, also voltage-dependent Na^+ channels (Na_v) can be regulated by H_2S. Native (from jejunum smooth muscle) and recombinant ($Na_v1.5$) Na_v currents are increased by

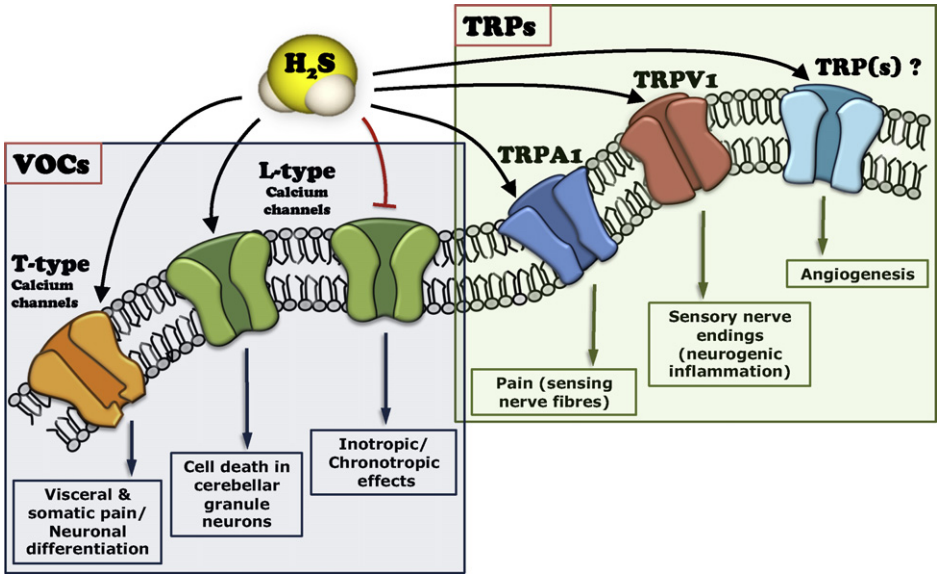

Fig. 1. Modulation of plasmalemmal Ca^{2+} channels by hydrogen sulfide. Hydrogen sulfide (H_2S) regulates the gating of a number of Ca^{2+}-permeable channels, including (1) L-type VOCs formed by either $Ca_V1.2$ or $Ca_V1.3$ subunits, (2) T-type VOCs composed by $Ca_V2.3$ subunits; (3) transient receptor potential ankyrin 1 (TRPA1); and (4) transient receptor potential vanilloid 1 (TRPV1). As more extensively described in the test, H_2S inhibits L-type VOCs in cardiac myocytes, whereas it activates them in neurons. Similarly, H_2S stimulates T-type VOCs, TRPA1 and TRPV1 both *in vitro* and *in vivo*. The identity of putative proangiogenic calcium-permeable channels (TRPs?), possibly modulated by H2S, is unknown.