Interaction of H$_2$S with Calcium Permeable Channels and Transporters

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A growing amount of evidence has suggested that hydrogen sulfide (H$_2$S), as a gasotransmitter, is involved in intensive physiological and pathological processes. More and more research groups have found that H$_2$S mediates diverse cellular biological functions related to regulating intracellular calcium concentration. These groups have demonstrated the reciprocal interaction between H$_2$S and calcium ion channels and transporters, such as L-type calcium channels (LTCC), T-type calcium channels (TTCC), sodium/calcium exchangers (NCX), transient receptor potential (TRP) channels, β-adrenergic receptors, and N-methyl-D-aspartate receptors (NMDAR) in different cells. However, the understanding of the molecular targets and mechanisms is incomplete. Recently, some research groups demonstrated that H$_2$S modulates the activity of calcium ion channels through protein S-sulfhydration and polysulfide reactions. In this review, we elucidate that H$_2$S controls intracellular calcium homeostasis and the underlying mechanisms.

1. Introduction

Hydrogen sulfide (H$_2$S) was thought for hundreds of years to be a toxic gas that smelled like rotten eggs, but the gas is now believed to be a molecule involved in intensive physiological and pathological processes [1], such as protecting the heart against acute myocardial infarction [2, 3] and ischemia/hypoxia injury, regulating blood pressure [4], mediating smooth-muscle relaxation [5], and inhibiting insulin release and renin activity [6, 7]. H$_2$S, as an endogenous gasotransmitter, can be mainly generated by pyridoxal-5’-phosphate- (PLP-) dependent cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), which interconverts the sulfuration from intracellular L-methionine and L-cysteine to produce H$_2$S [8]. In addition, 3-mercaptoppyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT) produce H$_2$S from cysteine through the combined actions of both enzymes [9].

An increasing amount of evidence has demonstrated that H$_2$S regulates cellular biological signaling through modulating calcium ion channels and their related transporters [10, 11], such as L-type calcium channels (LTCC), T-type calcium channels (TTCC), sodium/calcium exchangers (NCX), transient receptor potential (TRP), β-adrenergic receptors, and NMDA receptors. This review presents the current research on H$_2$S to better understand its regulation of calcium channels, with a special emphasis on mechanisms.

2. The Regulatory Mechanism of H$_2$S Interacting with Calcium ion Channels

2.1. Voltage-Dependent Calcium Channels (VDCC)

Ca$^{2+}$ serves as an important second messenger in both excitable and nonexcitable cells. Voltage-dependent calcium channels (VDCC), store-operated calcium channels (SOCs), and
G-protein coupled receptors (GPCRs) are responsible for calcium influx from extracellular fluids. Alterations in intracellular calcium levels trigger physiological responses, including cardiac muscle contraction, vascular dilation, hormone secretion, and neurotransmitter release [12–16].

The family of VDCCs includes L-, T-, N-, and P/Q-subtypes, which differ in their cellular and subcellular distributions and functional properties [17, 18]. For example, T-type calcium channels (TTCCs) are involved in regulating cellular excitability [19], and L- and P/Q-type channels mediate fast-evoked neurotransmitter release [20].

LTCCs are integral in excitation/contraction coupling and are one of the main channels for extracellular Ca$^{2+}$ influx in myocardial cells. In 2002, Zhao and Wang first reported that H$_2$S could directly inhibit calcium influx from LTCCs in smooth-muscle cells [21]. Moreover, in 2009, Sun et al. further demonstrated that H$_2$S, as a novel inhibitor of LTCC, has negative inotropic effects in rat cardiomyocytes [22]. In a recent study, Avanzato et al. investigated the role of H$_2$S in regulating VDCCs and the related functional effects on the cardiomyoblast cell line H9c2. They found that H$_2$S inhibits LTCCs and TTCCs in H9c2. Pretreatment with NaHS (a donor of H$_2$S) prevented cell death via H$_2$O$_2$ through inhibiting LTCCs. Their results were the first to demonstrate that H$_2$S protects rat cardiomyoblasts against oxidative stress through inhibition of LTCCs [23]. In addition, Tang et al. suggested that exogenous and endogenous H$_2$S inhibited pancreatic insulin secretion by inhibiting LTCCs activity. They confirmed that NaHS reversibly decreased LTCC current density in a concentration-dependent manner in CSE WT pancreatic beta cells. Furthermore, they observed that DL-propargylglycine (an inhibitor of CSE) increased the basal LTCC activity in beta cells from CSE WT mice, but not in pancreatic beta cells from CSE-KO mice. Pancreatic beta cells from CSE-KO mice displayed a higher LTCCs density than those from WT mice. These results suggested that a novel mechanism for regulating insulin secretion was related to the CSE/H$_2$S system, which controlled LTCC activity [24].

Recently, some data showed that exogenous and endogenous H$_2$S can modify cystein residues of different proteins through S-sulfhydration. The –SH from sulfhydryl donor is transformed to free cysteine sulfhydroxyl and forms covalent persulfide (–SSH) [25, 26]. In 2012, Zhang and his coworkers showed that NaHS inhibited the peak amplitude of the L-type calcium current in a concentration-dependent manner and could be partly inhibited by the oxidant sulfhydroly modifier diamide (DM). They explained that dithiothreitol (DTT), a reductant that transforms disulfide bridges into sulfhydroly groups in cysteine-containing proteins, could significantly reverse NaHS-induced inhibition of calcium current from LTCCs. Their results suggested that H$_2$S inhibited L-type calcium currents depending on the sulfhydroly group in rat cardiomyocytes [27] (Figure 1).

TTCCs are classified into three T-type channel subtypes, Cav3.1, Cav3.2, and Cav3.3. There have been reports about the T-type channels being activated by H$_2$S in neurons [28–30]. In the pain pathways, Cav3.2 in the peripheral terminals of nociceptors and dorsal horn spinal neurons appears to promote peripheral nociception and central nociceptive sensitization [28]. H$_2$S may function as a neuromodulator in sensory transmission. There is evidence that chemotherapy-induced neuropathic pain is blocked by ethosuximide, which is known to block TTCCs. Systemic administration of β-propargylglycine and β-cyanoalaneine, irreversible and reversible inhibitors of CSE, respectively, also abolished neuropathic pain. Okubo et al. demonstrated that Ca$_3$2 and CSE at the protein level are upregulated, which induced a significant increase in H$_2$S level. H$_2$S facilitated pain sensation by targeting Ca$_3$2.3 TTCCs. The H$_2$S/Ca$_3$3.2 pathway appears to play a role in the maintenance of surgically evoked neuropathic pain [31]. Intraplantar administration of NaHS causes mechanical hyperalgesia in rats, an effect reversed by mibefradil (a T-type Ca$^{2+}$ channel blocker), and also enhances membrane currents through the TTCC in NG 108-15 cells and mouse dorsal root ganglion neurons [29, 30]. Their data suggested that spinal and peripheral NaHS/H$_2$S facilitates the expression of Cav3.2 TTCCs in the primary afferent and/or spinal nociceptive neurons, leading to sensitization of nociceptive processing and hyperalgesia [31]. Sekiguchi et al. demonstrated that endogenous and exogenous hydrogen sulfide facilitate T-type calcium channel currents in Cav3.2-expressing HEK293 cells [32]. In contrast, Elies et al. reported an inhibitory effect with high doses of NaHS on Cav3.2-overexpressing HEK cells [33]. Their data were the first preliminary evidence that H$_2$S negatively modulates endogenously expressed TTCCs in a myoblast cell line. In spite of the opposite opinion in the effects of NaHS on TTCCs in different research groups, H$_2$S regulating the activity of TTCC has been confirmed widely. However, most of the evidence suggests that H$_2$S elevates the activities of TTCCs and increases the amplitudes of T-type Ca$^{2+}$ currents in different cell lines.

2.2 β-Adrenergic Receptors. Cardiac excitation-contraction coupling is under the direct control of the adrenergic nervous system. In the heart, the β-adrenergic receptor (AR), a G-protein coupled receptor, activates the associated adenyl cyclase (AC)-cAMP-protein kinase A (PKA) pathway [34]. β-Adrenoceptor-coupled stimulatory G proteins lead to an increased intracellular cAMP level and stimulate protein kinase A (PKA) to mediate phosphorylation of LTCCs and finally increase contractile function [35–37]. Some reports have observed that H$_2$S content in the heart was significantly reduced in a cardiac ischemia [38] and overstimulation of the β-adrenergic system by isoproterenol (ISO, β-adrenoceptor agonist) models [39]. Yong and his coworkers revealed that H$_2$S may negatively modulate β-adrenoceptor function via inhibiting adenylyl cyclase activity [40]. They found that ISO (10$^{-9}$–10$^{-6}$ M), in a concentration-dependent manner, increased the twitch amplitude of ventricular myocytes, which was attenuated by NaHS (10$^{-5}$–10$^{-3}$ M) in a dose-dependent manner. The amplitudes and maximal velocities (±dL/dt) for myocyte twitch
and EI-[Ca$^{2+}$], transient amplitudes were enhanced by ISO, forskolin (an adenylyl cyclase activator), 8-bromoadenosine-3',5'-cyclic monophosphate (an activator of protein kinase A), and Bay K-8644 (a selective LTCC agonist). Administration of NaHS (100 μM) significantly attenuated the effects of only ISO and forskolin. Moreover, NaHS reversed the ISO-induced cAMP increase and forskolin-stimulated adenylyl cyclase activity. Thus, they postulated that H$_2$S may negatively regulate β-AR function through inhibition of the cAMP/PKA pathway. In addition, some studies found that the plasma concentration of H$_2$S in patients with coronary heart disease [41] and in the setting of ISO overstimulation significantly decreased endogenous H$_2$S production, which implies that a reduced H$_2$S level caused by ischemia and β-adrenoceptor overstimulation may result in impairment of the negative modulation of H$_2$S on the β-adrenoceptor system and hence calcium overload.

2.3. Sodium Calcium Exchanger (NCX). The sodium calcium exchanger (NCX) is one of the key players in the regulation of intracellular calcium homeostasis. In a physiological condition, NCX, a nonselective cation channel, may induce the influx of 3 Na$^+$ into cells in exchange for the efflux of 1 Ca$^{2+}$ [42]. However, in pathological conditions, such as ischemia/reperfusion, hypoxia, and heart failure, NCX function could be reversed, with one Ca$^{2+}$ moving inward and three molecules of Na$^+$ going out of the cell [43]. H$_2$S may stimulate Ca$^{2+}$ influx into endothelial cells (ECs) by recruiting the reverse-mode for the NCX [44–46]. To confirm the role of NCX in NaHS-dependent Ca$^{2+}$ signaling, KB-R 7943 (20 μM), a selective inhibitor of the reverse-mode, was used in the experiment. Moccia and his coworkers’ data showed that NaHS failed to elicit a [Ca$^{2+}$]i elevation in ECs pretreated with KB-R 7943. In addition, the amplitude of the Ca$^{2+}$ response was significantly lower in ECs activated by the H$_2$S donor in the presence of KB-R 7943. Taken together, these findings hinted at NCX as a key mediator of NaHS-elicited Ca$^{2+}$ inflow in rat aortic ECs. To further determine the effect of sulfide signaling on the NCX, several studies investigated NCX expression and function in HeLa cells. They observed increased levels of NCX1 mRNA, protein, and activity after 24 h of GYY4137 (morpholin-4-ium-4-methoxyphenyl(morpholino) phosphinodithioate, a slow releasing H$_2$S donor) treatment. This increase was accompanied by elevated cAMP due to GYY4137 treatment, which was completely abolished when NCX1 was silenced. An increased cAMP level would point to upregulation of the β-adrenergic receptors. Thus, Cheng et al. investigated...
the relationship of \( \beta \)-adrenergic receptors with the NCX1 in the presence and/or absence of \( \text{H}_2\text{S} \) and determined the physiological importance of this potential communication using \( \text{GY1437} \) [47]. Indeed, \( \text{GY1437} \) increased expression of the \( \beta_1 \) and \( \beta_3 \) (but not \( \beta_2 \)) adrenergic receptors, suggesting that sulfide signaling played a role in regulating the NCX1 and \( \beta_1 \) and \( \beta_3 \) adrenergic receptors and their colocalization.

### 2.4. Transient Receptor Potential (TRP) Channels

A growing body evidence has shown that \( \text{H}_2\text{S} \) and neuronal excitation induce calcium ion influx in astrocytes, and the interaction between neurons and astrocytes regulates synaptic activity [48–50]. TRP channels were found to mediate the responses to \( \text{H}_2\text{S} \) in the urinary bladder and sensory neurons [51]. Although the effects of \( \text{H}_2\text{S} \) on transient receptor potential (TRP) channels are not completely clear, Kimura et al. demonstrated that polysulfides of \( \text{H}_2\text{S} \)-derived signaling molecules stimulated TRP channels in the brain [52]. They suggested that \( \text{H}_2\text{S} \) induced \( \text{Ca}^{2+} \) influx in astrocytes through generating polysulfides of TRP. They administered sodium polysulfides, \( \text{Na}_2\text{S}_3 \), in their experiments, which induced \( \text{Ca}^{2+} \) influx in a concentration-dependent manner. They also confirmed that this astrocyte response to \( \text{H}_2\text{S} \) was suppressed by the TRP channel blockers \( \text{La}^{3+} \) and \( \text{Gd}^{3+} \).

To further reveal the mechanism for \( \text{Na}_2\text{S}_3 \)-induced TRP channel opening, the TRPA1 channel inhibitors HC-030031 and AP-18 and TRPA1 siRNA were used. Their data showed that, in the presence of the inhibitors or TRPA1 siRNA, \( \text{Na}_2\text{S}_3 \) could not induce \( \text{Ca}^{2+} \) influx through the TRPA1 channel. Liu et al. showed that \( \text{H}_2\text{S} \) maintained mesenchymal stem cell function via regulation of \( \text{Ca}^{2+} \) channel sulfhydration [53]. They found that NaHS-treated bone marrow mesenchymal stem cells (BMSCs) induced \( \text{Ca}^{2+} \) influx with a limited contribution from intracellular \( \text{Ca}^{2+} \) storage. They also found that DTT, by reducing the disulfide bonds in proteins and increasing the number of residual sulfhydryl proteins, elevated NaHS-induced \( \text{Ca}^{2+} \) influx in BMSCs. Diamide, by reducing the number of sulfhydrlys and 2-sulfonato-methanethiosulfonate (MTSES), a nonpermeable reagent able to reduce free sulfhydryls only on the outer cytomembrane, could reduce NaHS-induced \( \text{Ca}^{2+} \) influx in BMSCs. These results revealed that free sulfhydrlys affect NaHS-induced \( \text{Ca}^{2+} \) influx. The above results suggested that polysulfides, as \( \text{H}_2\text{S} \)-derived bioactive molecules, stimulate TRP channels, providing a new molecular mechanism for sulfide-induced signaling.

### 2.5. N-Methyl-D-aspartate Receptors (NMDARs)

N-Methyl-D-aspartate receptors (NMDARs) form glutamate-gated ion channels that are widely expressed in the central nervous system and are highly permeable to calcium ions, which are essential for regulating synaptogenesis, use-dependent synaptic remodeling, and long-term plastic changes in synaptic strength [54]. \( \text{H}_2\text{S} \), as a neuromodulator, elevates the activity of N-methyl-D-aspartate (NMDA) receptors to facilitate the induction of hippocampal long-term potentiation (LTP), a synaptic model of memory formation [48, 55]. Nagai et al. demonstrated that \( \text{H}_2\text{S} \) enhances the neuronal response to glutamate and induces \( \text{Ca}^{2+} \) waves in astrocytes [49]. Glial cells communicate with surrounding cells by increasing the intracellular concentration of \( \text{Ca}^{2+} \) and propagating the signal as \( \text{Ca}^{2+} \) waves that occur in glia, and neurons show \( \text{Ca}^{2+} \) oscillations and intracellular \( \text{Ca}^{2+} \) waves. Because astrocytes elicit intracellular \( \text{Ca}^{2+} \) waves by electrical stimulation and application of NMDA in mixed cultures of neurons and astrocytes, astrocytes have been suggested to respond directly to a neurotransmitter released from neurons excited by NMDA or electrical stimulation [56–59]. \( \text{La}^{3+} \) and \( \text{Gd}^{3+} \) block \( \text{Ca}^{2+} \) waves and inhibit \( \text{Ca}^{2+} \) channels; \( \text{La}^{3+} \) and \( \text{Gd}^{3+} \) may inhibit the exocytosis of glutamate or some factor from neurons when neurons are stimulated by NMDA. However, \( \text{La}^{3+} \) and \( \text{Gd}^{3+} \) block \( \text{H}_2\text{S} \)-initiated waves in pure astrocyte culture, showing that \( \text{Ca}^{2+} \) is most likely involved in the propagation step. \( \text{H}_2\text{S} \) released in response to neuronal excitation may activate \( \text{Ca}^{2+} \) channels to induce \( \text{Ca}^{2+} \) waves in astrocytes. \( \text{H}_2\text{S} \) may therefore mediate signals between neurons and glia. \( \text{H}_2\text{S} \) is released from neurons or glia by neuronal excitation and increases the intracellular concentration of \( \text{Ca}^{2+} \) by activating \( \text{Ca}^{2+} \) channels in astrocytes and to a lesser extent causes release from intracellular \( \text{Ca}^{2+} \) stores. An elevated intracellular \( \text{Ca}^{2+} \) triggers the induction of \( \text{Ca}^{2+} \) waves that propagate to the neighboring astrocytes [60–63]. \( \text{H}_2\text{S} \) enhances the activity of NMDA receptors by reducing the cysteine disulfide bond in the hinge region of the ligand-binding domain of NMDA receptors, and polysulfides further enhance this activity by adding bound sulfane sulfur to the receptors. Polysulfides activate the TRPA1 channels in astrocytes to induce \( \text{Ca}^{2+} \) influx, which facilitates the release of the giotransmitter D-serine to enhance the activity of NMDA receptors. By these integrated mechanisms, \( \text{H}_2\text{S} \) along with polysulfides may facilitate the induction of LTP [64].

### 3. Conclusions and Perspective

An increasing amount of evidence has clearly demonstrated that \( \text{H}_2\text{S} \) is associated with relevant biological processes, such as cardiac systolic function, sensory transduction, antiapoptotic function, and neuroprotection [65]. These functions are closely related to \( \text{H}_2\text{S} \) regulating various calcium ion channels and transporters [66]. Many studies cited in this review investigated the fact that polysulfides of calcium ion channels, which are modified by \( \text{H}_2\text{S} \), have been found to elevate the activity of TRP, TTCC, and NMDARs and to inhibit LTCC through the mechanism of sulfhydration. Furthermore, \( \text{H}_2\text{S} \) could upregulate the activities of the NCX1 and \( \beta_1 \) and \( \beta_3 \) adrenergic receptors and their colocalization. Altered effects of \( \text{H}_2\text{S} \) on calcium ion channels under different pathophysiological conditions are being investigated. Extensive research on the mechanisms of \( \text{H}_2\text{S} \) modulation of calcium signaling will provide new insights into the physiological function of \( \text{H}_2\text{S} \).
Conflict of Interests

No conflict of interests, financial or otherwise, was declared by any of the authors.

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