Hydrogen Sulfide and T-Type Ca^{2+} Channels in Pain Processing, Neuronal Differentiation and Neuroendocrine Secretion

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Key Words

Hydrogen sulfide · $\text{Ca}_v3.2$ T-type calcium channel · Prostate · Pain

Abstract

Background: Hydrogen sulfide (H_2S), a gasotransmitter, is generated from L-cysteine by mainly 3 enzymes, cystathionine- γ -lyase (CSE), cystathionine- β -synthase, and 3-mercaptopyruvate sulfurtransferase in cooperation with cysteine aminotransferase. The H_2S -forming enzymes, particularly CSE, are overexpressed under the pathological conditions such as inflammation, neuronal or neuroendocrine differentiation and cancer development. Given that $\text{Ca}_v3.2$ T-type Ca^{2+} channels mediate some of the biological activity of H_2S , we focus on the role of the $\text{H}_2\text{S}/\text{Ca}_v3.2$ pathway in regulating the neuronal and neuroendocrine function. **Summary:** In the neuronal system, H_2S regulates the activity of various ion channels including $\text{Ca}_v3.2$. Exogenous and endogenous H_2S enhances the $\text{Ca}_v3.2$ channel activity, promoting somatic and visceral pain signaling. The $\text{H}_2\text{S}/\text{Ca}_v3.2$ pathway also facilitates neuritogenesis or neuronal differentiation. Interestingly, endogenous H_2S formed by CSE regulates secretory function by enhancing $\text{Ca}_v3.2$ channel activity in neuroendocrine-differentiated prostate cancer cells or carotid glomus cells. **Key Messages:** The $\text{H}_2\text{S}/\text{Ca}_v3.2$ path-

way may serve as therapeutic targets for treatment of intractable pain, neuronal injury, androgen-independent prostate cancer, cardiovascular diseases, etc. © 2016 S. Karger AG, Basel

Introduction

Like nitric oxide and carbon monoxide, hydrogen sulfide (H_2S) is now considered a gasotransmitter in the mammalian body. H_2S is generated endogenously from L-cysteine (L-Cys) mainly by 3 enzymes, cystathionine- γ -lyase (CSE), cystathionine- β -synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST) in cooperation with cysteine aminotransferase (CAT) [1–3]. Numerous physiological and pathophysiological roles of endogenous H_2S have been reported in the cardiovascular, neuronal, gastrointestinal, urinary, and endocrine systems [1, 3, 4]. There is also evidence that H_2S has the paradoxical actions on cancer cells, whereby lower (endogenous) production of H_2S tends to promote, while its higher production (from exogenously added H_2S donors) tends to inhibit cancer cell proliferation [5]. H_2S appears to target a variety of voltage-dependent and ligand-gated ion channels, such as ATP-sensitive K^+ (K_{ATP}) channels, voltage-gated T-type and L-type Ca^{2+} channels, transient

receptor potential ankyrin-1 (TRPA1) channels, large conductance Ca^{2+} -activated K^+ (BK) channels, cystic fibrosis transmembrane conductance regulator Cl^- channels, NMDA receptors, in addition to transcription factors and protein kinases [2, 3, 6, 7]. In the nociceptor neurons, H_2S enhances the function of $\text{Ca}_v3.2$ T-type Ca^{2+} channels [8–12] and TRPA1 channels [13, 14], leading to the neuronal excitation and then pain sensation or hyperalgesia/allodynia [3, 7, 15]. We have also shown that the acceleration of $\text{Ca}_v3.2$ channel activity by exogenous H_2S causes neurogenesis and neuronal differentiation in NG108-15 cells [9, 10, 16], and that the endogenous $\text{H}_2\text{S}/\text{Ca}_v3.2$ pathway regulates secretory function in the neuroendocrine-like differentiated human prostate cancer LNCaP cells [17]. Here, we thus focus on the roles of $\text{Ca}_v3.2$ T-type Ca^{2+} channels and H_2S in pain processing, neuronal differentiation and neuroendocrine secretion.

H_2S -Mediated Functional Acceleration of T-Type Calcium Channels

Low-voltage-activated Ca^{2+} channels, known as T-type Ca^{2+} channels, are classified into 3 different types, $\text{Ca}_v3.1$, $\text{Ca}_v3.2$ and $\text{Ca}_v3.3$ [18]. Unlike high-voltage-activated (HVA: L-, N-, P/Q-, and R-types) Ca^{2+} channels, T-type Ca^{2+} channels appear to consist of only $\alpha 1$ subunit and do not require coassembly with auxiliary subunits such as $\alpha 2\delta$, β and γ that are essential for HVA Ca^{2+} channels. Under the physiological conditions, T-type Ca^{2+} channels are activated by depolarization near a resting membrane potential, thereby regulating neuronal excitability. There is evidence for the involvement of T-type Ca^{2+} channels in secretion of hormones or neurotransmitters, muscle contraction and cell differentiation or proliferation [19, 20]. Among 3 isoforms of T-type Ca^{2+} channels, $\text{Ca}_v3.2$ appears to mediate some of biological actions of H_2S . H_2S donors, NaHS and Na_2S , facilitate T-type Ca^{2+} channel currents (T-currents) in NG108-15 (mouse neuroblastoma $\times$ rat glioma) cells, isolated dorsal root ganglion (DRG) neurons and neuroendocrine-like differentiated human prostate cancer LNCaP cells that naturally express $\text{Ca}_v3.2$ [9–11, 17], and also in $\text{Ca}_v3.2$ -transfected HEK293 cells [21]. The T-currents are suppressed by DL-propargylglycine (PPG), a CSE inhibitor, in neuroendocrine-differentiated LNCaP cells and $\text{Ca}_v3.2$ -transfected HEK293 cells that abundantly express CSE, an H_2S -forming enzyme, suggesting that the function of $\text{Ca}_v3.2$ is positively regulated by endogenous H_2S in those cells [17, 21]. These findings are

in agreement with recent evidence from an independent group that endogenous H_2S formed by CSE following hypoxia and H_2S donor-derived exogenous H_2S cause Ca^{2+} influx in glomus cells and sensory excitation of the carotid body in wild-type mice, and these effects of H_2S are greatly attenuated in $\text{Ca}_v3.2$ -knockout mice and by selective T-type Ca^{2+} channel blockers [22]. In physiological conditions, $\text{Ca}_v3.2$, but not $\text{Ca}_v3.1$ or $\text{Ca}_v3.3$, is considered to be tonically attenuated by zinc binding to the histidine residue at position 191 (His^{191}) in the second extracellular loop of domain I of the channel protein [23]. The His^{191} of $\text{Ca}_v3.2$ is conserved through different species including human, mouse and rat, and contributes to much higher sensitivity of $\text{Ca}_v3.2$ to inhibition with metals such as zinc, copper and nickel, compared to $\text{Ca}_v3.1$ or $\text{Ca}_v3.3$ [15, 20]. It is considered that typical zinc chelators and also some substances capable of interacting zinc, such as H_2S and L-Cys, cancel inhibition of channel activity by zinc binding to His^{191} of $\text{Ca}_v3.2$. In addition, $\text{Ca}_v3.2$, but not $\text{Ca}_v3.1$ or $\text{Ca}_v3.3$, is suppressed by ascorbic acid (vitamin C) via the metal-catalyzed oxidation at His^{191} [24]. The NaHS-induced facilitation of T-currents in NG108-15 cells is also attenuated by ascorbic acid [10]. There is a conflicting report showing that NaHS at low and high concentrations suppresses and enhances T-currents, respectively, in $\text{Ca}_v3.2$ -transfected HEK293 cells [12], although the physiological significance of the H_2S -induced T-current attenuation, if any, remains unclear. Nevertheless, we have never detected such inhibitory effect of H_2S donors in an extensive concentration range on T-currents in HEK293 cells transfected with $\text{Ca}_v3.2$ or other cells that naturally express $\text{Ca}_v3.2$ [7, 9–11, 16, 17, 21].

Roles of Endogenous H_2S and T-Type Calcium Channels in Pain Processing

The concentrations of endogenous H_2S in the mammalian tissues are regulated by 3 principal H_2S -producing enzymes, CSE, CBS, and 3-MST/CAT. It has been reported that CSE and CBS are upregulated in inflammatory conditions such as sepsis, pancreatitis and cystitis in rats or mice [25–28]. On the other hand, $\text{Ca}_v3.2$ targeted by H_2S is upregulated de novo or functionally in the DRG and/or spinal cord in laboratory animals with surgically induced neuropathic pain [15, 29–33]. In mice or rats, intraplantar and intracolonic administration of NaHS, an H_2S donor, evokes mechanical hyperalgesia/allodynia and visceral nociceptive behavior accompanied by re-

Fig. 1. A scheme for the pain processing by H_2S via $\text{Ca}_v3.2$ and TRPA1 in the sensory neurons.

ferred hyperalgesia, respectively, which are blocked by T-type Ca^{2+} blockers or gene silencing of $\text{Ca}_v3.2$ [8, 11, 29, 34, 35]. In the mice with cerulein-induced pancreatitis and with cyclophosphamide-induced cystitis, CSE protein is markedly upregulated in the pancreas and bladder, respectively, and the concomitant pancreatic and bladder pain are abolished by a CSE inhibitor or distinct T-type Ca^{2+} channel blockers, and by knockdown of $\text{Ca}_v3.2$ in DRG by intrathecal administration of the $\text{Ca}_v3.2$ -targeting antisense oligodeoxynucleotide [26, 27, 36]. The CSE inhibitor, T-type Ca^{2+} channel blockers and knockdown of $\text{Ca}_v3.2$ also suppress the neuropathic hyperalgesia induced by L5 spinal nerve injury in rats, and upregulation of $\text{Ca}_v3.2$ channels, but not CSE, at protein levels are detectable in the DRG following the development of neuropathy [31]. Paclitaxel-evoked painful neuropathy in rats is also suppressed by the inhibitors of CSE and T-type Ca^{2+} channels, while the expression levels of CSE and $\text{Ca}_v3.2$ proteins in the DRG and hindpaws do not change after paclitaxel treatment [37]. In both type I (streptozotocin (STZ) injection-induced diabetes) and type II (leptin-deficient (*ob/ob*) mice) diabetic mouse models, the diabetic neuropathy is considered to involve the up-regulation of $\text{Ca}_v3.2$ T-type Ca^{2+} channels in small-medi-

um size DRG neurons [38, 39]. There is also evidence that hyperalgesia/allodynia accompanying STZ-induced diabetic neuropathy in rats is suppressed by systemic administration of inhibitors of CSE (PPG) or CBS (hydroxylamine) [40]. The increased production of endogenous H_2S by CSE or CBS and possibly concomitant enhancement of $\text{Ca}_v3.2$ channel activity would thus appear to be involved in diabetic neuropathy. Together, studies on the roles of the endogenous $\text{H}_2\text{S}/\text{Ca}_v3.2$ pathway in pain processing might unveil novel therapeutic avenues for treatment of intractable pain including neuropathic and visceral pain (fig. 1). TRPA1 in addition to $\text{Ca}_v3.2$ mediates the pronociceptive effect of H_2S [15, 41] (fig. 1). We have shown that the somatic hyperalgesia following intraplantar injection of NaHS and prompt phosphorylation of ERK in the spinal dorsal horn following NaHS injection into the pancreatic duct are reduced by pharmacological inhibition or knockdown of not only $\text{Ca}_v3.2$ but also TRPA1 [34, 36]. It has been reported that H_2S activates TRPA1 through sulfhydrylation of the intracellular cysteine residue of this channel and that polysulfides are more potent in activating TRPA1 than H_2S [42]. The effect of polysulfides on $\text{Ca}_v3.2$ channels is still open to question, and needs to be examined in future.

Role of Endogenous H₂S and T-Type Calcium Channels in Neuronal Differentiation

H₂S may regulate differentiation of various cells [16, 43, 44]. In neuronal progenitor-like NG108-15 cells that abundantly express Ca_v3.2 channels, dibutyl cyclic AMP (db-cAMP), a membrane-permeable analogue of cAMP, causes neuronal differentiation characterized by neurite outgrowth and functional upregulation of HVA Ca²⁺ channels [45, 46], being dependent on Ca_v3.2 channel activity [45]. Interestingly, NaHS and Na₂S, H₂S donors, also cause similar neurite outgrowth and functional upregulation of HVA channels in NG108-15 cells [9, 10, 16]. The NaHS-evoked neuritogenesis is inhibited by BAPTA-AM, an intracellular Ca²⁺ chelator, or mibefradil, a T-type Ca²⁺ channel blocker, and by zinc chloride and ascorbic acid that inhibit Ca_v3.2, but not Ca_v3.1 or Ca_v3.3, as mentioned above, suggesting the involvement of Ca²⁺ influx through Ca_v3.2 channels in the H₂S-dependent neuronal differentiation [9, 10]. The NaHS-induced neuritogenesis is not affected by inhibition of other ion channels known as possible targets for H₂S, K_{ATP}, TRPA1 and HVA Ca²⁺ channels [10]. The activity of Src kinase appears to be required for the NaHS-evoked neuronal differentiation of NG108-15 cells [10].

Roles of Endogenous H₂S and T-Type Calcium Channels in Neuronal and Neuroendocrine Secretion

H₂S appears to regulate secretory function in the pancreas, hypothalamus and pituitary gland; for example, H₂S decreases insulin secretion via activation of K_{ATP} channels in rat insulinoma INS-1E cells or inhibition of L-type Ca²⁺ channels in mouse pancreatic β cells [47, 48], enhances 5-hydroxytryptamine release via activation of TRPA1 channels in clonal rat pancreatic delta RIN14B cells [49] and reduces the exocytosis of secretory granules via activation of K_{ATP} channels in rat pituitary tumor GH3 cells [50]. Interestingly, T-type Ca²⁺ channels trigger Ca²⁺ entry during small depolarization around the resting potential and concomitant vesicular exocytosis in the neuronal system, which is essential for synaptic neurotransmitter release at rest [51]. Thus, we next focus on the roles of the endogenous H₂S/Ca_v3.2 pathway in neuronal or neuroendocrine secretion in neuroendocrine-differentiated prostate cancer cells, glomus cells in the carotid body, and chromaffin cells in the adrenal medulla [17, 52, 53].

The H₂S/Ca_v3.2 Pathway Regulates Neuroendocrine Secretion in Human Prostate Cancer LNCaP Cells

The growth of prostate cancer is androgen-dependent in general, so that the hormone therapy by androgen deprivation or androgen receptor blockade is highly effective. However, the hormone therapy for prostate cancer patients is overridden in several years. Acquisition of the androgen-independent properties of prostate cancer is conceived as a major reason for the development of the hormone therapy tolerance [54]. There is evidence that neuroendocrine-like differentiation of prostate cancer cells contributes to the androgen-independent growth [55, 56]. In androgen-dependent human prostate cancer LNCaP cells, the neuroendocrine-like differentiation is induced by an elevation of intracellular cAMP levels, androgen deprivation or treatment with interleukin-6 in a subpopulation of the cells [57]. The differentiated cells show androgen-independent properties, and secrete the proliferative neuropeptides such as neurotensin and parathyroid hormone-related peptide (PTHrP), which promote the proliferation of surrounding prostate cancer cells [58, 59]. Ca_v3.2 T-type Ca²⁺ channels are abundantly expressed in neuroendocrine-like differentiated LNCaP cells, and mediate Ca²⁺-dependent secretion [17, 60–62], while the expression of L-type Ca²⁺ channels is not detected in those cells [63].

The protein expression and activity of H₂S-forming enzymes, CSE and CBS, in the prostate cancer LNCaP cells are much more than those in normal prostatic epithelial RWPE-1 cells [64]. The expression of these enzymes in LNCaP cells increases during neuroendocrine-like differentiation induced by treatment with db-cAMP plus isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor [17]. The H₂S donors, NaHS and Na₂S, further enhance the increased T-currents dependent on the upregulated Ca_v3.2 in the neuroendocrine-like differentiated LNCaP cells. Interestingly, the increased T-currents in the neuroendocrine-like LNCaP cells are partially attenuated by the treatment with the CSE inhibitors, PPG and β-cyano-L-alanine, but not a CBS inhibitor aminoxy acetic acid (AOAA), suggesting that endogenous H₂S produced by the upregulated CSE, but not CBS, enhances the activity of the overexpressed Ca_v3.2 channels in neuroendocrine-like LNCaP cells. In parallel with the upregulation of Ca_v3.2 channels, spontaneous Ca²⁺-dependent secretion increases following neuroendocrine-like differentiation with db-cAMP plus IBMX in LNCaP cells [60]. The increased spontaneous secretory function in the neuroendocrine-like LNCaP cells is abolished by mibefradil, a T-type Ca²⁺ channel blocker, or PPG, but not AOAA,

Fig. 2. Involvement of the H₂S/Cav3.2 T-type Ca²⁺ channel pathway in secretory function in neuroendocrine-like differentiated prostate cancer LNCaP cells.

whereas mibefradil does not affect the secretory response in undifferentiated LNCaP cells that do not abundantly express CSE and Cav3.2 [17]. Together, it is considered that, in neuroendocrine-differentiated prostate cancer LNCaP cells, H₂S generated by the upregulated CSE further enhances the channel activity of the upregulated Cav3.2 and causes the augmentation of the secretory function (fig. 2). Since the neuroendocrine-like differentiation of LNCaP cells by db-cAMP plus IBMX results in the upregulation of neurotensin and PTHrP, mitogenic neuropeptides [17], the enhanced secretory function brought about by the CSE/H₂S/Cav3.2 pathway may contribute to the increased secretion of those mitogenic factors in the differentiated cells, leading to androgen-independent proliferation in the surrounding undifferentiated cells (fig. 2). This hypothesis is in agreement with the evidence that the conditioned culture medium from the neuroendocrine-like differentiated LNCaP cells and neurotensin promote mitogenesis of androgen-independent prostate cancer PC3 cells [65]. Therefore, pharmacological inhibition of the CSE/H₂S/Cav3.2 pathway might be beneficial to prevent the acquisition of androgen-independent property of prostate cancer. However, the long-term inhibition of CSE might cause unexpected serious

biological events, because endogenous H₂S plays important physiological roles throughout the mammalian body including the cardiovascular, gastrointestinal and neuronal systems [1, 4]. In addition, CSE contributes to not only L-Cys metabolism to H₂S and pyruvate, but also L-cystathionine metabolism to L-Cys and α -ketobutyrate [3], so that the abnormal metabolism of L-Cys or L-cystathionine caused by CSE inhibitors might also limit their therapeutic use. In contrast, the pharmacological blockade of Cav3.2 T-type Ca²⁺ channels might be suitable for the treatment of prostate cancer. Actually, several blockers of T-type Ca²⁺ channel appear to inhibit the proliferation and migration of androgen-independent prostate cancer DU-145 cells [66]. Although there is no report concerning the development of selective blockers of Cav3.2 channels, ascorbic acid (vitamin C) and zinc block Cav3.2, but not Cav3.1 or Cav3.3 [23, 24]. Strikingly, it has been reported that the pharmacological dose of ascorbic acid suppresses tumor growth and metastases of implanted hormone-resistant prostate adenocarcinoma cells in rats [67].

Involvement of the H₂S/Cav3.2 Pathway in the Hypoxia-Induced Catecholamine Secretion in the Carotid Body and Adrenal Medulla

Hypoxia induces Ca²⁺-dependent release of catecholamines (CAs) in glomus cells of the carotid body, an organ sensing arterial blood O₂ concentration, which is essential for controlling the function of respiratory and cardiovascular systems [22]. The hypoxia-induced secretion of CAs is observed also in the adrenal medullary chromaffin cells [52]. It has been reported that CSE, an H₂S-forming enzyme, is expressed in glomus cells in the carotid body and adrenal medullary chromaffin cells, and that the hypoxia-evoked CA secretion is attenuated in CSE knockout mice or by the treatment with CSE inhibitors, indicating the critical role of endogenous H₂S formed by CSE in the respiratory and cardiovascular homeostatic control in response to hypoxia [22, 52]. Voltage-gated HVA and T-type Ca²⁺ channels are also expressed in glomus cells of the carotid body [68, 69], and the hypoxia-evoked elevation of intracellular Ca²⁺ concentration and CA secretion in the glomus cells are attenuated by inhibitors of L-type (nifedipine) or T-type (mibefradil and TTA-A2) Ca²⁺ channels, or in Cav3.2 knockout mice [22, 70]. Therefore, endogenous H₂S produced by CSE is considered to cause Ca²⁺ influx through both Cav3.2 T-type channels and L-type HVA channels in the carotid body, regulating the hypoxia-evoked CA secretion. Recently, it has also been reported that H₂S inhibits background K⁺ currents

through TWIK-related acid-sensitive potassium (TASK) channels in carotid body type 1 (glomus) cells, leading to membrane depolarization and elevation of the intracellular Ca^{2+} concentration via voltage-dependent Ca^{2+} channels [71]. Thus, in addition to the direct enhancement of $\text{Ca}_v3.2$ activity by H_2S , indirect activation of $\text{Ca}_v3.2$ following membrane depolarization by the H_2S inhibition of TASK channels might also contribute to H_2S -enhanced Ca^{2+} influx and CA secretion in response to hypoxia. The adrenal chromaffin cells of mammalian species express all types of voltage-gated Ca^{2+} channels, whereas CA secretion is mostly mediated by Ca^{2+} influx via HVA Ca^{2+} channels (L-, N-, P/Q- and R-types) [72]. On the other hand, T-type Ca^{2+} channels are poorly expressed or absent in adult chromaffin cells, but are functionally expressed in the embryonic and neonatal chromaffin cells [51, 72]. In addition, $\text{Ca}_v3.2$ T-type Ca^{2+} channels in adult chromaffin cells are upregulated in response to various long-term stressful stimuli, such as chronic hypoxia, β -adrenergic and high-frequency sympathetic stimulation and exposure to cAMP, and contribute to low-threshold CA secretion [19, 51]. On the other hand, NaHS, an H_2S donor, induces CA secretion in rat adrenal chromaffin cells [53]. CSE is detected in the chromaffin cells, and upregulated and involved in the hypoxia-evoked CA secretion in response to hypoxia [52]. Col-

lectively, the CSE/ H_2S / $\text{Ca}_v3.2$ pathway may play a role in the hypoxia-evoked CA secretion in adrenal chromaffin cells, as in glomus cells of the carotid body. This hypothesis has yet to be demonstrated by future more in-depth studies.

Conclusion

As described so far, the CSE/ H_2S / $\text{Ca}_v3.2$ T-type Ca^{2+} channel pathway plays roles in the pain processing, neuronal differentiation and neuronal or neuroendocrine secretion. Thus, pharmacological inhibition or acceleration of this pathway may be beneficial for treatment of intractable pain, neuronal or axonal injury, prostate cancer and impaired homeostatic regulation of the respiratory and cardiovascular systems.

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Disclosure Statement

The authors have no conflict of interest to declare.

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