Roles of the Hydrogen Sulfide/T-Type Calcium Channel System in Somatic and Visceral Pain Processing

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Abstract

Hydrogen sulfide (H\textsubscript{2}S), a gasotransmitter, is endogenously formed from \textit{l}-cysteine by certain enzymes including cystathionine-\gamma-lyase (CSE) in the mammalian body. H\textsubscript{2}S sensitizes Ca\textsubscript{v}3.2 T-type calcium channels, leading to excitation of sensory neurons followed by somatic hyperalgesia in rats and mice. The enhanced activity of the H\textsubscript{2}S/Ca\textsubscript{v}3.2 system is involved in the neuropathic pain/hyperalgesia induced by repeated administration of paclitaxel, an anti-cancer drug, or by spinal nerve injury. It is also noteworthy that the H\textsubscript{2}S-induced mechanical hyperalgesia requires activation of both Ca\textsubscript{v}3.2 and transient receptor potential A1 (TRPA1) channels in mice. H\textsubscript{2}S and Ca\textsubscript{v}3.2 T-type calcium channels are also involved in processing of visceral nociception including colonic, pancreatic and bladder pain. Endogenous H\textsubscript{2}S formed by upregulated CSE contributes to the pancreatitis-related pain. Further, the excitation of sensory nerves by H\textsubscript{2}S through T-type calcium channels exerts mucosal cytoprotection against colitis in rats. Together, endogenous H\textsubscript{2}S formed by CSE appears to stimulate sensory nerves by targeting Ca\textsubscript{v}3.2 T-type calcium channels and, in some cases, TRPA1 channels, leading to facilitation of somatic and visceral pain signals and also contributing to colonic mucosal cytoprotection. Thus, the CSE/H\textsubscript{2}S/Ca\textsubscript{v}3.2 system may serve as therapeutic targets for treatment of neuropathic or visceral pain and of colitis.

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Hydrogen sulfide (H\textsubscript{2}S) is now considered the third gasotransmitter after nitric oxide (NO) and carbon monoxide (CO), and plays a variety of roles in health and disease. H\textsubscript{2}S is formed from \textit{l}-cysteine through three distinct enzymatic pathways catalyzed by cystathionine-\gamma-lyase (CSE), cystathionine-\beta-synthase (CBS) and 3-mercaptopypyruvate sulfurtransferase (MST) along with cysteine aminotransferase (CAT) [1] (fig. 1). Like NO and CO, H\textsubscript{2}S is a highly lipophilic molecule able to freely penetrate the cell membrane [2], and targets multiple molecules including Ca\textsubscript{v}3.2 T-type calcium channels [3, 4], transient receptor potential A1 (TRPA1) channels [5] and ATP-sensitive potassium (K\textsubscript{ATP}) channels [6] in the mammalian body [7]. Some
of the biological effects of H$_2$S appear to result from S-sulphydration of the target proteins including K$_{ATP}$ channels [8, 9]. Here we focus on the roles of H$_2$S in processing of somatic and visceral pain signals and also in colonic mucosal cytoprotection.

Roles of H$_2$S in Processing of Somatic Pain Signals in Health and Disease

In 2007, we first reported that H$_2$S sensitizes/activates T-type calcium channels, leading to somatic mechanical hyperalgesia in rats [4]. The Ca$_{v}$3.2 isoform of T-type calcium channels is now considered responsible for the development of the H$_2$S-induced hyperalgesia [3] (fig. 1). The CSE/H$_2$S/Ca$_{v}$3.2 system appears to be involved in the pathophysiology of neuropathic pain induced by L5 spinal nerve cutting or by repeated administration of paclitaxel, an anti-cancer drug, since inhibitors of CSE or T-type calcium channels and silencing of Ca$_{v}$3.2 in the sensory neurons by intrathecal administration of the antisense oligodeoxynucleotides or small interfering RNA abolish the neuropathic hyperalgesia in both models [10, 11]. Expression levels of Ca$_{v}$3.2 channels in the dorsal root ganglion are dramatically upregulated in the L5 spinal nerve injury model, but not the paclitaxel model (table 1). However, the paclitaxel-treated rats exhibit increased levels of endogenous H$_2$S that sensitizes/activates Ca$_{v}$3.2 [11], and decreased levels of endogenous ascorbic acid, known to inhibit Ca$_{v}$3.2 [12], in the hindpaw (table 1). Thus, apart from the underlying detailed mechanisms, the peripheral H$_2$S/Ca$_{v}$3.2 system is functionally upregulated in animals with neuropathy, and considered a potential therapeutic target for neuropathic pain (fig. 1). We have then focused on ascorbic acid that inhibits Ca$_{v}$3.2, but...
not Cav₃.1 or Cav₃.3 [13], and found that topical application of disodium isostearyl 2-Ο-L-ascorbyl phosphate, a skin-permeable, amphiphilic prodrug of ascorbic acid, as well as intraplantar administration of ascorbic acid reverses the neuropathic hyperalgesia induced by paclitaxel and also by L5 spinal nerve injury in rats [12] (fig. 1). It is also noteworthy that in addition to sensitizing Cav₃.2, H₂S targets and activates TRPA1 channels in sensory neurons [5, 14] contributing to the development of mechanical hyperalgesia in mice [15] (fig. 1).

### Roles of H₂S in Processing of Visceral Pain Signals and in the Pathophysiology of Pancreatitis-Related Pain

The H₂S/Cav₃.2 system is also considered to be involved in processing of visceral pain signals, especially colonic [16], pancreatic [17] and bladder pain [18] (fig. 1). We have shown that intracolonic administration of NaHS, an H₂S donor, causes visceral pain-like nociceptive behavior followed by referred hyperalgesia in the lower abdomen in mice [16]. The luminal H₂S-induced colonic pain and referred hyperalgesia are abolished by pretreatment with T-type calcium channel blockers. Intracolonic NaHS also causes prompt phosphorylation of ERK in the superficial layers of the spinal dorsal horn in mice, implying excitation of nociceptors [16]. The function of Cav₃.2, but not Cav₃.1 or Cav₃.3, is known to be tonically suppressed by endogenous Zn²⁺ that binds to the histidine residue at position 191 in the Cav₃.2 molecule, and chelating Zn²⁺ sensitizes or enhances the function of Cav₃.2 [19]. Most interestingly, intracolonic administration of Zn²⁺ chelators mimics the colonic pronociceptive effects of intracolonic NaHS, and the NaHS-induced colonic pain and referred hyperalgesia are prevented by ZnCl₂ in mice [20]. Therefore, it is likely that luminal NaHS/H₂S interacts with endogenous Zn²⁺ and cancels Zn²⁺ inhibition of Cav₃.2, leading to colonic pain or referred hyperalgesia in mice.

### Table 1. Expression levels of Cav₃.2 and CSE proteins and concentrations of H₂S and ascorbic acid in DRG and hindpaw tissues of the rats subjected to L5SNC or paclitaxel treatment

<table>
<thead>
<tr>
<th></th>
<th>L5SNC</th>
<th>Paclitaxel</th>
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<tbody>
<tr>
<td></td>
<td>DRG</td>
<td>hindpaw</td>
</tr>
<tr>
<td>Cav₃.2 upregulated</td>
<td>n.d.</td>
<td>unchanged</td>
</tr>
<tr>
<td>CSE unchanged</td>
<td>n.d.</td>
<td>unchanged</td>
</tr>
<tr>
<td>H₂S n.d.</td>
<td>n.d.</td>
<td>unchanged</td>
</tr>
<tr>
<td>Ascorbic acid n.d.</td>
<td>n.d.</td>
<td>changed</td>
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n.d. = Not determined; L5SNC = L5 spinal nerve cutting; DRG = dorsal root ganglion; CSE = cystathionine-γ-lyase.
The CSE/H$_2$S/Cav3.2 system plays an important role in processing of pancreatic pain and in the maintenance of pancreatitis-related pain in mice (fig. 1). Infusion of NaHS into the pancreatic duct causes prompt phosphorylation of ERK and delayed expression of Fos in the superficial layers of the spinal dorsal horn in rats and/or mice, implying excitation of nociceptors [17, 21]. Both the ductal NaHS-induced ERK phosphorylation and Fos expression are prevented by pretreatment with T-type calcium channel blockers. In mice with acute pancreatitis induced by repeated administration of cerulein, CSE protein is greatly upregulated in the pancreatic tissue, and the referred hyperalgesia in the upper abdomen accompanying the pancreatitis is prevented by CSE inhibitors and reversed by T-type calcium channel blockers [17]. Thus, activation of T-type calcium channels by endogenous H$_2$S the production of which elevates following upregulation of CSE during the development of pancreatitis is considered to participate in the development and/or maintenance of the pancreatitis-related pain.

**Involvement of the Excitation of Sensory Neurons in the H$_2$S-Induced Colonic Mucosal Cytoprotection**

Apart from the pronociceptive role of H$_2$S in the mouse colon, there is evidence that luminal NaHS/H$_2$S protects colonic mucosa against the colonic inflammation and ulceration induced by trinitrobenzene sulfonic acid (TNBS) in rats [22]. There is conflicting evidence for the protective and pro-inflammatory roles of capsaicin-sensitive sensory nerves in rats with TNBS-induced colitis [23–25]. Given that luminal H$_2$S is capable of stimulating sensory nerves in mice [16, 20], as described above, it is likely that the protective effect of luminal NaHS/H$_2$S against TNBS-induced colitis in rats is attributable to excitation of sensory nerves. Most recently, we have found that the protective effects of intracolonic administration of NaHS against the TNBS-induced colonic mucosal damage are reduced by ablation of capsaicin-sensitive sensory nerves and by pretreatment with a T-type calcium channel blocker in rats [26], suggesting that the effects of NaHS/H$_2$S are dependent on excitation of C-fiber sensory nerves and activation of T-type calcium channels. Our study has also demonstrated that intracolonic administration of NaHS causes prompt phosphorylation of ERK in the superficial layers of the spinal dorsal horn in rats in the early stage of colitis (1–3 days after TNBS treatment), an effect prevented by pretreatment with the T-type calcium channel blocker, although it does not evoke spinal ERK phosphorylation in naïve rats [26]. Thus, the NaHS/H$_2$S-induced excitation of sensory nerves appears to occur through activation of T-type calcium channels only in the presence of colitis in rats, in contrast to the evidence that intracolonic NaHS/H$_2$S induces spinal ERK phosphorylation even in naïve mice [16]. The higher sensitivity of sensory nerves to NaHS/H$_2$S in rats with colitis may be attributable to the upregulation of Ca$_v$3.2 expression in the dorsal root ganglion.
of rats in the early stage of TNBS-induced colitis [26]. Together, we propose that luminal NaHS/H$_2$S exerts colonic mucosal cytoprotection in rats treated with TNBS through excitation of sensory nerves by activating Ca$_v$3.2 T-type calcium channels that are upregulated during the development of colitis (fig. 2). Similarly, the neurally mediated cytoprotective effect of H$_2$S has been reported in mice with ethanol-induced gastric damage [27].

**Conclusion and Future Perspective**

Endogenous H$_2$S formed by CSE stimulates sensory nerves by targeting Ca$_v$3.2 T-type calcium channels and, in some cases, TRPA1 channels, leading to facilitation of somatic and visceral pain signals and also contributing to colonic mucosal cytoprotection. Selective inhibitors of CSE and/or blockers of Ca$_v$3.2 may be available as novel analgesics for treatment of persistent pain including neuropathic pain and visceral pain accompanying colitis and pancreatitis, whilst H$_2$S donors may serve as cytoprotective agents against gastrointestinal inflammation including colitis.
References


