Molecular Basis of Regulating High Voltage-Activated Calcium Channels by S-Nitrosylation*

Meng-Hua Zhou, Alexis Bavencoffe, and Hui-Lin Pan1
From the Department of Anesthesiology and Perioperative Medicine, Center for Neuroscience and Pain Research, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030

Nitric oxide (NO) is involved in a variety of physiological processes, such as vasoregulation and neurotransmission, and has a complex role in the regulation of pain transmission and synaptic transmission. We have shown previously that NO inhibits high voltage-activated Ca2+ channels in primary sensory neurons and excitatory synaptic transmission in the spinal dorsal horn. However, the molecular mechanism involved in this inhibitory action remains unclear. In this study, we investigated the role of S-nitrosylation in the NO regulation of high voltage-activated Ca2+ channels.

There exist several types of HVA Ca2+ channels, including L-, N-, P/Q-, and R-types. L-type Ca2+ channels are not only present in cardiac and smooth muscles but also expressed in neurons and endocrine cells where they regulate a multitude of processes, including the release of hormones and neurotransmitters and gene expression (5, 6). N-type channels are distributed in the brain and peripheral nervous system and are the major subtypes present in nociceptive dorsal root ganglion (DRG) neurons (7–10). P/Q-type channels are involved in neurotransmitter release at synaptic terminals (11, 12). The Cavβ subunits (Cavβ1-Cavβ4) are abundantly expressed in excitable tissues such as the brain, heart, and muscles. Cavβ3 is the predominant partner of Cav2.2 (N-type), and Cavβ4 is the most prevalent partner of Cav2.1 (P/Q-type) in the brain (3, 13). HVA Ca2+ channels are essential for the nociceptive transmission (14, 15), and changes in HVA Ca2+ channel properties in DRG neurons may be involved in the development of chronic neuropathic pain (16–18).

Nitric oxide (NO) plays a complex role in the modulation of pain. Although some studies suggest that NO is pro-nociceptive (19, 20), NO can also reduce pain and nociceptive transmission (21–23). Indeed, our previous study has shown that NO inhibits HVA Ca2+ channel currents in DRG neurons and attenuates excitatory synaptic transmission in the spinal dorsal horn (24). However, the molecular mechanism underlying this inhibitory action is still unclear. NO exerts ubiquitous signaling via post-translational modification of cysteine residues, a reaction termed S-nitrosylation. Because nitrosothiols are exceptionally labile because of their reactivity with intracellular reducing reagents such as ascorbic acid, S-nitrosylation functions as a reversible post-translational modification analogous to phosphorylation. S-Nitrosylation is involved in regulation of NMDA receptors (25) and ryanodine receptor/Ca2+ release channels (26). In DRG neurons, NO-induced HVA Ca2+ channel inhibition is resistant to the guanylate cyclase inhibitor (24). However, N-ethylmaleimide, a specific alkylating agent of cysteine sulfhydryl, prevents the inhibitory effect of the NO precursor l-arginine and the NO donor S-nitroso-N-acetyl-DL-penicillamine (SNAP) on HVA Ca2+ channels (24). Thus, NO likely inhibits HVA Ca2+ channels through cGMP-indepen-
dent pathways such as the nitrosylation of free sulphhydryl groups of cysteine residues.

In this study, we determined the role of S-nitrosylation in the effects of NO on various types of reconstituted HVA Ca\(^{2+}\) channels. We present new molecular evidence showing cysteine modification as a distinct mechanism for regulation of N-type channels by NO.

**Experimental Procedures**

**Cell Culture and Transfection**—Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco/Life Technologies) supplemented with 10% fetal bovine serum (Sigma) at 37 °C in 5% CO\(_2\). For transfection experiments, 1.2 \(\times\) 10\(^4\) cells were plated on poly-d-lysine-coated coverslips in each well of a 24-well plate. After 24 h, we used PolyJet DNA In Vitro transfection reagent (SignaGen Laboratories, Gaithersburg, MD) to transiently transfected the cells with various combinations of Cav\(_{\alpha}\) (Cav1.2, Cav2.1, or Cav2.2), \(\alpha_\delta\delta_1\), and Cav\(_\beta\) (Cav\(_\beta_1\)–Cav\(_\beta_4\)) subunits. The sources of cDNAs for rat Cav\(_\alpha\), \(\alpha_\delta\delta_1\), and Cav\(_\beta\) subunits were reported previously (27, 28). A Cav2.2–V5 construct was made by inserting a rat Cav2.2 fragment into a pCDNA6 V5-FLAG vector (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The surface protein samples were probed with the anti-V5 antibody (1:1,000 dilution; EMD Millipore, Billerica, MA) using Western blot analysis. Na\(^{+}/K\(^{-}\)–ATPase antibody (1:1,000 dilution; EMD Millipore, Billerica, MA) using Western blot analysis, Na\(^{+}/K\(^{-}\)–ATPase, a plasma membrane protein, was used as a loading control. The amounts of Cav2.2 proteins were normalized by the protein band of Na\(^{+}/K\(^{-}\)–ATPase. The mean value of Cav2.2 protein levels in HEK293 cells treated with the vehicle was defined as 1 (27).

**Site-directed Mutagenesis**—Point mutations were performed using the QuikChange Site-directed Mutagenesis Kit (Stratagene, Santa Clara, CA) or the In-Fusion HD Cloning Kit (Clontech) according to the manufacturer’s instructions.

**Data Analysis**—The HVA Ca\(^{2+}\) current data were analyzed using Origin 8 software (Microcal Software, Northampton, MA). Conductance-voltage (G-V) curves were fitted using the Boltzmann equation, 

\[
\frac{G}{G_{\text{max}}} = \frac{1}{1 + \exp \left( \frac{(V_{0.5} - V_{m})}{k} \right)}
\]

where \(G\) is the membrane conductance, \(V_m\) is the test potential, and \(V_{0.5}\) is the voltage for 50% activation or inactivation of HVA Ca\(^{2+}\) currents, and \(k\) is a voltage-dependent slope factor. Western immunoblotting data were quantified by LI-COR Image Studio software (LI-COR Biosciences, Lincoln, NE). We used the Student’s \(t\) test to compare the SNAP or MTSEA effects on HVA Ca\(^{2+}\) currents between two groups. For comparing the differences in the SNAP effects on N-type channel currents between the wild-type and various mutant groups, we used the \(\chi^2\)-square test with Yates correction for continuity. Values are given as mean \(\pm\) S.E., with \(n\) indicating the number of cells used for the electrophysiological recording or the number of independent repeats for biochemical experiments. Differences were considered statistically significant if the \(p\) value was less than 0.05.

**Results**

**Differential Effects of SNAP on N-type, P/Q-type, and L-type Channels**—To determine whether NO differentially inhibits different HVA Ca\(^{2+}\) channels, we compared the inhibitory effects of the NO donor SNAP on N-type (Cav2.2), P/Q-type (Cav2.1), and L-type (Cav1.2) channels in HEK293 cells cotransfected with \(\alpha_\delta\delta_1\) and different Cav\(_\beta\) (Cav\(_\beta_1\), Cav\(_\beta_2\), Cav\(_\beta_3\), or Cav\(_\beta_4\)) subunits. The whole cell \(I_{\text{Na}}\) were elicited by a depolarizing pulse to 0 mV for 200 ms from a holding potential of −90 mV. Bath application of 100 \(\mu\)M SNAP caused a rapid and large inhibition of the current amplitude of N-type Ca\(^{2+}\) channels reconstituted with Cav\(_\beta_1\) (42.46 ± 7.12%, \(n = 7\)) or Cav\(_\beta_3\) (33.27 ± 3.28%, \(n = 7\); Fig. 1). In contrast, SNAP only slightly inhibited N-type Ca\(^{2+}\) channel currents reconstituted with Cav\(_\beta_2\) (9.97 ± 1.84%, \(n = 7\)) or Cav\(_\beta_4\) (15.20 ± 1.82%, \(n = 7\); Fig. 1). Upon SNAP washout, the reduced HVA Ca\(^{2+}\) currents often persisted for more than 10 min. However, the inhibitory effect of SNAP on HVA Ca\(^{2+}\) currents was rapidly reversed by bath application of the reducing agent DTT (5 mm, Fig. 1A) (25, 29). DTT alone had no significant effect on N-type currents (Fig. 1B).

Bath application of SNAP produced only a small inhibitory effect on P/Q-type currents in HEK293 cells coexpressing with Cav\(_\beta_1\) (7.95 ± 2.53%, \(n = 8\)) or Cav\(_\beta_3\) (8.72 ± 1.69%, \(n = 6\)) and had no significant effect on P/Q-type currents reconstituted with Cav\(_\beta_2\) or Cav\(_\beta_4\) (Fig. 2). Also, SNAP inhibited L-type currents reconstituted with Cav\(_\beta_1\) (15.41 ± 0.87%, \(n = 7\)) but had no significant effect on L-type currents reconstituted with Cav\(_\beta_2\), Cav\(_\beta_3\), or Cav\(_\beta_4\) (Fig. 2). Thus, compared with P/Q-
type and L-type channels, N-type channels reconstituted with Cavβ1 or Cavβ3 were much more sensitive to inhibition by NO. In the following experiments, we focused our analysis on NO modulation of N-type channels.

**SNAP Causes a Depolarizing Shift of Voltage-dependent Activation of N-type Channels**—To examine the SNAP effect on voltage-dependent activation of N-type channels, the membrane potential was held at \(-90\) mV. \(I_{Na}\) currents were elicited by a series of depolarizing pulses for 150 ms from \(-70\) to \(50\) mV with an interval of \(10\) mV. HEK293 cells expressing Cav2.2, \(\alpha_2\delta_1\), and Cavβ1, Cavβ3, or Cavβ4 subunits were tested. Bath application of \(100 \mu M\) SNAP caused a significant depolarizing shift of voltage-dependent N-type channel activation (i.e. reducing the channel sensitivity to depolarizing voltages) and slightly increased the slope factors when Cav2.2 was coexpressed with \(\alpha_2\delta_1\) and Cavβ1, Cavβ3, or Cavβ4 subunits (Fig. 3A, Table 1). SNAP had no significant effect on voltage-dependent N-type channel activation in HEK293 cells coexpressing Cav2.2, \(\alpha_2\delta_1\), and Cavβ2 or Cavβ4 subunits. D, summary data show inhibition of N-type channel currents with different Cavβ subunits by \(100 \mu M\) SNAP. *, \(p < 0.05\), compared with the baseline control before SNAP application.

**SNAP Has No Effect on the Membrane Surface Expression of Cav2.2**—Because the inhibitory effect of NO on N-type currents may be associated with reduced Cav2.2 trafficking to the plasma membrane, we next analyzed the effect of SNAP on Cav2.2 surface expression. HEK293 cells transfected with V5-tagged Cav2.2, Cavβ3, and \(\alpha_2\delta_1\) were treated with \(100 \mu M\) SNAP or vehicle for 5 min. Cell surface proteins were then isolated and subjected to Western blotting analysis. Na+/K+-ATPase, a plasma membrane protein, was used as a loading control. The Ca2.2 surface protein levels did not differ significantly between SNAP- and vehicle-treated groups (Fig. 4).

**SNAP Inhibits N-type Channels through S-Nitrosylation**—The SNAP effect on HVA Ca\(^{2+}\) channels in DRG neurons and transfected HEK293 cells is independent of cGMP pathways (24, 29). To determine whether S-nitrosylation is involved in the inhibitory effect of NO on N-type currents, we used MTSEA, a membrane-permeable sulfhydryl-specific modifying reagent (25), which can rapidly react with thiols to form mixed disulfides, thereby preventing subsequent S-nitrosylation of proteins. HEK293 cells expressing Cav2.2, Cavβ3, and \(\alpha_2\delta_1\) subunits were pretreated with \(2.5 \text{ mM MTSEA}\) for \(1\) min before whole cell recordings. Treatment with MTSEA alone did not...
significantly change the amplitude of N-type currents. MTSEA treatment completely blocked the inhibitory effect of 100 μM SNAP on the amplitude of N-type channels (Fig. 5). These results suggest that NO inhibits N-type channels through S-nitrosylation.

Intracellular Cysteine Residues of N-type Channels Are Involved in S-Nitrosylation Modification—Our results with the sulfhydryl-specific modifying reagent indicate that NO may interact with cysteine residues to produce its effects on N-type channels. We next used site-directed mutagenesis to identify the possible cysteine residues involved in S-nitrosylation modifications using site-directed mutagenesis.

We mutated the seven cysteine residues individually on the Cav2.2 subunit as well as Cys-346 on the Cav3 subunit to alanine. Mutating two cysteine residues (Cys-277 and Cys-1688) in the pore loop of Cav2.2 largely diminished the I_{\text{Ba}} currents in HEK293 cells. The maximum current amplitudes produced by C277A and C1688A were 43.92 ± 16.00 pA (n = 7) and 29.21 ± 3.69 pA (n = 8), respectively. Nevertheless, mutating other cysteine residues in Cav2.2 produced wild-type-like N-type currents. In HEK293 cells coexpressing Cav\(\beta_1 + \alpha_\delta_1\) and C805A, C930A, C1835A, or C2145A mutant of Cav2.2, the inhibitory effect of SNAP on N-type currents was diminished (Fig. 7, Table 2). In HEK293 cells coexpressing Cav\(\beta_3\), C805A, and C1045A mutant, the inhibitory effect of SNAP on N-type currents was reduced by about 60% compared with that in wild-type Cav2.2 (Fig. 7, Table 2).

In contrast, mutating Cys-1065, a non-predicted cysteine residue in the II-III intracellular loop, to alanine did not significantly alter the inhibitory effect of SNAP (Fig. 7B, Table 2). Furthermore, SNAP had no significant effect on voltage-dependent activation or inactivation of N-type currents reconstituted with the C805A or C930A in HEK293 cells (Fig. 8, Table 1).

Mutating Cys-346 to alanine in the Cav3 subunit generated wild-type-like N-type currents in HEK293 cells coexpressing Cav2.2 and C805A. However, the inhibitory effect of SNAP on N-type currents reconstituted with the C346A mutant was reduced by about 50% compared with that in wild-type Cav3 (16.02 ± 3.13% versus 32.09 ± 3.21%; Fig. 7, Table 2). Taken together, our findings reveal that NO inhibits N-type channels through S-nitrosylation of intracellular cysteine residues in both \(\alpha_\delta\) and Cav\(\beta_3\) subunits.

**Discussion**

NO is a pleiotropic cell signaling molecule that controls diverse biological processes. S-Nitrosylation is the covalent modification of a protein cysteine thiol by an NO group to generate an S-nitrosothiol, which can occur rapidly without the action of any enzymes (25, 31, 32). In the cardiovascular system, S-nitrosylation seems to be involved in post-translational modification of several ion channels, including Nav1.5, L-type channels, Kv1.5, and Kv4.3 (33). NO can inhibit NMDA receptor currents through S-nitrosylation in the central nervous system (34), and mutating Cys-399 on the GluN2A subunit removes NO sensitivity (25). However, it has not been clear whether S-nitrosylation is involved in regulating N-type and P/Q-type \(Ca^{2+}\) channels by NO. In this study, we systematically determined the role of S-nitrosylation in the inhibition of HVA \(Ca^{2+}\) currents by NO. Compared with L-type and P/Q-type channels, N-type channels coassembled with Cav\(\beta_1\) or Cav\(\beta_3\) subunits...
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![Graphs showing voltage-dependent activation and inactivation of N-type channels](image)

FIGURE 3. Effect of SNAP on voltage-dependent activation and inactivation of N-type channels. A, voltage-dependent activation curves of N-type channels reconstituted with Cav2.2, α\textsubscript{1}δ1, and different Cavβ subunits. Voltage-dependent activation curves were obtained by plotting the normalized conductance as a function of the command potential recorded. B, voltage-dependent inactivation curves of N-type channels reconstituted with Cav2.2, α\textsubscript{1}δ1, and different Cavβ subunits. Voltage-dependent inactivation curves were obtained using inactivation protocols. Data points were fitted using the Boltzmann equation. \( V_{0.5} \) and slope factors are shown as the mean ± S.E. listed in Table 1.

| Specific subunit combination          | \( V_{0.5} \) | Slope factor |
|---------------------------------------|--------------|-------------|
| Voltage-dependent activation          |              |             |
| Cav2.2 + β1 + αδ1 (SNAP)              | -13.23 ± 2.04| 3.03 ± 0.91 |
| Cav2.2 + β1 + αδ1 (SNAP)              | -3.68 ± 4.68*| 5.58 ± 1.54*|
| Cav2.2 + β3 + αδ1 (SNAP)              | -12.13 ± 2.53| 3.69 ± 1.16 |
| Cav2.2 + β3 + αδ1 (SNAP)              | -2.48 ± 2.72*| 6.85 ± 8.00*|
| Cav2.2 + β4 + αδ1 (SNAP)              | -15.44 ± 2.06| 5.23 ± 0.71 |
| Cav2.2 + β4 + αδ1 (SNAP)              | -13.20 ± 1.48| 6.84 ± 0.79 |
| Cav2.2 C805A + β3 + αδ1 (SNAP)        | -8.89 ± 2.42  | 5.29 ± 0.50 |
| Cav2.2 C805A + β3 + αδ1 (SNAP)        | -9.49 ± 4.21  | 7.06 ± 0.64 |
| Cav2.2 C930A + β3 + αδ1 (SNAP)        | -1.07 ± 0.64  | 3.55 ± 0.57 |
| Cav2.2 C930A + β3 + αδ1 (SNAP)        | -1.40 ± 0.84  | 5.74 ± 0.44 |
| Voltage-dependent inactivation         |              |             |
| Cav2.2 + β1 + αδ1 (SNAP)              | -48.94 ± 1.16| 7.27 ± 0.52 |
| Cav2.2 + β1 + αδ1 (SNAP)              | -49.19 ± 1.04| 8.63 ± 0.53 |
| Cav2.2 + β3 + αδ1 (SNAP)              | -47.45 ± 1.05| 7.23 ± 0.45 |
| Cav2.2 + β3 + αδ1 (SNAP)              | -51.42 ± 1.04| 6.68 ± 0.89 |
| Cav2.2 + β4 + αδ1 (SNAP)              | -47.73 ± 1.04| 10.69 ± 0.86|
| Cav2.2 + β4 + αδ1 (SNAP)              | -46.84 ± 1.77| 8.72 ± 1.55 |
| Cav2.2 C805A + β3 + αδ1 (SNAP)        | -55.21 ± 0.60| 11.30 ± 0.54|
| Cav2.2 C805A + β3 + αδ1 (SNAP)        | -56.15 ± 0.76| 11.16 ± 0.62|
| Cav2.2 C930A + β3 + αδ1 (SNAP)        | -47.78 ± 1.53| 9.89 ± 1.17 |
| Cav2.2 C930A + β3 + αδ1 (SNAP)        | -48.93 ± 1.07| 11.83 ± 0.92|

*\( p < 0.05 \), compared with the corresponding control (before SNAP application).

showed prominent sensitivity to NO. The NO donor SNAP rapidly inhibited HVA Ca\textsuperscript{2+} currents, and this effect was readily reversed by DTT. Also, SNAP failed to inhibit HVA Ca\textsuperscript{2+} currents in the presence of MTSEA, which covalently modifies protein sulphydryl groups. The sulphydryl side chain (-SH) of cysteine forms a covalent disulfide bond with sulfur in MTSEA (35), and this chemical reaction can prevent subsequent S-nitrosylation by NO. Notably, the modification by MTSEA does not affect ion channel function if the exposure time is less than 8 min (25, 29, 36). These findings demonstrate that N-type

FIGURE 4. SNAP does not affect Cav2.2 membrane surface expression. A, original gel image shows the Cav2.2 protein levels on the membrane surface. For the vector-only group, HEK293 cells were cotransfected with pcDNA6 V5-FLAG vector + Cavβ3 + αδ1 and were used as a blank control for the SNAP group. HEK293 cells were cotransfected with Cav2.2-V5 + Cavβ3 + αδ1 and treated with 100 μM SNAP for 5 min before cell surface biotinylation. \( B \), summary data show that SNAP had no significant effect on the surface protein levels of Cav2.2-V5 (\( n = 4 \) independent experiments). The amounts of Cav2.2 proteins were normalized by Na\textsuperscript{+}/K\textsuperscript{+}-ATPase proteins on the same gel. The mean value of Cav2.2 treated with the vehicle was defined as 1.
channels with Cavβ1 or Cavβ3 subunits are particularly sensitive to NO via S-nitrosylation modulation. We found that in N-type channels co-assembled with Cavβ1 or Cavβ3 subunits, the inhibitory effect of SNAP was associated with a significant depolarizing shift of voltage-dependent activation of N-type channels. However, SNAP treatment had no significant effect on the Cav2.2 protein abundance of the membrane surface. Cavβ subunits are critical for channel trafficking and surface expressions (1, 3). The trafficking of Ca²⁺ channels is controlled by numerous processes, including co-assembly with auxiliary subunits, ubiquitin ligases, and interactions with other membrane proteins (37). Ca²⁺ channel trafficking typically takes more than 10 min and does not change the channel kinetic properties (38, 39). Because the inhibitory effect of SNAP on HVA Ca²⁺ channels occurred rapidly and associated with the depolarizing shift of channel activations (i.e., reduced voltage-dependent activation) in our study, NO likely inhibits N-type Ca²⁺ channels through S-nitrosylation-induced changes in channel conformation and gating properties. Also, because SNAP failed to alter voltage-dependent activation of N-type channels when cysteine S-nitrosylation sites (i.e., C805A and C930A mutants) were mutated, our data suggest that the shift in voltage dependence likely accounts for all of the inhibitory effect of NO on N-type channels.

In this study, we also identified key structural motifs essential for the inhibitory gating of N-type Ca²⁺ channels via cysteine S-nitrosylation. Sequence alignment analysis indicated that the consensus motifs of S-nitrosylation are much more abundant in Cav2.2 than in Cav1.2 and Cav2.1. This structural distinction is consistent with our recording data showing that SNAP had a much more pronounced effect on N-type channels than on P/Q-type and L-type channels. Thus, the prominent sensitivity to NO is a characteristic of N-type channels. We found that several intracellular cysteine residues, including Cys-805 and Cys-930 in the II-III loop and Cys-1835 and Cys-2145 in the C terminus, critically mediate the S-nitrosylation modification of the N-type channels. Mutating Cys-1045 in the II-III loop also significantly reduced NO sensitivity of N-type channels. In concord with our findings, polynitrosylation modification is known to contribute to the activation of the ryanodine receptor (40). Multiple consensus motifs of S-nitrosylation also exist in NMDA receptors (25). The cytoplasmically accessible cysteine sites may produce an additive effect and transmit cysteine modifications to functionally critical domains of N-type channels to elicit their conformational changes. In addition, site-directed mutagenesis analysis showed that mutating Cys-346 of the Cavβ3 subunit decreased the NO inhibitory effect by ~50% compared with the wild-type Cavβ3. These data indicate that in addition to Cavα1 subunits, the Cavβ subunit is also involved in redox modification of the N-type channel by NO. Our findings indicate that polynitrosylation modification is an important
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Inhibitory effects of SNAP on N-type Ca\(^{2+}\) channels reconstituted in HEK293 cells expressing wild-type Cav2.2, and Cavβ3 or their mutants

| Specific subunit combination | SNAP inhibition (%) | n |
|-----------------------------|---------------------|---|
| Wild-type Cav2.2 + β3 + α\(_d\) | 32.09 ± 3.21\(^a\) | 7 |
| Cav2.2 C805A + β3 + α\(_d\) | 8.30 ± 2.17\(^b\) | 10 |
| Cav2.2 C930A + β3 + α\(_d\) | 5.05 ± 1.79\(^c\) | 7 |
| Cav2.2 C1045A + β3 + α\(_d\) | 12.53 ± 1.50\(^d\) | 7 |
| Cav2.2 C1065A + β3 + α\(_d\) | 29.75 ± 1.31\(^e\) | 6 |
| Cav2.2 C1835A + β3 + α\(_d\) | 5.00 ± 2.36\(^f\) | 6 |
| Cav2.2 C2145A + β3 + α\(_d\) | 6.54 ± 1.39\(^g\) | 8 |
| Cav2.2 + β3 C346A + α\(_d\) | 16.02 ± 3.13\(^h\) | 8 |

\(^a\) p < 0.05, compared with the baseline control.
\(^b\) p < 0.05, compared with the wild-type controls.

FIGURE 7. Critical cysteine residues involved in S-nitrosylation control of N-type channels. A, original current traces show the SNAP effects on N-type channel currents reconstituted with wild-type Cav2.2, Cavβ3, and their mutants in HEK293 cells. B, summary data show the effect of SNAP on N-type channel currents reconstituted with wild-type Cav2.2, Cavβ3, and their mutants. The percentage of inhibition of N-type channels by SNAP is shown as the mean ± S.E. in Table 2. * p < 0.05, compared with the baseline control. # p < 0.05, compared with wild-type controls.

In summary, our study provides new information for our understanding of HVA Ca\(^{2+}\) channel regulation by S-nitrosylation at the molecular level. Our findings reveal that cytoplasmically accessible cysteine residues serve as “NO sensors” of HVA Ca\(^{2+}\) channels. This mechanism is important for the feedback regulation of Ca\(^{2+}\) signals by NO in neurons. N-type channels are expressed at high levels in both DRG neurons and their presynaptic terminals and are critically involved in triggering the neurotransmitter release from primary afferent terminals (9, 10, 41). N-type Ca\(^{2+}\) channels are also recognized as a crucial target for treating painful conditions. Both Cav2.2 and Cavβ3 subunits whose cysteine residues undergo S-nitrosylation probably conduct NO-induced inhibition of N-type channels in native DRG neurons. Thus, S-nitrosylation may play a critical role in feedback regulation of N-type channels by NO following stimulation of endogenous neuronal NO synthase in native primary sensory neurons (21, 24). The S-nitrosylation sites on N-type channels could be targeted for pain treatment.

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