Supplementary Information

Supplementary Method

Measurement of Ca²⁺ Transients

Ca²⁺ transients were measured in CSLV neurons with a Leica digital fluorescence microscope (DM IL LED; Leica Microsystems, Wetzlar, Germany) and a digital CCD camera (Zyla 4.2 sCMOS; Andor Technology, Belfast, UK). Neurons were washed or maintained with an extracellular solution (ECS) in a small-volume (0.2 ml) perfusion chamber at room temperature. Neurons were loaded with 5 μ M fura-2 AM (Molecular Probes; Eugene, OR, USA) for 30 min at 37°C in tissue culture medium and then rinsed with ECS and allowed to deesterify for at least 30 min before use. The recording chamber was perfused continuously with ECS or the test chemicals by a gravity-fed valve control system (VC-66CS; Warner Instruments, Hamden, CT, USA). Dual images (340- and 380-nm excitation, 510-nm emission) were collected, and pseudocolored ratiometric images were monitored during the experiments. The image signals were continually analyzed at 2-s intervals during the experiments by using the MetaFluor® software (Universal imaging; West Chester, PA, USA). An increase in 340/380 ratio [Δ Ratio (F340/F380)] was measured as the difference between the peak amplitude of Ca²⁺ transients (4-s average) and the 30-s average at baseline.



Figure S1. Effects of HC-030031 (20 μ M, 16.5 min) on the NaHS (100 μ M, 5 min)-induced potentiation of the Ca²⁺ transients evoked by adenosine in rat capsaicin-sensitive lung vagal (CSLV) neurons. **(A)** and **(B)**: experimental records illustrating the effects of pretreatments with vehicle or HC-030031, respectively, on the NaHS-potentiated Ca²⁺ transient evoked by adenosine (20 μ M, 30 s). Adenosine was applied before and 5 min after the NaHS perfusion. Capsaicin (1 μ M, 30 s) was applied to identify the CSLV neurons at the end of the both experiments. **(C)**: group data showing HC-030031 (n=10), but not its vehicle (n=10), eliminated the NaHS-induced potentiating effects on the adenosine-evoked Ca²⁺ transients. Data are means ± SE. ^a, significantly different from before NaHS (*P* < 0.05); *, significant difference when corresponding data between vehicle and HC-030031. Ado, adenosine; Cap, capsaicin.



Figure S2. Effect of HC-030031 on capsaicin-induced Ca²⁺ transients in rat capsaicin-sensitive lung vagal (CSLV) neurons (n=10). Capsaicin (0.1 μ M, 30 s)-evoked Ca²⁺ transients were compared before and after pretreatment of HC-030031 (20 μ M, 16.5 min) or its vehicle. Time between 2 consecutive capsaicin challenges was 20 min. Data are means ± SE.



Figure S3. Effects of AP-18 (a TRPA1 antagonist; 40 μ M, 6.5 min) on the NaHS (100 μ M, 5 min)induced potentiation of capsaicin-evoked Ca²⁺ transients in rat capsaicin-sensitive lung vagal (CSLV) neurons. **(A)** and **(B)**: experimental records illustrating the effects of pretreatments with vehicle or AP-18, respectively, on the NaHS-potentiated Ca²⁺ transient evoked by capsaicin (0.1 μ M, 30 s). A KCI solution (60 mM, 30 s) was applied to test the cell vitality at the end of both experiments. **(C)**: group data showing AP-18 (n =15), but not its vehicle (n=20), eliminated the NaHS-induced potentiating effects on the capsaicin-evoked Ca²⁺ transients. Data are means ± SE. ^a, significantly different from before NaHS (*P* < 0.05); *, significant difference when corresponding data between vehicle and AP-18. Cap, capsaicin.



Figure S4. Allyl isothiocyanate (AITC; a TRPA1 agonist, 30 μ M, 2.5 min)-induced potentiation of the Ca²⁺ transients evoked by capsaicin in rat capsaicin-sensitive lung vagal (CSLV) neurons. **(A)** and **(B)**: experimental records illustrating the effects of pretreatments with vehicle or AITC, respectively, on the capsaicin-induced Ca²⁺ transients. Capsaicin (0.1 μ M, 30 s) was applied before and 5 min after the AITC or vehicle perfusion. A KCl solution (60 mM, 30 s) was applied to test the cell vitality at the end of both experiments. **(C)**: group data showing AITC (n=22), but not its vehicle (n=22), potentiated the capsaicin-induced Ca²⁺ transients. Data are means ± SE. ^a, significantly different from before perfusion (*P* < 0.05); *, significant difference when corresponding data between vehicle and AITC.