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Short communication

Selective sensitization of C-fiber nociceptors by hydrogen sulfide

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ABSTRACT

We examined the effects of intraplantar (i.pl.) administration of NaHS, an H_2S donor, known to cause T-type Ca^{2+} channel (T-channel)-dependent mechanical hyperalgesia, on responsiveness to electric stimulation with 5, 250 and 2000 Hz sine waves (SW) that selectively excites C, Aδ and Aβ fibers, respectively. NaHS, given i.pl., caused behavioral hypersensitivity to SW stimulation at 5 Hz, but not 250 or 2000 Hz, in rats. NaHS also enhanced phosphorylation of spinal ERK following 5 Hz SW stimulation. Three distinct T-channel blockers abolished the NaHS-induced behavioral hypersensitivity to 5 Hz SW stimulation. Thus, H_2S selectively sensitizes C-fiber nociceptors via T-channels.

Hydrogen sulfide (H_2S), a gasotransmitter, is produced from L-cysteine by cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) or 3-mercaptopyruvate sulfurtransferase (3MST) in combination with cysteine aminotransferase (CAT) in the mammalian body (1). We have demonstrated, for the first time to our knowledge, that intraplantar (i.pl.) or intrathecal administration of H_2S donors including NaHS causes hyperalgesia by enhancing the activity of $Ca_V3.2$ T-type Ca^{2+} channels in rats and mice (2–4). It is now clear that transient receptor potential ankyrin-1 (TRPA1) channels in addition to $Ca_V3.2$ contribute to the pronociceptive effects of H_2S (4–6). There is plenty of evidence for the involvement of the $H_2S/Ca_V3.2$ pathway in neuropathic, colonic, pancreatic and bladder pain (6–10). Studies using acutely dissociated rat sensory neurons have shown that $Ca_V3.2$ is most abundantly expressed in a T-type Ca^{2+} channel-rich (T-rich) C-fiber subpopulation among C-type and Aδ nociceptors, in addition to D-hair mechanosensory neurons (11). Interestingly, transcutaneous electrical sine-wave stimuli at frequencies of 2000, 250 and 5 Hz selectively activate Aβ, Aδ and C afferent fibers, respectively (12). The present study aimed at identifying the most sensitive nociceptors to H_2S in the peripheral tissue *in vivo*, using sine-wave electric stimulation at distinct frequencies.

Male Wistar rats (9–12 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan). All procedures were approved by the Committee for the Care and Use of Laboratory Animals at Kindai University, and were in accordance with the guidelines of the Committee for Research and Ethical Issues of IASP [www.iasp-pain.org/Education/Content.aspx?ItemNumber=1217]. To determine the threshold for behavioral responses to sine-wave electric stimulation, as reported elsewhere (12,13), the conscious rat was hold in a wire-meshed cylinder, and a stimulation electrode was fixed in the right hindpaw. After acclimatization, the hindpaw was stimulated with sine-waves at 5, 250 and 2000 Hz for 0.6 s (namely, 3, 150 and 1200 sine-waves, respectively) at 10 s-intervals, using a stimulus generator (STG2004, Multi Channel Systems MCS GmbH, Reutlingen, Germany). The intensity of sine-waves at each frequency was increased in 5 μA-steps. The current required to induce escape behavior was determined as the nociceptive threshold before and after i.pl. administration of NaHS (Kishida Chemical Industries, Osaka, Japan) in a volume of 10 μl. The data are presented as the percentage of the baseline threshold and/or as AUC (area under the curve) for the time course of the nociceptive thresholds. In the immunohistochemical experiments, the rats were anesthetized with i.p. sodium pentobarbital (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) at 40 mg/kg, and received i.pl. NaHS in a volume of 100 μl and/or electrical stimulation. For rapid fixation of the spinal cord, the rats were perfused transcardially with 30 ml of saline, 30 ml of 1% paraformaldehyde (Merck KGaA, Darmstadt, Germany) and then 300 ml of 4% paraformaldehyde in a phosphate buffer (pH 7.4), 2 or 20 min after i.pl.

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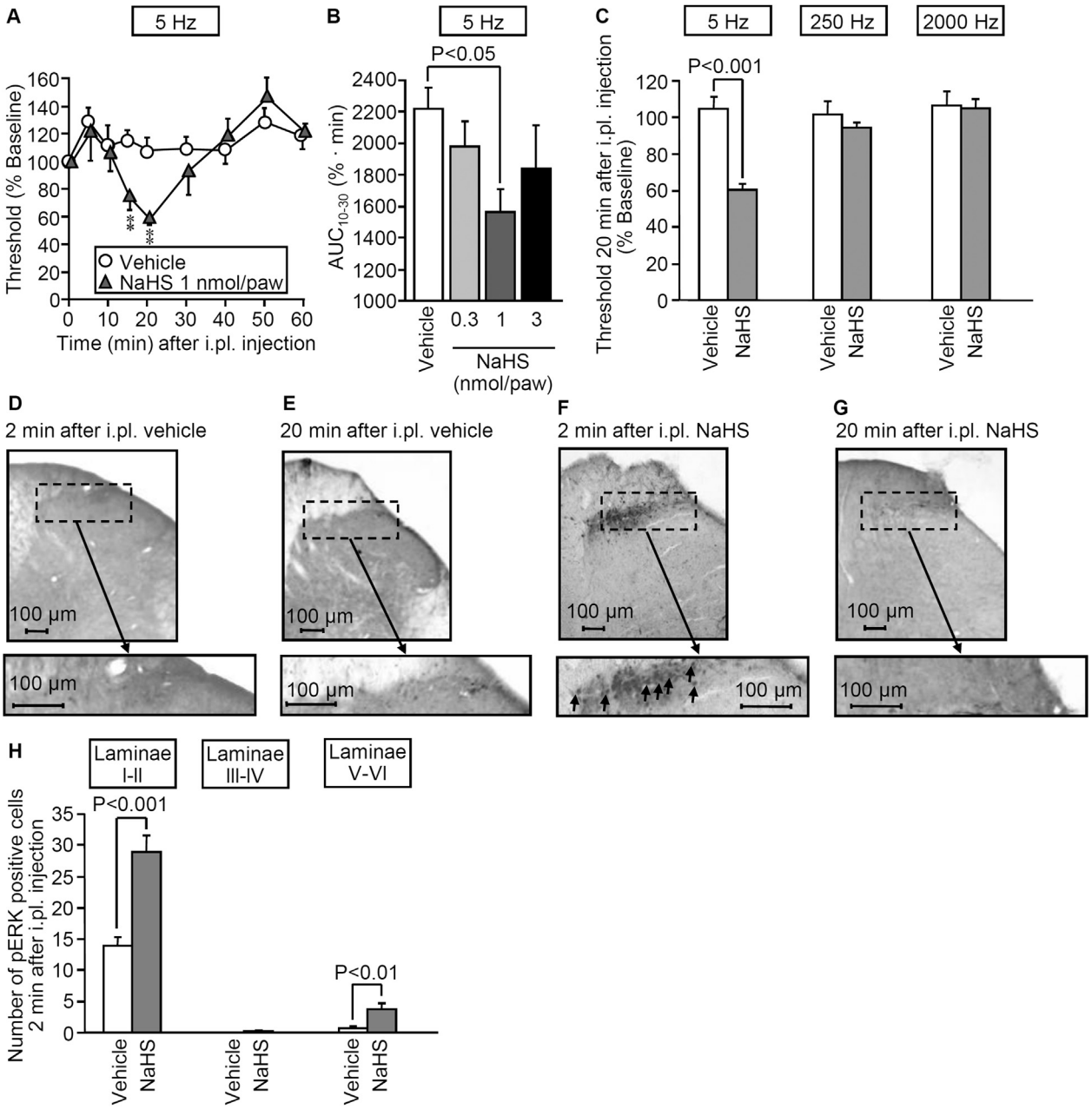

Fig. 1. Intraplantar administration of NaHS causes behavioral hypersensitivity to sine-wave stimulation at 5 Hz and transient phosphorylation of ERK in the superficial layers of the spinal dorsal horn in rats. (A) Time-dependent change of nociceptive threshold to 5 Hz sine-wave stimulation (SWS) after i.pl. NaHS at 1 nmol/paw. $^{**}P < 0.01$ vs. vehicle. (B) AUC of the time–threshold curve between 10 and 30 min (AUC_{10-30}) after i.pl. NaHS at 0.3, 1 or 3 nmol/paw. (C) Comparison of the nociceptive threshold to 5-Hz SWS, 20 min after i.pl. NaHS at 1 nmol/paw. The baseline threshold (μA): 41.9 ± 5.0 (5 Hz); 73.6 ± 12.4 (250 Hz); 96.4 ± 7.9 (2000 Hz). (D–G) Typical microphographs of immunostaining of phosphorylated ERK (pERK) 2 min (D, F) or 20 min (E, G) after i.pl. vehicle (D, E) or NaHS at 1 nmol/paw (F, G). Arrows indicate pERK-positive cells. (H) The number of pERK-positive cells in laminae I–II, III–IV and V–VI of the L4–L5 spinal cord 2 min after i.pl. vehicle or NaHS at 1 nmol/paw. Data show the mean ± SEM for 4–11 rats (A–C) and 20 slices from 4 rats (H).

NaHS or immediately after the 2-min electric stimulation. The phosphorylated ERK in the L4–L5 spinal dorsal horn, as a prompt marker for nociceptor excitation, was stained with a rabbit polyclonal antibody against human phospho-p44/p42 mitogen-activated protein (MAP) kinase (Thr202/Tyr204) (Cell Signal. Tech., Beverly, MA, USA), as reported previously (14). The rat received i.pl. or i.p. administration of NNC55-0396, mibefradil or ethosuximide (Sigma–Aldrich, St. Louis, MO, USA), T-type Ca^{2+} channel blockers, 10 min before i.pl. NaHS. Results are represented as mean ± SEM. Statistically analyses were performed by Student's t-test for two-group data or by ANOVA followed by Tukey's test for multiple comparisons, and significance was set at a $P < 0.05$.

NaHS, when administered i.pl. at 1 nmol/paw, significantly decreased the nociceptive threshold in response to stimulation with 5 Hz sine-waves in conscious rats (Fig. 1A, B), the time course and effective dose range being consistent to the NaHS-induced mechanical hyperalgesia (2). In contrast, i.pl. NaHS did not change the sensitivity to stimulation with 250 or 2000 Hz sine-waves (Fig. 1C). In the anesthetized rats, prompt phosphorylation of ERK in the spinal superficial layers, laminae I–II, that receive