Histamine Modulation of Acute Nociception Involves Regulation of Na_v1.8 in Primary Afferent Neurons in Mice

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Keywords

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SUMMARY

Aims: To explore the role of histamine in acute pain perception and its possible mechanisms. **Methods:** Pain-like behaviors induced by four types of noxious stimuli (hot-plate, tail-pressure, acetic acid, and formalin) were accessed in mice. Na_v1.8 expression and functions in primary afferent neurons were compared between histidine decarboxylase knock-out (HDC^{-/-}) mice and their wild-types. **Results:** HDC^{-/-} mice, lacking in endogenous histamine, showed elevated sensitivity to all these noxious stimuli, as compared with the wild-types. In addition, a depletion of endogenous histamine with α -fluoromethylhistidine (α -FMH), a specific HDC inhibitor, or feeding mice a low-histamine diet also enhanced nociception in the wild-types. Na_v1.8 expression in primary afferent neurons was increased both in HDC^{-/-} and in α -FMH-treated wild-type mice. A higher Na_v1.8 current density, a lower action potential (AP) threshold, and a higher firing rate in response to suprathreshold stimulation were observed in nociception-related small DRG neurons of HDC^{-/-} mice. Na_v1.8 inhibitor A-803467, but not TTX, diminished the hyperexcitability and blocked repetitive AP firing of these neurons. **Conclusion:** Our results indicate that histamine participates in acute pain modulation in a dose-related manner. The regulation of Na_v1.8 expression and the excitability of nociceptive primary afferent neurons may be involved in the underlying mechanisms.

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Introduction

Many lines of evidence show that histamine, an inflammatory mediator and a strong regulator of other chemical agents, participates in pain modulation. In the peripheral nervous system, histamine directly sensitizes nociceptors and facilitates pain transmission, or indirectly causes pain *via* H₁ receptors [1–3], in addition to specifically activating a subset of unmyelinated C-fibers and triggering itch [4]. On the contrary, the role of histamine in the central nervous system is controversial. Some studies support its action as a dose-dependent analgesic [5–7], while others suggest that the spinal and cerebral histaminergic system facilitates nociception [8–10]. Therefore, modulation of acute pain

perception by histamine seems complicated because its effects on pain sensation might depend not only on the site of action, but probably also on other factors like doses.

Voltage-gated sodium channels (VGSCs) in primary afferent neurons are essential for peripheral input. Based on the sensitivity to tetrodotoxin (TTX) blockade, VGSCs are classified into two groups: TTX-sensitive (TTX-s) and TTX-resistant (TTX-r). Na_v1.8 is a type of TTX-r VGSCs and is expressed predominantly in small-diameter nociceptive neurons in the dorsal root ganglia (DRG) [11]. About 80% of the inward current underlying the action potential (AP) upstroke in these cells is mediated by Na_v1.8 [12]. Na_v1.8-null mice show no response to noxious mechanical stimuli, decreased thermal and visceral nociception, and delayed

development of inflammatory hyperalgesia [13–15]. These results indicate that Na_v1.8 plays a crucial role in pain perception and is an important molecular target in the study of pain [16].

It has been demonstrated that histamine depolarizes neurons directly or increases membrane depolarization in primary sensory, supraoptic, and basal nucleus neurons [1,17,18]. One of the underlying ionic mechanisms is that histamine increases TTX-r Na⁺ conductance by acting on histamine H₁ receptors [19]. Moreover, H₁ receptor antagonists diphenhydramine and terfenadine block TTX-sensitive sodium channels, although this local anesthetic-like effect may not be completely due to an interaction with H₁ receptors [20–22]. These studies suggest that histamine may modulate sodium channels. However, it remains yet unclear whether or not histamine plays a role in modulating Na_v1.8.

Therefore, in this study, we used histidine decarboxylase (HDC) knockout (HDC^{-/-}) mice and their wild-type (HDC^{+/+}) littermates fed a diet rich or low in histamine to determine the role of histamine in acute pain sensation and its effect on Na_v1.8 in DRG neurons.

Materials and Methods

Animals

All experiments were in accordance with the guidelines of The International Association for the Study of Pain [23] and were approved by the Animal Care and Use Committee of Zhejiang University. Efforts were made to minimize the number of animals used and their suffering. HDC^{-/-} mice were kindly provided by Professor Ohtsu, Tohoku University, Japan. Mice aged 8–12 weeks were kept under a 12-h light–dark cycle (lights on from 08:00 to 20:00). Water and chow were given *ad libitum*. Some mice were fed regular chow which contains a high concentration of histamine (135.3 μg/g), while some were fed chow low in histamine (0.5 μg/g), starting from 15 days before experiments until sacrifice.

Behavioral Tests

The behavioral tests were carried out between 10:00 and 17:00 after habituation in testing environment for 20 min. The temperature for the hot-plate test was set at 52.5 ± 0.1°C. The latency to a withdrawal response (hindpaw lick, flick or lift) was measured, and a cut-off time of 30 seconds was used to minimize tissue damage. The average latency from three separate trials was recorded as the threshold for thermal nociception. In Randall–Selitto tail pressure test, a linearly incremental pressure was applied to the tail 3 cm proximal to the end. The pressure required to elicit struggle was determined, and the cut-off value was set at 280 g to avoid tissue damage. The average pressure from three separate trials was considered the threshold for mechanical nociception. In the acetic acid writhing test, the number of writhes was counted during the period of 3–20 min after an intraperitoneal injection of 0.01 mL/g of 1% acetic acid. In the formalin test, after a subcutaneous injection of 1% formalin (20 μL) into the plantar area of the right hindpaw of mice, the duration of paw licking was determined at 0–5 min (early phase, neurogenic) and 15–40 min (late phase, inflammatory).

α-Fluoromethylhistidine (α-FMH, kindly provided by Professor C. Kamei, Okayama University, Japan) was given to HDC^{+/+} mice

(50 mg/kg, i.p.) once daily for 6 days. The hot-plate test was performed 4 h after the last treatment.

Electrophysiological Recordings

L4 and L5 DRGs were rapidly dissected from the mice and then incubated at 37°C for 25 min in DMEM/F-12 (Invitrogen, Carlsbad, CA, USA) supplemented with 2 mg/mL collagenase (type II; Sigma-Aldrich, St. Louis, MO, USA) and 1 mg/mL trypsin (type I, Sigma). The cellular suspension was incubated at 37°C under 5% CO₂ and 90% humidity for 1 h, and then, 2 mL of medium supplemented with 10% fetal bovine serum was added. The cells were incubated for 2–8 h before recording.

For voltage-clamp recording, the external solution contained (in mM, pH 7.4) 70 NaCl, 70 choline chloride, 20 TEA-Cl, 1 MgCl₂, 1 CaCl₂, 0.1 CdCl₂, 10 HEPES, and 10 glucose. The internal solution contained (in mM, pH 7.2) 140 CsF, 10 NaCl, 1 EGTA, and 10 HEPES. Pipettes had resistances of 1–3 MΩ when filled with the pipette solution. Before seal formation, the pipette potential was adjusted to zero, and the voltage was not corrected for liquid junction potential. After achieving the whole-cell recording configuration, capacity transients were cancelled and series resistance was compensated (>80%). The cell capacitance was read directly from the amplifier. Currents were low-pass filtered at 6 kHz and digitally sampled at 20 kHz. For recording Na_v1.8 currents, 300 nM TTX was added to the external solution to inhibit TTX-s sodium currents, and membrane potential was held at -60 mV to inactivate the TTX-r Na_v1.9 current [24]. Na_v1.8 currents were activated by applying 50-ms test pulses to potentials between -55 and 60 mV in 5-mV increments. The interval between individual pulses was 10 second. The peak current density (pA/pF) of Na_v1.8 was calculated and plotted versus command voltage. To generate the activation curve, the sodium conductance (*G*) was calculated using the equation: $G = I/(V_m - E_{Na})$, where *I* is the peak current amplitude, *V_m* is the membrane potential, and *E_{Na}* is the equilibrium potential for Na⁺ and determined by calculating the theoretical value. The Boltzmann equation used to fit the activation curve was of the following form: $G/G_{max} = 1/(1 + \exp[(V_{1/2} - V_m)/k])$, where *G_{max}* is the maximum *G*, *V_{1/2}* is the potential at half-maximum of Na_v1.8 activation, and *k* is the slope of the curve. Steady-state inactivation was measured by applying a protocol consisting of a 500-ms prepulse ranging from -70 to 10 mV (in 5-mV increments), followed by a test pulse to 0 mV. Data were fit with the Boltzmann function: $I/I_{max} = 1/(1 + \exp[(V - V_{1/2})/k])$, where *I_{max}* is the maximal peak current, *V* is the prepulse membrane potential, *V_{1/2}* is the potential at half-maximal activation, and *k* is the slope factor.

For current-clamp recording, the standard external solution contained (in mM, pH 7.4) 150 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 D-glucose, and 10 HEPES. The pipette solution contained (in mM, pH 7.3) 120 KCl, 30 NaCl, 5 EGTA, 1 MgCl₂, 0.5 CaCl₂, 10 HEPES, and 2 Mg-ATP. Pipettes had resistances of 3–5 MΩ when filled with pipette solution. The whole-cell configuration was obtained in voltage-clamp mode before proceeding to the current-clamp recording mode. With or without TTX to block TTX-s VGSCs (300 nM; Sigma), or A-803467 (300 nM; Sigma) to block Na_v1.8 selectively [25] in the bath solution, current threshold was determined by a series of depolarizing current injections

(increased in 10-pA steps) at which the first AP was elicited. Firing rate was calculated by quantifying the number of repetitive APs evoked in response to a 500-pA, 500-ms depolarizing current injection. A-803467 (500 nM) was applied in the bath solution to test whether Na_v1.8 contributes to repetitive AP firing [25,26].

Immunohistochemistry

Under anesthesia by chloral hydrate (400 mg/kg, i.p.), mice were perfused intracardially with phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde. L4/L5 DRGs and the sciatic nerve were dissected, and cryostat sections were cut at 8 μ m. The sections were first incubated with rabbit anti-Na_v1.8 (Millipore; 1:300, Billerica, MA, USA) and mouse anti-NF200 (diluted 1:750; Abcam, Cambridge, UK) or anti-peripherin (1:400; Sigma) IgG and then with anti-rabbit IgG-Alexa Fluor 568 (1:100; Invitrogen) and anti-mouse IgG-Alexa Fluor 488 (Invitrogen; 1:400). Images were captured under identical illumination and exposure conditions by a fluorescence microscope (Olympus, Tokyo, Japan). The fluorescence intensity of each neuron, excluding the nuclear region, was measured using ImageJ (v1.37, US National Institute of Health, Bethesda, MD, USA).

Western Blotting

Under chloral hydrate anesthesia, mice were perfused intracardially with PBS (pH 7.4). L4/L5 DRGs and the sciatic nerve were removed, then homogenized, and lysed in homogenization buffer.

After concentration determination and denaturization, protein samples were separated by SDS-PAGE gels and then transferred onto a nitrocellulose membrane. The membranes were incubated with mouse anti-Na_v1.8 (Abcam; 1:300) or mouse anti-GAPDH (Kangchen; 1:5000, Shanghai, China) IgG and were visualized with horseradish peroxidase-conjugated goat anti-mouse IgG (Odyssey; 1:5000, LI-COR, Lincoln, NE, USA). The ratio between Na_v1.8 and GAPDH was calculated and then normalized to the values measured in control mice.

Statistical Analysis

Electrophysiological curves were plotted and fitted using Origin software (OriginLab Corp., Northampton, MA, USA). All data are presented as mean $\pm$ SEM. Statistical analysis was performed with SPSS for Windows (v16.0; SPSS Inc., Chicago, IL, USA). Comparison between groups was made using *t*-test. For all analyses, the tests were two-sided and *P* < 0.05 was considered statistically significant.

Results

Decrease in Histamine Level Elevated Sensitivity to Acute Pain in Mice

HDC^{-/-} mice, lacking in endogenous histamine, showed hypersensitivity in the hot-plate test, the Randall–Selitto tail-pressure

Figure 1 Modulation by histamine of acute pain sensitivity to various noxious stimuli. (A and E) paw withdrawal threshold to noxious heat in the hot-plate test at 52.5°C. (B) tail withdrawal threshold to noxious pressure in the Randall–Selitto test. (C) number of writhes in the acetic acid test. (D) total time of paw biting, licking, and shaking behaviors in the early and late phases of the formalin test. HDC^{+/+} and HDC^{-/-} mice in A–D were fed a regular or a low-histamine diet. (E) HDC^{+/+} mice were repeatedly treated with α -FMH. *n* = 8/group. **P* < 0.05 and ****P* < 0.001 compared with the wild-type; #*P* < 0.05 and ###*P* < 0.001 compared with each other.

test, and the writhing test (Figure 1A–C; $P < 0.001$ or 0.05), as compared with their wild-types. Moreover, HDC^{-/-} mice spent more time licking the injected paw in the early phase of the formalin test, but in the late phase, they showed shorter licking duration than the wild-type ($P < 0.05$; Figure 1D).

After feeding mice low-histamine diet for 15 days, the histamine content in the serum, spinal cord, and cerebral cortex were markedly reduced in wild-type and HDC^{-/-} mice (Table 1). HDC^{+/-} mice became more sensitive to all tested types of noxious stimuli than those fed a regular diet, while HDC^{-/-} mice were more sensitive in the hot-plate and formalin tests (phase 1) (Figure 1A–D). In addition, once-daily treatment for 6 days with α -FMH, which is a specific and irreversible inhibitor of HDC, the key enzyme of histamine synthesis, significantly elevated the sensitivity to noxious heat stimulus in wild-type mice ($P < 0.001$; Figure 1E).

Up-Regulation of Na_v1.8 Expression in Small Primary Afferent Neurons in HDC^{-/-} Mice and α -FMH-Treated HDC^{+/-} Mice

Immunohistochemical studies revealed a marked augmentation in Na_v1.8 protein expression in the L4/L5 DRG neurons of HDC^{-/-} mice (Figure 2). The total Na_v1.8 immunofluorescent staining intensity in mutated mouse neurons increased by about 26% compared with the wild-type. Further analysis showed that Na_v1.8 up-regulation occurred both in peripherin- and NF200-positive neurons of HDC^{-/-} mice ($P < 0.001$; Figure 2A–F). Compared with the wild-type, the staining intensity of Na_v1.8 in NF200-negative neurons increased by 25% in mutated mice ($P < 0.001$), but that in peripherin-negative neurons was 8.7% less ($P < 0.05$). In addition, the percentage of Na_v1.8-positive neurons that were colabeled with peripherin increased from 37.7% (162/430) in HDC^{+/-} mice to 63.6% (253/398) in HDC^{-/-} mice ($P < 0.001$). There was no difference between the two genotypes in the percentage of Na_v1.8 and NF200 double-labeled neurons (40.8% vs. 44.1%). Western blot analysis revealed that Na_v1.8 protein was increased by 42.6% in the DRG neurons of HDC^{-/-} mice (Figure 2G). The up-regulation of Na_v1.8 expression in HDC^{-/-} mice was also observed in the sciatic nerve trunk ($P < 0.05$; Figure 2H–J).

Moreover, chronic treatment of α -FMH unregulated the expression of Na_v1.8 in DRG neurons of wild-type mice by approximately 30% (Figure 3A–D). This increase consisted of up-regulation both in peripherin-positive (increased by 55.4%; $P < 0.001$) and in NF200-positive and NF200-negative neurons (increased by 32.4% and 24.1%, respectively; $P < 0.001$; Figure 3E,F). Na_v1.8 expression in peripherin-negative neurons was

decreased by 13.3% after α -FMH treatment ($P < 0.01$; Figure 3E). The percentage of Na_v1.8-positive neurons that were colabeled with peripherin increased to 65.6% (263/401) in the α -FMH-treated group from 38.1% (137/360) in the saline-treated group ($P < 0.001$). Quantitative analysis by Western blotting showed that α -FMH increased the amount of Na_v1.8 protein by 54% (Figure 3G).

Increased Na_v1.8 Current Density in Small DRG Neurons of HDC^{-/-} Mice

The mean capacitance of small DRG neurons was 19.06 ± 6.28 pF ($n = 63$) in HDC^{-/-} and 18.74 ± 7.79 pF ($n = 58$) in HDC^{+/-} mice, and the input resistance was 570.40 ± 33.40 and 554.30 ± 38.20 M Ω , respectively, with no difference between groups. In the presence of 300 nM TTX, Na_v1.8 sodium currents were activated by applying 50-ms depolarizing voltage commands between -55 and 60 mV (in 5-mV increments) from a holding potential of -60 mV. Representative inward Na_v1.8 currents recorded in DRG neurons from HDC^{+/-} and HDC^{-/-} mice are shown in Figure 4A. The average peak inward current was larger in the HDC^{-/-} group than in the wild-type. Plots of peak $I_{Na,1.8}$ density versus command voltage showed a significant enhancement in HDC^{-/-} mice (Figure 4B).

The I–V curve revealed that the activation threshold of Na_v1.8 in both genotypes was -30 mV. $V_{1/2}$ of the activation curve was -11.90 ± 0.88 mV for neurons from HDC^{-/-} and -10.06 ± 1.63 mV for those from HDC^{+/-} mice ($P > 0.05$, Figure 4C). The slope factor k of the two genotypes was similar (5.56 ± 0.32 for HDC^{+/-} vs. 5.75 ± 0.42 for HDC^{-/-}). $V_{1/2}$ of the steady-state inactivation curve was -31.03 ± 0.89 in the HDC^{-/-} group, similar to that in the HDC^{+/-} group, which was -29.60 ± 0.27 . The k values for the two genotypes were not different (4.70 ± 0.11 for HDC^{+/-} vs. 4.71 ± 0.18 for HDC^{-/-}; Figure 4D).

Hyperexcitability of Small DRG Neurons in HDC^{-/-} Mice

Under current clamp, we found that the resting membrane potential was -64.11 ± 0.52 mV for small DRG neurons from wild-type mice and -63.91 ± 0.56 mV from HDC^{-/-} mice. The current threshold for evoking an AP was 35.94 ± 4.36 pA in DRG neurons from HDC^{-/-} mice, much lower than that in wild-type neurons, which was 62.50 ± 6.29 pA ($P < 0.01$; Figure 5A–C). A-803467 (300 nM), the selective blocker of Na_v1.8, diminished the reduction in AP threshold in HDC^{-/-} mice (Figure 5D–F).

Table 1 Histamine contents in tissues of wild-type C57BL/6J (HDC^{+/-}) and HDC^{-/-} mice fed regular diet and low-histamine diet, respectively ($n = 5$ /genotype)

Genotype	Diet	Blood serum (ng/mL)	Spinal cord (ng/g)	Cortex (ng/g)
HDC ^{+/-}	Regular diet	1150 $\pm$ 244	294 $\pm$ 72	108 $\pm$ 31
	Low-histamine diet	302 $\pm$ 56	165 $\pm$ 32	61 $\pm$ 16
HDC ^{-/-}	Regular diet	381 $\pm$ 96	42 $\pm$ 14	16 $\pm$ 7
	Low-histamine diet	52 $\pm$ 17	29 $\pm$ 11	Not detectable

Figure 2 Expression of Nav1.8 in primary afferent neurons of HDC^{+/+} and HDC^{-/-} mice. A-D, double staining of Nav1.8 with peripherin (A and B) and NF200 (C and D) in L4/L5 DRG neurons. Arrows in merged images indicate colabeled cells (scale bars, 50 μ m). (E and F) normalized intensity of Nav1.8 in total, and neurons labeled with peripherin NF200, respectively. (G) Western blot analysis of Nav1.8 in DRG neurons. (H and I) immunohistochemical staining of Nav1.8 and normalized fluorescence intensity of Nav1.8 in the sciatic nerve trunk (scale bar, 400 μ m) (J) Western blot analysis of Nav1.8 in the sciatic nerve trunk. *** P < 0.001 and * P < 0.05, compared with the wild-type or control.

In the presence of 300 nM TTX, APs still could be evoked in DRG neurons of both genotypes (Figure 5G,H). However, the injection current required for evoking an AP was markedly increased to 97.50 ± 15.44 pA in the wild-type, while that in HDC^{-/-} mice was slightly increased to 41.25 ± 3.87 pA (P < 0.01; Figure 5I). No difference was found between the two genotypes in amplitude, duration, half-width duration, and maximum decay slope of the APs, no matter whether with or without

TTX (data not shown). Moreover, during the injection of supra-maximal depolarizing current pulses (500 pA, 500 ms), the rate of repetitive firing was higher in the HDC^{-/-} group (22.15 ± 2.20 Hz) than in the wild-type (14.00 ± 2.83 Hz) (P < 0.05; Figure 6A-C). A-803467 (500 nM) blocked the repetitive AP firing in both HDC^{-/-} and wild-type groups (Figure 6D-F). However, the amplitude and the shape of evoked APs were similar in the two genotypes.

Figure 3 Expression of Na_v1.8 in L4/L5 DRG neurons of HDC^{+/±} mice after repeated α-FMH treatment. (A–D) double staining of Na_v1.8 with peripherin (A and B) and NF200 (C and D). Arrows in merged images indicate colabeled cells (scale bars, 50 μm). (E and F) normalized intensity of Na_v1.8 in total neurons, and neurons labeled with peripherin and NF200, respectively. (G and H) Western blot analysis of Na_v1.8 in DRG neurons of both groups. n = 6/group. ****P* < 0.001, ***P* < 0.01, and **P* < 0.05, compared with the saline-treated group.

Discussion

The role of histamine in pain modulation remains controversial. We proposed that its effects may be related to its tissue levels. To verify this hypothesis, we first tested the pain sensitivity of HDC^{-/-} mice. These mice had significantly lower histamine levels than the wild-type, even when they were fed a regular diet (Table 1). We found that HDC^{-/-} mice were more sensitive to various types of acute noxious stimuli, manifested as shorter latencies in the hot-plate and Randall–Selitto tests, more writhes in the acetic acid test, and longer duration of licking in the early phase of the formalin test. Similarly, wild-type mice fed a low-histamine diet also showed an enhanced nociception. Moreover, a depletion of endogenous histamine by α-FMH in wild-type mice facilitated thermal nociception. These behavioral results accordantly support our hypothesis that sensitivity to acute pain is related to histamine levels in tissues.

Interestingly, our findings that HDC^{-/-} mice were hypernociceptive when fed a histamine-rich diet, but showed no difference from the wild-type when fed a low-histamine diet were inconsistent

with those of Yoshida et al. [10]. As Yoshida et al. fed mice with histamine-free chow, one possible explanation for this discrepancy is that different levels of histamine in the diet induced different levels of histamine in tissues. This may provide further evidence of the dose-related influence of histamine on pain perception. Based on our results and those of Yoshida et al., we speculated that the overall effect of histamine on acute pain sensitivity is dual, depending on the tissue concentrations. In a range of lower concentrations, histamine is proalgesic; once reaching the peak effect, further increase in the concentration may induce analgesia.

We noticed that in HDC^{-/-} mice, although the low-histamine diet induced hypersensitivity in the hot-plate and formalin tests (phase 1) as did in the wild-type, this treatment did not change their pain thresholds in tail pressure and writhing tests. We do not have further data to explain this phenomenon, but it is possible that long-term deficiency of endogenous histamine in HDC^{-/-} mice may induce different responses to manipulation of histamine levels. Moreover, it is worth mentioning that HDC^{-/-} mice, no matter fed either diet, showed less pain behavior than HDC^{+/±} mice in the late phase of the formalin test. It is known that the

Figure 4 Measurement of Na_v1.8 characteristics in small DRG neurons. **(A)** representative traces of Na_v1.8 currents recorded from DRG neurons from HDC^{+/+} (black traces) and HDC^{-/-} (red traces) mice. A series of 50-ms depolarizing voltage commands between -55 and 60 mV (in 5-mV increments) were applied from a holding potential of -60 mV. **(B)** peak $I_{Na_v1.8}$ density-voltage relationship of neurons from HDC^{+/+} (n = 15) and HDC^{-/-} (n = 14) mice. **(C)** no significant difference in the activation curves of Na_v1.8 between HDC^{-/-} (n = 10) and HDC^{+/+} mice (n = 13). **(D)** no significant difference in the steady-state inactivation curves of Na_v1.8 between HDC^{-/-} and HDC^{+/+} mice (n = 7/genotype). This curve was made using a double-pulse protocol consisting of a 500-ms prepulse ranging from -70 to 10 mV (in 5-mV increments), followed by a test pulse to 0 mV (insert).

early and late phases of the formalin test measure the neurogenic and the inflammatory pain responses, respectively. As histamine itself is an inflammatory mediator and knockout of the HDC gene produces abnormal mast cells [27], it is understandable that HDC^{-/-} mice with low histamine levels showed reduced inflammatory pain sensitivity.

We further found an up-regulation of Na_v1.8 expression in primary afferent neurons both in HDC^{-/-} mice and in α -FMH-treated HDC^{+/+} mice. Electrophysiological experiments revealed that the peak current density of Na_v1.8 was higher in HDC^{-/-} mice, while acute treatment of cultured DRG neurons with histamine did not affect Na_v1.8 current (data not shown). These findings indicate that long-term low levels of endogenous histamine promoted the expression of Na_v1.8 in primary afferent neurons. In addition, no difference was detected in either the activation or

inactivation properties of Na_v1.8 between two genotypes, indicating that histamine modulation of Na_v1.8 does not involve the channel characteristics. Because Na_v1.8 plays a crucial role in pain perception, the up-regulation of Na_v1.8 in HDC^{-/-} mice and α -FMH-treated HDC^{+/+} mice is strongly supportive of the behavioral observation that these animals were hypernociceptive, although the contribution of histamine reduction in the central nervous system cannot be ruled out.

Several lines of evidence have demonstrated that Na_v1.8 up-regulation increases the excitability of peripheral neurons, either in physiological or in pathophysiological (inflammatory or neuropathic) states [28,29], while down-regulation of Na_v1.8 by single mutation or the antisense oligodeoxynucleotides reverses CFA or nerve injury-induced heat and mechanical hypersensitivity [30–32]. In the present study, we also found that the current intensity

Figure 5 Changes in excitability of small DRG neurons of HDC^{-/-} mice. (A) representative trace of an AP evoked by 70-pA current injection in a DRG neuron from an HDC^{+/+} mouse, as well as the subthreshold response to 60-pA injection. (B) representative trace of an AP evoked by 40-pA current injection in a DRG neuron from an HDC^{-/-} mouse, as well as the subthreshold response to 30-pA injection. (C) averaged current threshold for evoking an AP (n = 16 for HDC^{-/-} and n = 18 for HDC^{+/+}). (D and E) representative traces of AP responses to current injection at the indicated intensities under A-803467 blockade in neurons from an HDC^{+/+} and an HDC^{-/-} mouse, respectively. (F) averaged current threshold for evoking an AP under A-803467 blockade (n = 7/genotype). (G and H) representative traces of the AP response to current injection at the indicated intensities under TTX blockade in neurons from an HDC^{+/+} and an HDC^{-/-} mouse, respectively. I, averaged current threshold for evoking an AP under TTX blockade (n = 8 for HDC^{-/-} and n = 10 for HDC^{+/+}). ***P* < 0.01 and **P* < 0.05, compared with the wild-type.

required for AP initiation was approximately 27 pA lower and the firing rate of APs in response to suprathreshold stimulation was 8 Hz higher in DRG neurons from HDC-null mice. These results indicate that primary afferent neurons from mutated mice were easier to activate and fired more spikes in response to suprathreshold stimulation. This hyperexcitability was abolished by the selective Na_v1.8 blocker A-803467, but not TTX, strongly suggesting the pivotal contribution of Na_v1.8. With our finding that A-803467 blocked continuous AP firing in both HDC-null and wild-type mice, and others showing Na_v1.8 is involved in continuous firing activity [12,33], the increased rate of repetitive firing also supported the up-regulation of Na_v1.8. Therefore, the hyperexcitability of small DRG neurons from HDC^{-/-} mice sug-

gests that the overexpression of Na_v1.8 was functional and may be associated with the hypernociception observed in behavioral tests.

In addition, as reported [34,35], the present study found that Na_v1.8 staining was rich in, but not restricted to peripherin-positive DRG neurons. The analysis of expression profile revealed Na_v1.8 was unregulated not only in peripherin-positive, but also in NF200-positive DRG neurons, but decreased in peripherin-negative neurons both in mutated and α -FMH-treated mice. Although peripherin and NF200 are conventionally considered to be markers for small and large DRG neurons, respectively, there might be overlap in the sizes of neurons labeled with these markers according to the studies of Perry et al. and Goldstein et al. [36,37]. Our results suggest that long-term low histamine

Figure 6 Repetitive firing of small DRG neurons in response to a 500-pA (500 ms) depolarizing current pulse. (A and B) representative firing responses of DRG neurons from an HDC^{+/+} and an HDC^{-/-} mouse, respectively. (C) averaged firing rate in neurons with repetitive firing ($n = 11$ for HDC^{+/+} and 13 for HDC^{-/-}, respectively). (D and E) representative firing responses of DRG neurons from an HDC^{+/+} and an HDC^{-/-} mouse under A803467 (500 nM) blockade, respectively. (F) averaged firing rate in neurons with repetitive firing under A803467 (500 nM) blockade ($n = 15$ for HDC^{+/+} and 13 for HDC^{-/-}, respectively).

levels induce an up-regulation of Na_v1.8 in peripherin-positive and peripherin and NF200 double-labeled neurons, but a slight down-regulation in peripherin-negative neurons. This redistribution of Na_v1.8 implies that endogenous histamine plays an important role in maintaining the normal Na_v1.8 expression profile in primary afferent neurons. Further investigation is needed to elucidate the mechanisms underlying histamine regulation of Na_v1.8.

In summary, our results suggest that histamine modulates sensitivity to acute pain depending on its levels in tissues. This effect may be related to the regulation of Na_v1.8 and the excitability of nociceptive primary afferent neurons.

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Conflict of Interest

The authors declare no conflict of interest.

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