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Cellular Basis of Itch Sensation

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Itch and pain are two distinct sensations. Although our previous study suggested that gastrin-releasing peptide receptor (GRPR) is an itch-specific gene in the spinal cord, a long-standing question of whether there are separate neuronal pathways for itch and pain remains unsettled. Here we selectively ablated lamina I neurons expressing GRPR in the spinal cord of mice. These mice showed profound scratching deficits in response to all of the itching (pruritogenic) stimuli tested, irrespective of their histamine-dependence. In contrast, pain behaviors were unaffected. Our data also suggest that GRPR⁺ neurons are different from the spinothalamic tract (STT) neurons which have been the focus of the debate. Together, the present study suggests that GRPR⁺ neurons constitute a long-sought labeled line for itch sensation in the spinal cord.

Itch has long been considered to be a sub-modality or sub-quality of pain (1-4), because both sensations share many similarities (5). Whether itch and pain, two distinct sensations, are mediated by distinct neural circuits has been the subject of controversy (6-8). In the spinal cord, arguments for the “labeled line” came from electrophysiological recordings in cat showing the presence of a small subset of histamine-responsive, mechanically, thermally and mustard oil insensitive lamina I STT neurons (9). Recent studies in primates, however, found that histamine-sensitive STT neurons were all responsive to noxious mechanical and chemical stimuli, notably capsaicin, arguing against the “labeled line” for itch (10,11). Although our previous data suggested that GRPR is an itch-specific gene in the spinal cord (12), they could not be extrapolated to imply that GRPR⁺ neurons are itch-specific, simply because neurons expressing one sensory modality-specific gene may also express other sensory modality-specific genes as often seen in sensory neurons (13). One way to address this issue is to selectively ablate a subset of itch-signaling neurons and assess whether pain behaviors are altered in the absence of these neurons. We selectively ablated GRPR⁺ neurons in the spinal cord of mice by intrathecal administration of bombesin-saporin (bombesin-sap), a toxin-coupled to bombesin that binds with high affinity to GRPR and results in GRPR internalization and cell death (fig.S1) (14,15).

We first determined the optimal dose and time course of bombesin-sap treatment. Ablation of GRPR⁺ neurons reduced pruritogen-induced scratching behaviors in a dose-dependent manner (fig. S2). Most of GRPR⁺ neurons (>75%) were lost two weeks after single intrathecal injection of bombesin-sap (400 ng, Fig. 1. A to C). To determine the specificity of bombesin-sap treatment, we analyzed several subpopulations of neurons in the spinal cord by using lamina-specific molecular markers. Expression of neuromedin U receptor 2 (NMUR2) and prodynorphin was not affected in lamina I of mice treated with bombesin-sap (Fig. 1. D to F,

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and fig. S3), nor was neurokinin 1 receptor (NK1, Fig. 1. G to I), a lamina I and III gene expressed in subset of neurons known for their involvement in nociceptive transmission (15, 16). Expression of lamina II markers such as neurotensin and PKC γ in the bombesin-sap group was also not affected (Fig. 1. J to O), nor was the projection of primary afferents (fig. S4). Saporin conjugated to a random peptide sequence (blank-sap) did not show cytotoxicity effects (fig. S4-5).

We next examined scratching behaviors of mice treated with bombesin-sap in response to intradermal injection of a panel of histamine-dependent pruritogenic agents. Unlike the control mice which exhibited vigorous scratching response after intradermal injection of histamine, bombesin-sap-treated mice showed profound scratching deficits (reduced by 77%, Fig. 2A). Scratching behaviors evoked by compound 48/80 (17) and serotonin (5-HT) were also dramatically reduced relative to the control mice (by 79% and 88% respectively, Fig. 2. B and C and fig. S9D). We further examined scratching behavior evoked by endothelin-1 (ET-1) whose pruritogenic effect is partially dependent on histamine (18). While ET-1 elicited robust scratching behavior, mice treated with bombesin-sap exhibited scarce scratching responses (reduced by 84%, Fig. 2D).

We next evaluated scratching behavior elicited by two histamine-independent pruritogenic agents: an agonist of the protease-activated receptor 2 (PAR2) (19) and chloroquine which causes pruritus when used as an anti-malaria drug in humans (20). Bombesin-sap-treated mice showed significantly reduced scratching responses compared with the control mice in either of these tests (reduced by 71% and 85% respectively, Fig. 2. E and F). To ascertain whether GRPR⁺ neurons are also important for chronic itch, for which no effective treatment is available (21,22), we examined mice treated with diphenylcyclopropenone (DCP), a topical immunotherapy agent used in the treatment of alopecia areata which often results in severe side effects including eczematous skin, contact dermatitis as well as intense itching in both patients and mice (23,24). Mice treated with DCP showed increased and persistent scratching behavior (Fig. 2G). In contrast, scratching responses of the bombesin-sap-treated mice to DCP were nearly lost (Fig. 2G), despite their normal motor function (fig. S6). The remarkable phenotype raises a question of whether certain subsets of non-GRPR⁺ neurons which may express other bombesin-like peptide receptors (14) might have also been ablated by bombesin-sap, thereby contributing to the loss of scratching response. Because our molecular analysis is constrained by a very limited number of lamina I-specific markers, it is likely that a loss of a small subset of non-GRPR⁺ neurons which are important for itch may have escaped detection. To examine this possibility, we compared scratching behaviors of GRPR mutant mice treated with bombesin-sap and with blank-sap, and found that two groups showed comparable scratching responses to a variety of pruritogenic agents (fig. S7), demonstrating that protecting GRPR⁺ neurons from being ablated by the GRPR mutation completely abolished the effect of bombesin-sap. Thus, even though some non-GRPR⁺ neurons that could also bind to bombesin-sap may have been lost, they are unlikely to be involved in itch signal transmission.

Although we have shown previously that GRPR is not important for nociceptive transmission, we have been unable to exclude the possibility that GRPR⁺ neurons are involved in pain (12). To test whether GRPR⁺ neurons are required for pain sensation, innocuous and noxious mechanical sensitivity of mice was examined by von Frey filaments and the Randall-Selitto test, and no significant differences were found between two groups (Fig. 3. A and B). Acute thermal pain as assessed by the paw withdrawal (Hargreaves), water immersion and hot-plate tests also remained unaltered in the bombesin-sap group (Fig. 3. C, D and E). We further tested inflammatory pain responses, and found that licking and flinching behaviors elicited by intraplantar injection of 5% formalin in both phases were indistinguishable between the treated group and the control group (Fig. 3F). Mustard oil and capsaicin, which elicit a sensation of burning pain, also evoked comparable licking and flinching behaviors between groups (Fig. 3.

G and H). Consistently, persistent inflammatory pain of longer duration was evaluated using complete Freund's adjuvant (CFA) model, and no difference in mechanical hypersensitivity was found between groups (fig. S8A). Lastly, we asked whether there was an alteration of neuropathic pain in these mice using the partial sciatic nerve injury (PNI) model, and mechanical allodynia in bombesin-sap treated mice appeared normal (fig. S8B).

Our results are unexpected in several aspects. In marked contrast to the findings that the response of GRPR mutant mice to histamine-dependent pruritogenic stimuli was either not affected or modestly reduced (fig. S9) (12), bombesin-sap treated mice showed nearly complete loss of scratching responses to both histamine-dependent and -independent pruritogenic stimuli, suggesting that GRPR⁺ neurons may contain a repertoire of itch-specific signaling molecules which are programmed differentially to transmit pruritogenic signals with distinct underlying mechanisms. Given recent identification of two separate itch pathways in STT neurons (11), it will be interesting to determine whether GRPR⁺ neurons comprise distinct subpopulations with discrete pruritic responsiveness. Another surprising finding is that several lines of evidence suggest that GRPR⁺ and STT neurons are two non-overlapping subpopulations. First, expression of NK1, which is expressed in approximately 80% of STT neurons (25,26), is normal in bombesin-sap-treated mice. And the ablation of NK1⁺ cells compromised chronic pain behaviors in rats (15,16), whereas a loss of GRPR⁺ neurons did not. Second, a lesion of the STT pathway always impaired both itch and pain (27,28). Moreover, histamine- or cowhage-responsive STT neurons in primates also responded to capsaicin (10, 11). By contrast, our comprehensive pain behavioral analysis reveals that despite their critical roles in both acute and chronic itch, GRPR⁺ neurons are dispensable for both innocuous and noxious stimuli across a broad range of sensory modalities, notably including capsaicin-evoked behavior. Taken together, GRPR⁺ neurons represent a previously unrecognized subpopulation of lamina I neurons that confers the specificity of itch sensation. A detailed understanding of the anatomic basis of GRPR⁺ neurons (interneurons versus projection neurons) and their relationship with STT neurons will require further study. Nevertheless, our study supports the labeled line for itch in the spinal cord, and provides an important cellular basis for elucidating how a pruritogenic stimulus, being received by cutaneous sensory receptors, conveyed by primary afferents, differentiated and transmitted by the spinal cord, is ultimately perceived by the brain as a major sensation that is distinguishable from pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Graham DT, Goodell H, Wolff HG. J Clin Invest 1951;30:37. [PubMed: 14803555]
2. Rothman S. Physiol Rev 1941;21:357.
3. Tuckett, RP. Itch: Mechanisms and Management of Pruritus. Bernhard, JD., editor. McGraw-Hill, Inc.; 1994. p. 1-22.
4. von Frey M. Arch Neerland. Physiol 1922;7:142.
5. Schmelz M. Neurosci Biobehav Rev. 2008
6. Shelley WB, Arthur RP. AMA Arch Derm 1957;76:296. [PubMed: 13457411]
7. McMahon SB, Koltzenburg M. Trends Neurosci 1992;15:497. [PubMed: 1282750]

8. Schmelz M. *Nat Neurosci* 2001;4:9. [PubMed: 11135635]
9. Andrew D, Craig AD. *Nat Neurosci* 2001;4:72. [PubMed: 11135647]
10. Simone DA, et al. *J Neurophysiol* 2004;91:213. [PubMed: 14715718]
11. Davidson S, et al. *J Neurosci* 2007;27:10007. [PubMed: 17855615]
12. Sun YG, Chen ZF. *Nature* 2007;448:700. [PubMed: 17653196]
13. Jordt SE, McKemy DD, Julius D. *Curr Opin Neurobiol* 2003;13:487. [PubMed: 12965298]
14. Jensen RT, Battey JF, Spindel ER, Benya RV. *Pharmacol Rev* 2008;60:1. [PubMed: 18055507]
15. Mantyh PW, et al. *Science* 1997;278:275. [PubMed: 9323204]
16. Nichols ML, et al. *Science* 1999;286:1558. [PubMed: 10567262]
17. Kuraishi Y, Nagasawa T, Hayashi K, Satoh M. *Eur J Pharmacol* 1995;275:229. [PubMed: 7539379]
18. Trentin PG, Fernandes MB, D'Orleans-Juste P, Rae GA. *Exp Biol Med* 2006;231:1146.
19. Steinhoff M, et al. *J Neurosci* 2003;23:6176. [PubMed: 12867500]
20. Sowunmi A, Walker O, Salako LA. *Lancet* 1989;2:213. [PubMed: 2568535]
21. Paus R, Schmelz M, Biro T, Steinhoff M. *J Clin Invest* 2006;116:1174. [PubMed: 16670758]
22. Wahlgren CF. *J Dermatol* 1999;26:770. [PubMed: 10635621]
23. van der Steen PH, van Baar HM, Perret CM, Happle R. *J Am Acad Dermatol* 1991;24:253. [PubMed: 2007671]
24. Seike M, Ikeda M, Kodama H, Terui T, Ohtsu H. *Exp Dermatol* 2005;14:169. [PubMed: 15740588]
25. Todd AJ, McGill MM, Shehab SA. *Eur J Neurosci* 2000;12:689. [PubMed: 10712649]
26. Al-Khater KM, Kerr R, Todd AJ. *J Comp Neurol* 2008;511:1. [PubMed: 18720412]
27. Bickford R. *Clin. Sci* 1938;3:337.
28. Hyndman OR, Wolkin J. *Arch Neurol Psychiatry* 1943;50:129.

Fig. 1.

Selective ablation of GRPR⁺ neurons in the spinal cord. Comparison of molecular expression (C, F, I, L and O; black bars: bombesin-sap group; white bars: blank-sap group) in the superficial spinal cord of mice treated with blank-sap (A, D, G, J, M) and bombesin-sap (B, E, H, K, N). (A-B) GRPR expression in lamina I detected by *in situ* hybridization (ISH). (C) Approximately 80% of GRPR⁺ neurons were lost in the bombesin-sap group compared to the blank-sap group (** $P < 0.01$). (D-E) NMUR2 expression in lamina I detected by ISH. (F) The number of NMUR2⁺ neurons was similar between the bombesin-sap and blank-sap groups ($P > 0.05$). (G-H) NK1 receptor expression in the dorsal spinal cord detected by immunocytochemistry. (I) The density of NK1 signal in lamina I was comparable between the bombesin-sap and blank-sap groups ($P > 0.05$). (J-K) PKC γ in lamina II layer detected by immunocytochemistry. (L) The number of PKC γ ⁺ neurons was comparable between groups.

($P > 0.05$). (**M-N**) Neurotensin expression in Lamina II detected by ISH. (**O**) The number of neurotensin⁺ neurons was comparable between the bombesin-sap and blank-sap groups ($P > 0.05$). Scale bar: A, 100 μm (A, B, D, E, G, H, J, K, M, N). Student's *t*-test. $n = 4\sim 6$ for each group. Sap, saporin. Data with error bars represent mean $\pm$ SEM.

Fig. 2.

Selective ablation of GRPR⁺ neurons abolished scratching behaviors. (A) Histamine-evoked scratching behavior in mice treated with bombesin-sap was almost lost compared with the blank-sap control (500 μ g/50 μ l, $P < 0.001$). (B) Compound 48/80-evoked scratching behavior in bombesin-sap-treated mice was almost absent (100 μ g/50 μ l, $P < 0.05$). (C) 5-HT-evoked scratching behavior in bombesin-sap-treated mice was largely abolished (10 μ g/50 μ l, $P < 0.001$). (D) ET-1-evoked scratching behavior (25 ng/50 μ l) in bombesin-sap-treated mice was largely gone ($P < 0.001$). (E) The PAR2 agonist-evoked scratching behavior in bombesin-sap-treated mice was almost absent (SLIGRL-NH₂, 100 μ g/50 μ l; $P < 0.001$). (F) Chloroquine-evoked scratching behavior in bombesin-sap-treated mice was also absent (200 μ g/50 μ l) ($P <$

0.001). (G) Scratching behavior evoked by DCP was nearly blocked in mice treated with bombesin-sap compared with blank-sap ($P < 0.001$). Two-way repeated measured analysis of variance (ANOVA). $n = 6\sim 9$ for each group. Sap, saporin; i.d., intradermal; ET-1, endothelin-1; DCP, diphenylcyclopropanone. Data with error bars represent mean $\pm$ SEM.

Fig. 3.

Normal pain behaviors in mice treated with bombesin-sap. (A) Mechanical sensitivity in bombesin-sap-treated mice as measured by paw withdrawal threshold upon exposure to von Frey filaments was comparable to mice treated with blank-sap. $P > 0.05$. (B) The tail-flick latencies in the Randall-Selitto test did not show significant difference between groups. $P > 0.05$. (C-E) Responses to noxious thermal stimulation measured by the paw withdrawal latency (Hargreaves test, C), the water immersion latency (D), and hot plate test (E) were indistinguishable between groups. $P > 0.05$. (F) Spontaneous pain responses in first (0~10 min) and second phase (10~60 min) of the formalin test was comparable between mice treated with blank-sap and bombesin-sap. $P > 0.05$. (G) Spontaneous pain response in mustard oil test was comparable between groups. $P > 0.05$. (H) Spontaneous pain response induced by intraplantar injection of capsaicin (0.1%) was comparable between groups. $P > 0.05$. Student's t -test. $n = 6\sim9$ for each group. Sap, saporin. Black bars: the bombesin-sap group; white bars: the blank-sap group. Data with error bars represent mean $\pm$ SEM.