Journal of Dermatological Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Letter to the Editor

Specific activation of inhibitory interneurons in the spinal dorsal horn suppresses repetitive scratching in mouse models of chronic itch

Chronic itch is a debilitating symptom of inflammatory skin conditions, such as atopic and contact dermatitis and dry skin, and affects millions of individuals worldwide. There are currently no effective medications that directly and effectively suppress itch itself. Understanding the mechanism of chronic itch and developing an effective treatment is a major clinical challenge. Somatosensory information, including itch from the periphery, is processed by neural circuits in the spinal dorsal horn (SDH) [1]. Recent evidence has revealed the existence of a neuronal pathway selective for itch processing [1]. However, the mechanisms underlying chronic itch are poorly understood. A recent study has shown that mice deficient in the transcription factor BHLHB5 lose a subpopulation of SDH inhibitory interneurons and display itch-related behaviors, including scratching [2]. This behavioral phenotype reduces following transplantation of embryonic γ-aminobutyric acid (GABA)-ergic precursor neurons into the SDH [3]. Thus, SDH inhibitory interneurons may play a role in chronic itch. However, whether acute and specific stimulation of inhibitory interneurons that are intrinsically integrated into SDH neuronal circuits alleviates chronic itch remains to be determined.

To specifically stimulate SDH inhibitory interneurons *in vivo*, we used designer receptors exclusively activated by designer drugs (DREADD) technology [4] in which the excitatory DREADD, hM3Dq (a modified human muscarinic Gq protein-coupled receptor) is expressed in a specific cell-population. The hM3Dq-expressing cells can be exclusively activated by clozapine-N-oxide (CNO), which does not activate endogenously expressed muscarinic receptors. To express hM3Dq precisely in SDH inhibitory interneurons, we used the flip-excision (FLEX) switch technology [5] (Fig. 1a): an adeno-associated virus (AAV) vector carrying hM3Dq and mCherry (AAV2/9-EF1α-FLEX-hM3Dq-P2A-mCherry) was microinjected into the cervical SDH of *vesicular GABA transporter* (*Vgat*)-*Cre* mice (*Vgat-Cre*;AAV-hM3Dq^{FLEX} mice hereafter). In these mice, a Cre-dependent genetic inversion switch occurs in *Vgat-Cre*$^{+}$ neurons (GABAergic inhibitory neurons) and hM3Dq is expressed (Fig. 1a). For expressing hM3Dq in the cervical SDH, where somatosensory inputs from the upper back skin is received, we first developed a method for minimally-invasive microinjection into the cervical SDH by modifying a previous method for the lumbar levels [6]. Under anesthesia, an incision was made in the neck, and a small opening was made in the muscles around the interspace between the C4 and C5 vertebrae (Fig. 1b). A microcapillary was inserted into the SDH parenchyma through a small window. An injected blue dye remained localized around the cervical segment C3 to C5 (Fig. 1b). In *Vgat-Cre*;AAV-hM3Dq^{FLEX} mice, mCherry fluorescence was observed only in the cervical SDH ipsilateral to the AAV-microinjected side (Fig. 1c). All mCherry^{+} cells were labeled by the neuronal marker NeuN (Fig. 1d), but not by markers of microglia (ionized calcium-binding adaptor molecule 1, Iba1) (Fig. 1e) or astrocytes (glial fibrillary acidic protein, GFAP) (Fig. 1e), indicating that hM3Dq was expressed specifically in cervical SDH neurons. For functional assay, we performed whole-cell patch-clamp recordings using spinal cord slices taken from *Vgat-Cre*;AAV-hM3Dq^{FLEX} mice and found that CNO application induced mCherry^{+} SDH neurons to fire action potentials (Fig. 1f), suggesting that CNO activates hM3Dq-expressing GABAergic neurons in the SDH.

To examine the effect on chronic itch, we applied diphenylcyclopropenone (DCP), a reagent known to produce chronic itch related to contact dermatitis (see Supplementary information), to the back skin of *Vgat-Cre*;AAV-hM3Dq^{FLEX} mice. On day 35 (Fig. 2a), we counted the number of incidents of scratching behavior with the left hindpaw (the hM3Dq-expressing side). DCP-treated *Vgat-Cre*;AAV-hM3Dq^{FLEX} mice had skin lesions of the rostral back (Supplementary Fig. 1) displayed scratching behavior (Supplementary Movie 1), and the number of scratching incidents was clearly increased (Fig. 2b). The incidents were similar to DCP-treated wild-type mice (infected with the mock-virus AAV-mCherry^{FLEX}) (30520.6 ± 6913.4). On day 36, we administered saline or CNO (10 mg/kg) to DCP-treated *Vgat-Cre*;AAV-hM3Dq^{FLEX} mice and found that CNO markedly suppressed scratching behavior (Fig. 2b and c). The suppressive effect of CNO persisted at least for 24 h after the administration. In *Vgat-Cre*;AAV-mCherry^{FLEX} mice, CNO had no effect on the DCP-induced scratching [pre-CNO, 23284.8 ± 8704.8; post-CNO, 23026.4 ± 6500.8; $n = 5$]. In addition, CNO did not affect the DCP-induced dermatitis on day 37, but suppressed at day 39 (Supplementary Fig. 2), suggesting that suppression of scratching behavior by GABAergic stimulation results in partially resolving the skin lesions. Furthermore, we tested in a mouse model of dry skin. Scratching behavior of *Vgat-Cre*;AAV-hM3Dq^{FLEX} mice treated with water following acetone/ether (1:1) mixture (AEW) to their rostral back skin was suppressed by CNO (Fig. 2d), indicating that stimulation of GABAergic interneurons also suppresses dry skin-induced itch.

Using DREADD technology, we demonstrate for the first time that repetitive scratching behavior in models of chronic itch, contact dermatitis and dry skin, was suppressed by the specific stimulation of GABAergic inhibitory interneurons that are intrinsically integrated into SDH circuits. The impact of inhibitory SDH interneurons in chronic itch is supported by recent findings that Bhlhb5-deficient mice, which lose inhibitory interneurons, exhibit enhanced pruritogen-evoked scratching [2], and that the pharmacological blockade of inhibitory signals in SDH neurons suppresses an inhibitory effect of scratching on itch-related spontaneous firing

http://dx.doi.org/10.1016/j.jdermsci.2017.05.017

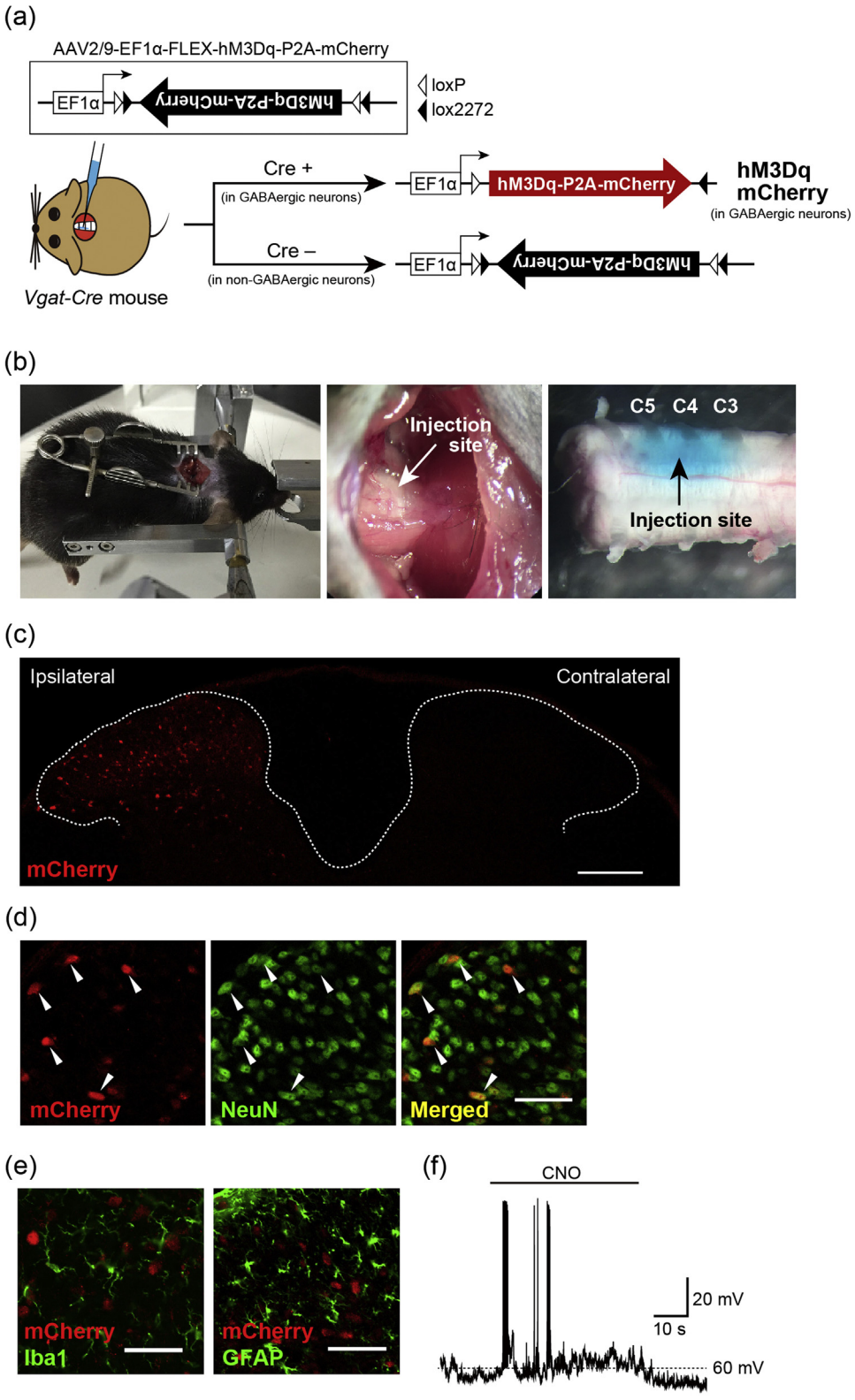

Fig. 1. Specific expression of hM3Dq in *Vgat-Cre*$^{+}$ inhibitory interneurons in the cervical SDH. (a) Schematic illustration of the FLEX switch system. AAV2/9 vector carrying EF1α and an inverted hM3Dq-P2A-mCherry sequence flanked by loxP and lox2272 on both sides. Cre-dependent genetic inversion switch of the hM3Dq-P2A-mCherry sequence occurs in Cre-expressing GABAergic inhibitory neurons of *Vgat-Cre* mice. (b) Photographs of the immobilization of the mouse (left), the microinjection site (middle) and of the cervical spinal cord removed after microinjection of Evans blue dye (right). The dye was localized at the C3–C5 segments. (c) Expression of mCherry in the cervical SDH of *Vgat-Cre*;AAV-hM3Dq^{FLEX} mice. Scale bar, 200 μm. (d,e) Immunostaining of mCherry^{+} cells (red) in the C3 SDH using the cell-type markers (green), NeuN (d), Iba1 (e, left) or GFAP (e, right). Scale bars, 50 μm. (**g**) Representative firing pattern of an mCherry^{+} SDH neuron in a spinal cord slice.

Please cite this article in press as: K. Kanehisa, et al., Specific activation of inhibitory interneurons in the spinal dorsal horn suppresses repetitive scratching in mouse models of chronic itch, J Dermatol Sci (2017), http://dx.doi.org/10.1016/j.jdermsci.2017.05.017