Neuroscience Letters 627 (2016) 51–60

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Electrophysiological properties of brain-natriuretic peptide- and gastrin-releasing peptide-responsive dorsal horn neurons in spinal itch transmission

Fumiya Kusube[a,b], Mitsutoshi Tominaga[a], Hiroaki Kawasaki[a], Fumiyuki Yamakura[c], Hisashi Naito[d], Hideoki Ogawa[a], Yasuhiro Tomooka[b], Kenji Takamori[a,e,*]

[a] Institute for Environmental and Gender Specific Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan
[b] Department of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku, Tokyo 125-8585, Japan
[c] Juntendo University Faculty of International Liberal Arts, Tokyo 113-8421, Japan
[d] Institute of Health and Sports Science & Medicine, Juntendo University, 1-1 Hiragagakuendai, Inzai, Chiba 270-1695, Japan
[e] Department of Dermatology, Juntendo University Urayasu Hospital, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan

HIGHLIGHTS

- The BNP-Npra and GRP-GRPR signaling pathways of spinal itch transmission differ.
- Spinal BNP may contribute little directly to histaminergic itch.
- Multiple neurotransmitters are involved in spinal itch transmission.

ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 18 May 2016
Accepted 25 May 2016
Available online 25 May 2016

Keywords:
BNP
Dorsal horn
GRP
In vivo electrophysiology
Itch

ABSTRACT

Spinal itch transmission has been reported to be mediated by at least two neuronal populations in spinal dorsal horn, neurons expressing brain-natriuretic peptide (BNP) receptor (Npra) and gastrin-releasing peptide (GRP) receptor (GRPR). Although Npra-expressing neurons were shown to be upstream of GRPR-expressing neurons in spinal itch transmission, the roles of BNP and GRP in the spinal neurotransmission of histamine-dependent and −independent itch remains unclear. Using *in vivo* electrophysiology and behavior analysis, this study examined the responses of chloroquine (histamine-independent pruritogen)-responsive and histamine-responsive dorsal horn neurons to spinal applications of BNP and GRP. Electrophysiologically, 9.5% of chloroquine-responsive neurons responded to BNP, 33.3% to GRP, and 4.8% to both, indicating that almost half of chloroquine-responsive neurons were unresponsive to both BNP and GRP. In contrast, histamine-responsive neurons did not respond to spinal BNP application, whereas 30% responded to spinal GRP application, indicating that 70% of histamine-responsive neurons were unresponsive to both BNP and GRP. Behavioral analyses showed differences in the time-course and frequency of scratching responses evoked by intrathecal BNP and GRP. These findings provide evidence that most BNP-Npra and GRP-GRPR signaling involve different pathways of spinal itch transmission, and that multiple neurotransmitters, in addition to BNP and GRP, are involved in spinal itch transmission. The electrophysiological results also suggest that spinal BNP contributes little to histaminergic itch directly.

1. Introduction

Itch is defined as a sensation in the skin that causes a reflex to scratch. Itch sensation is detected by nerve endings in the skin, whose cell bodies are located in the dorsal root ganglia (DRG) and trigeminal ganglia. These neurons differ widely in somata sizes, expression of ion channels and receptors, innervation areas, and electrophysiological properties [9]. Small-diameter DRG neurons

Abbreviations: BNP, B-type natriuretic peptide; CQ, chloroquine; DRG, dorsal root ganglia; GRP, gastrin-releasing peptide; GRPR, gastrin-releasing peptide receptor; i.d., intradermal; i.t., intrathecal; Npra, B-type natriuretic peptide receptor; NS, nociceptive-specific; WDR, wide dynamic range.

* Corresponding author at: Institute for Environmental and Gender Specific Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan.
E-mail address: ktakamor@juntendo.ac.jp (K. Takamori).

http://dx.doi.org/10.1016/j.neulet.2016.05.051
0304-3940/

with unmyelinated axons (C-fibers) are the major neuronal types that mediate itch as well as pain [4]. The sensations of itch and pain are distinct, and each can elicit different behavioral responses, such as scratching to remove irritants and withdrawal to avoid tissue injury.

Recent studies have reported that itch is mediated by at least two neuronal populations in murine dorsal horn: neurons expressing B-type natriuretic peptide (BNP) receptor (Npra) and those expressing gastrin-releasing peptide (GRP) receptor (GRPR). BNP was shown to be an important neuropeptide for spinal itch transmission [11]. BNP is thought to activate spinal Npra-expressing neurons, which release GRP. GRP, in turn, is thought to activate GRPR-expressing neurons, which mediate the transmission of itch information from the skin to the brain. Moreover, BNP was found to be expressed in and secreted from DRG, whereas GRP was not expressed in murine sensory ganglia [6].

However, a combination of molecular, anatomic and behavioral analyses showed that the BNP-Npra system is involved in both itch and pain, and that this system does not function upstream of the GRP-GRPR dedicated neuronal pathway [10]. More recently, GRP mRNA and peptides were found to be expressed in murine DRG neurons [3]. Thus, the exact spinal pathway mediating itch is currently unclear. This study was therefore performed to investigate the roles of BNP and GRP in spinal itch transmission. Specifically, the *in vivo* electrophysiological properties of murine chloroquine-responsive and histamine-responsive dorsal horn neurons to spinal applications of BNP and GRP were investigated, and the scratching behaviors evoked by intrathecal injection of BNP and GRP were analyzed.

2. Materials and methods

2.1. Animals

One hundred and two male C57BL/6NCrSlc mice, aged 6–8 weeks and weighing 19–28 g, were obtained from Japan SLC Inc. (Hamamatsu, Japan). The mice were housed in a room maintained under a 12-h light:12-h dark cycle, with food and tap water provided *ad libitum*. The study protocol was approved by the Institutional Animal Care and Use Committee at Juntendo University Graduate School of Medicine and Graduate School of Sports and Health Science.

2.2. In vivo *electrophysiology*

Mice were anesthetized by intraperitoneal injection of 60 mg/kg sodium somnopentyl (Kyoritsu Seiyaku Corp., Tokyo, Japan), and then prepared for single-unit recording from the lumbosacral spinal cord as described previously [2]. During single-unit recording, anesthesia was maintained by supplemental intraperitoneal injections of 10–20 mg/kg/h sodium somnopentyl. A tungsten microelectrode (FHC Inc., Bowdoin, ME, USA) was inserted into the spinal cord using a micromanipulator (Scientifica, East Sussex, UK). Extracellular single-unit activity was recorded, amplified, digitized (Powerlab; ADInstruments Inc., Colorado Springs, CO, USA), and displayed online using Chart 5 Chart 5software (ADInstruments Inc.) [2]. The exposed lumbosacral spinal cord was bathed continuously with artificial cerebrospinal fluid (Krebs: 117 mM NaCl, 3.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 11 mM glucose, equilibrated with 95% O_2 and 5% CO_2 at 37 °C).

A chemical search strategy was used to identify and isolate chloroquine- and histamine-responsive units [2] . To maximize the chance of isolating a chloroquine- or histamine-responsive dorsal horn neuron, the search strategy assumed that these neurons gave rise to ascending projections or served as interneurons in segmental scratch-reflex circuitry, with no attempt made to distinguish between these possibilities. Briefly, ~0.25 μL chloroquine (Sigma-Aldrich, St. Louis, MO, USA) (100 μg/μL) or histamine dihydrochloride (Sigma-Aldrich) (50 μg/μL) was injected intradermally into the ventral hind paw, and a spontaneously active unit in the superficial lumbar dorsal horn (depth <300 μm) was isolated. All units isolated in this study were located in the superficial dorsal horn 0–160 μm below the surface. After the spontaneous activity had waned, 1 μL chloroquine (100 μg/μL) or histamine (50 μg/μL) was injected into the same site through the same 30 gauge needle (Nipro Corp., Osaka, Japan). Only units showing a >30% increase in firing in response to the second microinjection of chloroquine or histamine were selected for further study. Responses were usually recorded for at least 30 min, although in many units, firing declined over a shorter period.

Spinal superfusion of BNP and GRP was performed as described previously with slight modifications [2]. After chloroquine- or histamine-responsive firing was recorded for 30 min, BNP, at a final concentration of 150 μM (GenScript Corp., Piscataway, NJ, USA), was applied directly through the bath system to the spinal cord for 3 min. Responses were usually recorded for at least 30 min, followed by the application of GRP, at a final concentration of 150 μM (Tocris Cookson, Avonmouth, UK). In some experiments, the order of application of GRP and BNP was reversed. Units exhibiting a >30% increase in firing in response to each application were regarded as neuropeptide reactive units.

Units were classified as wide dynamic range (WDR)-type if they responded in a graded manner to an innocuous mechanical stimulation (cotton) and a noxious pinch, or as nociceptive-specific (NS) if they responded to a noxious pinch and to the 6.0 g von Frey stimulus, but not to cotton stimuli, as described [1].

2.3. Scratching behavior

Scratching behavior was measured as described [7]. The lumbar fur of each mouse was clipped with an electric shaver. Mice were placed individually in acrylic cages composed of four cells (13 × 9 × 35 cm). A camcorder (Model HC-W850M; Panasonic, Osaka, Japan) was positioned above the cages to record behavior. After an acclimation period of at least 1 h, followed by intrathecal (i.t.) injection of saline (vehicle), BNP (1 nmol in 5 μL) or GRP (1 nmol in 5 μL), administered by lumbar puncture under sevoflurane (Maruishi Pharmaceutical Co., Osaka, Japan) anesthesia. Each mouse was subsequently placed in a cage, and scratching behavior was recorded on video for 180 min with no experimenters present in the observation room. The videos were replayed, and the number of scratching bouts counted. A scratching bout was defined as raising and lowering a leg, as described; grooming, licking and biting movements were not counted [7].

2.4. Statistical analysis

Results were compared using two-way unpaired *t*-tests. All statistical analyses were performed using Prism 5 software (GraphPad Software, La Jolla, CA, USA). In all analyses, $P < 0.05$ was defined as statistically significant.

3. Results

3.1. Responses of spinal chloroquine-responsive neurons to BNP and GRP

The *in vivo* electrophysiological analyses consisted of the application of BNP and GRP to 42 chloroquine-responsive superficial