



Neuropharmacology and analgesia

Peripheral gabapentin regulates mosquito allergy-induced itch in mice

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ABSTRACT

The antipruritic activity of gabapentin, an anticonvulsant, was studied in a mouse model of allergic itch. In mice sensitized by an extract of the salivary glands of the mosquito (ESGM), an intradermal injection of ESGM elicited scratching and increased peripheral nerve firing. Oral or intradermal administration of gabapentin at the ESGM injection site inhibited ESGM-induced scratching and peripheral nerve firing. However, gabapentin did not affect histamine-induced scratching. The distributions of immunoreactivity to the voltage-dependent calcium channel $\alpha_2\delta$ -1 subunit, a site of gabapentin action, and the histamine H_1 receptor differed in the mouse dorsal root ganglia. The $\alpha_2\delta$ -1 subunit was mainly found in neurons that were 15–20 μm in diameter, whereas the H_1 receptor was mainly in 20–30 μm neurons. In addition, $\alpha_2\delta$ -1 subunit immunoreactivity co-localized with that of transient receptor potential vanilloid 1 (TRPV1). These results suggest that gabapentin regulates allergic itch by acting on the calcium channel $\alpha_2\delta$ -1 subunit in peripheral TRPV1-positive neurons.

1. Introduction

Itch is a skin sensation that provokes the desire to scratch and is a chief complaint among patients with pruritic diseases. Histamine H_1 receptor antagonists are employed as first-line treatments of itch. While these drugs are effective in urticaria, many other pruritic diseases respond poorly to H_1 receptor antagonists (Wahlgren, 1991). Thus, the development of new drugs for the treatment of antihistamine-resistant pruritus is required.

Gabapentin [1-(aminomethyl)cyclohexanecarboxylic acid] is an anticonvulsant used to treat seizures associated with epilepsy. Gabapentin is a ligand of the $\alpha_2\delta$ -1 subunit of the voltage-dependent calcium channel and inhibits the activation of the channel (Gee et al., 1996), leading to neurotransmitter release and attenuation of postsynaptic excitability (Fink et al., 2002). Clinical studies have reported that gabapentin alleviates neuropathic pain, including postherpetic neuralgia and pain arising from spinal cord injury, reflex sympathetic dystrophy, and diabetic neuropathy (Backonja et al., 1998; Gore et al., 2007; Mellick et al., 1995; Rosenberg et al., 1997; Rosner et al., 1996; Tzellos et al., 2008). This is a central analgesic effect that involves activation of the descending pain regulatory pathway (Takasaki et al., 2001, 2002;

Tanabe et al., 2005). More recently, gabapentin was also reported to show anti-pruritic activity in brachioradial pruritus, uremic pruritus, and itch induced by burns (Manenti et al., 2005; Mendham, 2004; Winhoven et al., 2004). On the other hand, gabapentin did not produce any antipruritic activity in patients with cholestasis (Bergasa et al., 2006). Thus, the mechanism underlying the antipruritic activity of gabapentin remains unclear.

Mosquito bites induce a cutaneous reaction that involves wheal, flare, and itch. We have developed a mouse model of mosquito allergy-induced itch (Ohtsuka et al., 2001). Although they have no apparent response to the first mosquito bite, mice show marked scratching following repeated mosquito bites or injections of an extract of the salivary glands of the mosquito (ESGM). The H_1 receptor antagonist, terfenadine, diminishes plasma extravasation after mosquito bites without altering mosquito bite-induced scratching (Ohtsuka et al., 2001, 2003). This allergic itch reaction has been suggested to be mediated by 5-lipoxygenase metabolites (Kuraishi et al., 2007). It has also been suggested that sensory neurons expressing transient receptor potential vanilloid-1 (TRPV1), but not H_1 receptors, are involved in the signaling of mosquito allergy-induced itch (Nakano et al., 2008b, 2008c). In the present experiments, we used a mouse model of mosquito allergy to

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investigate the efficacy of gabapentin against allergic itch, and its site of action for the regulation of pruritus.

2. Materials and methods

2.1. Animals

Male ICR mice (Japan SLC, Shizuoka, Japan) were used in this study. The mice were 5 weeks old at the start of the experiments. They were housed in a room under controlled temperature ($22 \pm 2^\circ\text{C}$), humidity ($55 \pm 10\%$), and light (light on 07:00–19:00 h) conditions. Food and water were freely available. The procedures in the animal experiments were approved by the Committee for Animal Experiments at the University of Toyama and were conducted in accordance with the guidelines of the Japanese Pharmacological Society.

2.2. Agents

Gabapentin was synthesized by H.O. and H.T. and dissolved in regular tap water for oral administration and in a physiological saline for intradermal injection. Histamine (Wako Pure Chemical Industries Ltd., Osaka, Japan) and diphenhydramine hydrochloride (Wako Pure Chemical Industries Ltd.) was dissolved in a physiological saline. When these drugs were administered orally and intraperitoneally, they were administered in a volume of 0.1 mL/10 g body weight. When these drugs were administered intradermally, they were administered in a volume of 50 μL per site.

2.3. Sensitization and challenge

The thoraxes, including the salivary glands, were isolated from female mosquitoes (*Aedes albopictus*), homogenized in distilled water, and centrifuged at $9000 \times g$ for 30 min as described previously (Ohtsuka et al., 2001). The resultant ESGM was lyophilized and kept at -80°C until use. Prior to administration, ESGM was dissolved in saline. It was injected intradermally into the caudal or rostral back for sensitization or challenge, respectively, twice a week for 4 weeks; the dose and volume of injection per site were 10 μg and 50 μL , respectively.

2.4. Behavioral observation

The hair was clipped over the rostral part of the mouse back. The next day, ESGM or histamine was injected intradermally. The animals were put into an acrylic cage composed of four equal-sized cells ($13 \times 9 \times 40$ cm) for at least 1 h for acclimatization. Immediately after the intradermal injection, they were put back into the same cells and their behavior was videotaped for 1 h with personnel kept out of the observation room. The frequency of scratching around the injected site by the hind paws was counted by video playback. The mice stretched either of its hind paws towards the injection site, leaned their head toward it, rapidly scratched several times for about 1 s, and lowered the hind paw; a series of these movements was counted as one bout of scratching (Kuraishi et al., 1995).

The spontaneous locomotor activity was measured for 30 min using an activity wheel (SW-20, Melquest Ltd., Toyama, Japan).

2.5. Recording of peripheral nerve activity

The activity of the peripheral nerve branch was recorded as previously described (Ohtsuka et al., 2003; Maekawa et al., 2000). Briefly, under urethane (1.7 g/kg, i.p.) anesthesia, the rostral back skin was turned inside out. The peripheral nerve branch was then exposed, dissected free from surrounding tissues, and maintained in a mineral oil pool. Nerve activity was recorded extracellularly using bipolar electrodes of silver wire and an AC bioelectric amplifier (AB651J; Nihon Kohden, Tokyo) with a band-pass filter of 150–3000 Hz. The receptive

field was determined by mechanical stimulation of the skin. After recording the nerve activity, the noise threshold was determined and the frequency of nerve action potentials was calculated from the spike-height histogram using data analysis software (PowerLab/8s; AD Instruments Pty, Castle Hill, Australia). A solution of ESGM (10 μg) and histamine (100 nmol) in a volume of 50 μL was injected intradermally into the receptive field.

2.6. Immunohistochemistry

Mice were anesthetized with pentobarbital and perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The bilateral dorsal root ganglion (DRG) was removed at the C8–T2 levels, post-fixed for 4 h in the same fixative, and kept overnight in cold PBS containing 30% sucrose. The DRG was cut into 16 μm section using a cryostat. The sections were incubated overnight at 4°C with rabbit antiserum to the H_1 receptor (1:100; Calbiochem, La Jolla, CA, USA) or rabbit antiserum to the $\alpha_2\delta$ -1 subunit (1:200; Sigma, St. Louis, LO, USA). Prior to incubation with the $\alpha_2\delta$ -1 subunit antiserum, antigen unmasking was performed by heat treatment for 12 min in 10 mM citrate buffer (pH 6.0, 90°C). These sections were then incubated with Alexa Fluor-488 donkey anti-rabbit IgG (1:1000; Molecular Probes, Grand Island, NY, USA) at room temperature for 2 h. For doublelabeling, sections were incubated overnight at 4°C with goat antiserum to TRPV1 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) after incubation with $\alpha_2\delta$ -1 subunit antiserum. These sections were then incubated with Alexa Fluor-488 donkey anti-rabbit IgG (1:1000; Molecular Probes) and Alexa Fluor-594 donkey anti-rabbit IgG (1:1000; Molecular Probes). Digital images were captured on a confocal laser scanning microscope (Bio-Rad, Hercules, CA, USA). To determine the H_1 receptor- and $\alpha_2\delta$ -1 subunit-positive neuron profile, we only counted neurons with clearly visible nuclei.

2.7. Data processing

All data were presented as means $\pm$ the standard error of the mean. Statistical significance was analyzed using one-way analysis of variance followed by Dunnett's multiple comparison test or Student's *t*-test; $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of gabapentin on mosquito allergy- and histamine-induced scratching

An intradermal injection of ESGM (10 μg /site) elicited scratching in ESGM-sensitized mice (Fig. 1A). The maximal scratching responses were observed during the initial 10-min period after the injection and subsided within 40 min.

When administered orally, 30 mg/kg gabapentin significantly inhibited ESGM-induced scratching in the sensitized mice (Fig. 1B). Intradermal injection of gabapentin at 100 μg /site into the same region injected with ESGM also significantly suppressed this reaction (Fig. 1C). However, injection of gabapentin (10–100 μg /site) into the caudal back did not inhibit the scratching induced by the intradermal administration of ESGM into the rostral back (Fig. 1D). In contrast, neither systemic (3–30 mg/kg orally; Fig. 1E) nor local (10–100 μg /site intradermally; Fig. 1F) administration of gabapentin ameliorated the scratching induced by histamine (100 nmol/site).

A sedative antihistamine diphenhydramine hydrochloride (30 mg/kg intraperitoneally) inhibited spontaneous locomotor activity (Fig. 2A). However, gabapentin (30 mg/kg orally) did not affect spontaneous locomotor activity, suggesting that gabapentin did not have a sedative effect at the doses used in the present study (Fig. 2B).

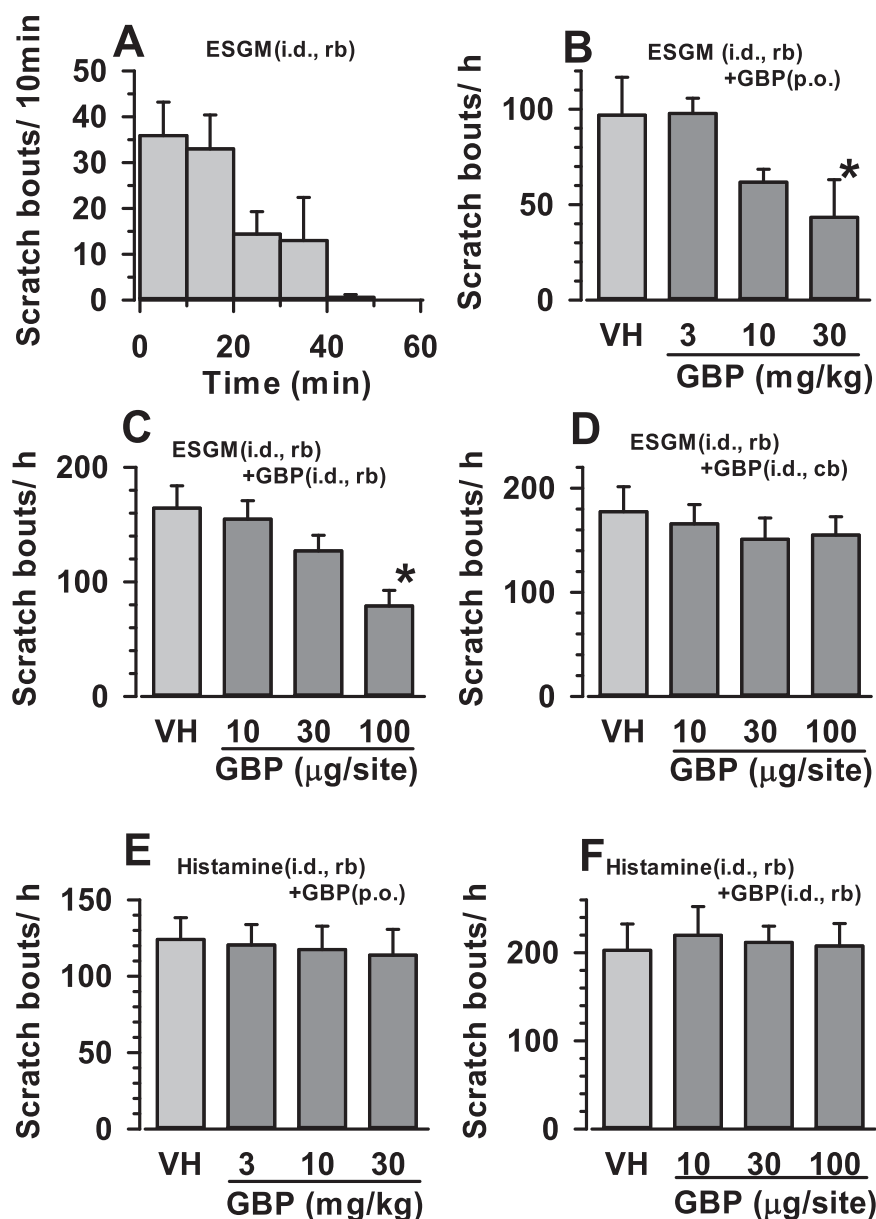


Fig. 1. Gabapentin-mediated effects on scratching induced by mosquito allergens or histamine. Extract of the salivary glands of the mosquito (ESGM; 10 µg/site) or histamine (100 nmol/site) was injected intradermally into the rostral back (rb) of sensitized or naïve mice, respectively. (A) Time-course of scratching following injection of ESGM in the sensitized mice. (B, E) Effects of systemic administration of gabapentin (GBP) on the scratching induced by ESGM or histamine. GBP or vehicle (VH) was administered orally 1 h before the injection of ESGM or histamine. (C, D, F) Effects of local injection of GBP on the scratching induced by ESGM or histamine. GBP or VH was injected intradermally into rb (C, F) or caudal back (cb) (D) of the mouse 30 min before the injection of ESGM or histamine. The dashed line shows the data obtained from naïve mice injected with ESGM (average of the data from eight mice). Values are the means and standard errors of the mean for 8–16 animals; * $P < 0.05$, when compared with VH (Dunnett's multiple comparison test).

3.2. Effects of gabapentin on peripheral nerve branch firing

An intradermal injection of ESGM markedly increased the firing of the peripheral nerve in sensitized mice; this activity was high during the initial 5-min period and had subsided by 60 min after the injection (Fig. 3A–C). In non-sensitized mice, ESGM does not increase nerve firing

(Kuraishi et al., 2007; Ohtsuka et al., 2003). Oral (30 mg/kg) (Fig. 3B) or intradermal (100 µg/site) (Fig. 3C) administration of gabapentin, but not vehicle, markedly inhibited ESGM-induced nerve firing.

Intradermal injection of histamine also markedly increased the firing of the peripheral nerves in naïve mice; this activity was high during the initial 5-min period and had subsided by 30 min after the

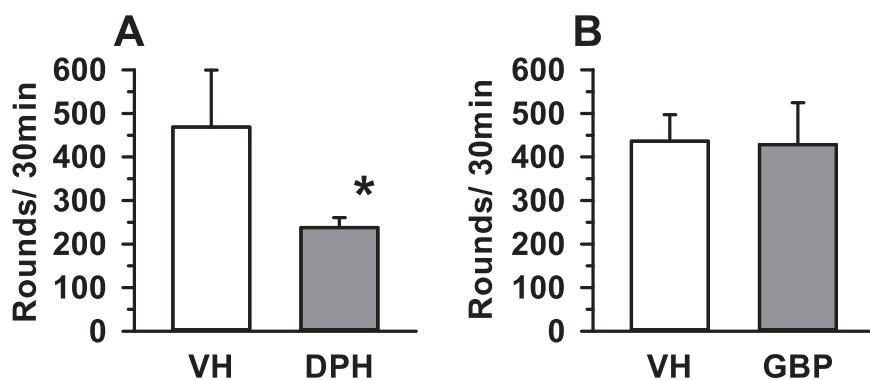


Fig. 2. Effects of gabapentin on spontaneous locomotor activity in mice. The spontaneous locomotor activity levels were measured for 30 min (A) Diphenhydramine hydrochloride (DPH, 30 mg/kg) or vehicle (VH) was administered intraperitoneally 30 min before measuring the locomotor activity. (B) Gabapentin (GBP, 30 mg/kg) or VH was administered orally 1 h before measuring the locomotor activity. Values are the means and standard errors of the mean for 4 animals; * $P < 0.05$, when compared with VH (Student's *t*-test).

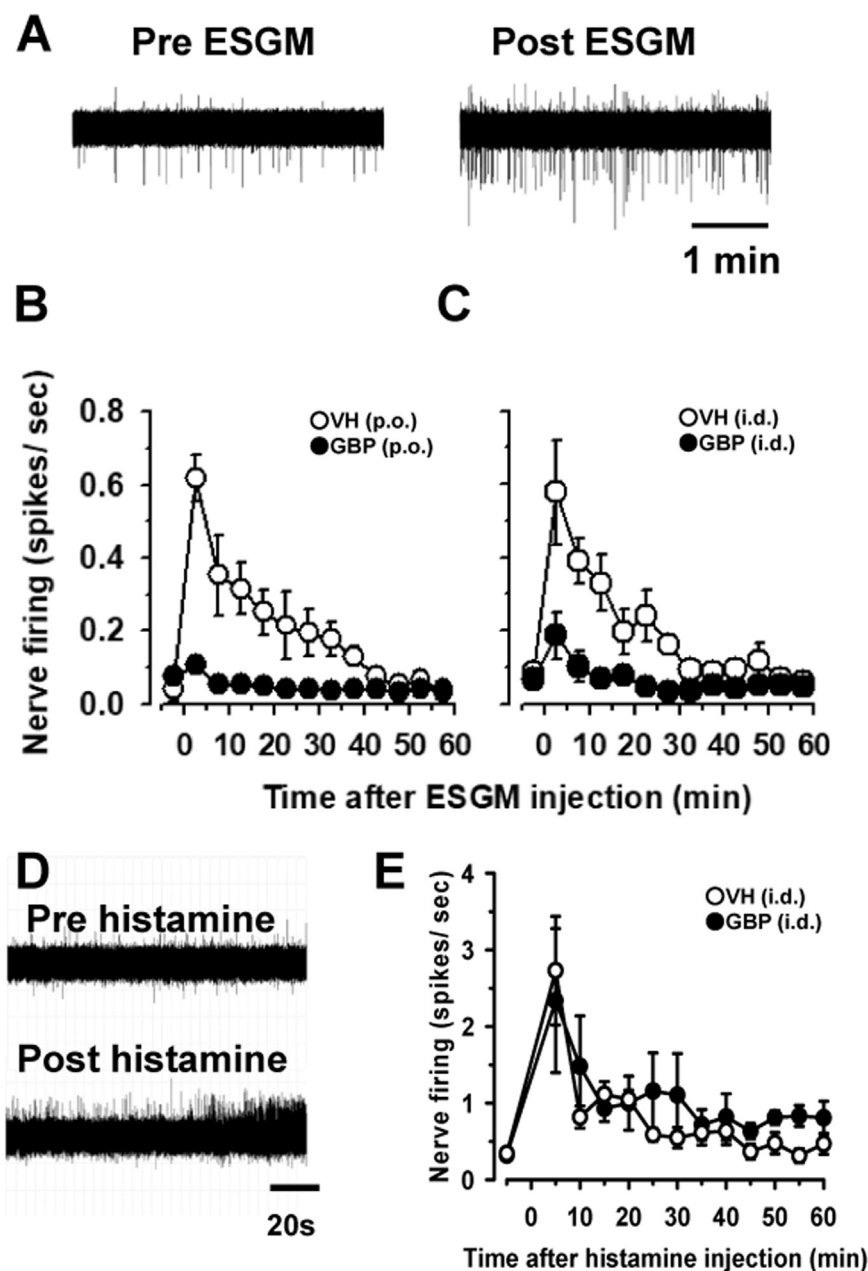


Fig. 3. Effect of gabapentin on peripheral nerve activity induced by mosquito allergens or histamine. Extract of the salivary glands of the mosquito (ESGM; 10 μ g/site) and histamine (100 nmol/site) was injected intradermally into the rostral back of the sensitized mouse and naïve mouse, respectively. Nerve activity was recorded extracellularly using bipolar electrodes. (A) Typical traces of peripheral nerve firing for 4 min before (Pre ESGM) and after (Post ESGM) the injection of ESGM. Effects of systemic (B) and local (C) gabapentin (GBP) on the increase in peripheral nerve firing following an injection of ESGM. (D) Typical traces of peripheral nerve firing for 1 min before (Pre histamine) and after (Post histamine) the injection of histamine. GBP was administered orally (30 mg/kg, p.o.) (B) or intradermally (100 μ g/site, i.d.) (C, E) 1 h or 30 min before pruritogen injection, respectively. Vehicle = VH. Values are the means and standard errors of the mean for 6–8 (B, C) or 4 (E) animals. Two-way repeated measures analysis of variance: main effects of drug treatment, (B) $F_{(1,13)} = 51.8$, $P < 0.001$; (C) $F_{(1,12)} = 27.34$, $P < 0.001$.

injection (Fig. 3D,E). In naïve mice, saline (the vehicle for histamine) did not increase nerve firing (Andoh and Kuraishi, 2014). Intradermal injection of gabapentin (100 μ g/site) did not inhibit histamine-induced nerve firing (Fig. 3E).

3.3. Distribution of the voltage-dependent calcium channel $\alpha_2\delta$ -1 subunit, H_1 histamine receptor, and TRPV1 in mouse DRG

Immunoreactivity to the $\alpha_2\delta$ -1 subunit was mainly distributed in neurons with a small ($\leq 15 \mu$ m) or medium (> 15 and $\leq 25 \mu$ m) diameter; the peak distribution was in neurons that were 15–20 μ m in diameter (Fig. 4A, C). In contrast, H_1 receptors were distributed in medium and large ($> 25 \mu$ m) neurons; the highest levels were detected in neurons that were 20–30 μ m in diameter (Fig. 4B, C).

Analysis of TRPV1 (Fig. 5A–C) revealed that 85% of neurons that were positive for $\alpha_2\delta$ -1 immunoreactivity also expressed TRPV1 (Fig. 5D). Conversely, 64% of TRPV1-immunoreactive neurons were positive for $\alpha_2\delta$ -1 (Fig. 5D).

4. Discussion

The present study showed that systemic administration of gabapentin inhibited itch-related responses induced by mosquito allergy. Local injection of gabapentin at the same region as the mosquito allergen, but not at a different injection site, also attenuated the itch response. In addition to the systemic administration, local administration of gabapentin also inhibited peripheral nerve firing, suggesting that the anti-pruritic effect of gabapentin was mainly mediated by peripheral activity.

Gabapentin suppressed both itch and peripheral nerve firing induced by mosquito allergy, but not histamine. Similar results have been reported for an immunosuppressant, tacrolimus (Nakano et al., 2008c). Our preliminary experiments have shown that proteinase-activated receptor-2 (PAR₂) is involved in the allergic itch response to mosquito allergens (Akiyama, Andoh, and Kuraishi; unpublished observation). The proto-oncogene, c-Fos, is an immediate early gene and c-fos protein is rapidly and transiently expressed by dorsal horn neurons exposed to

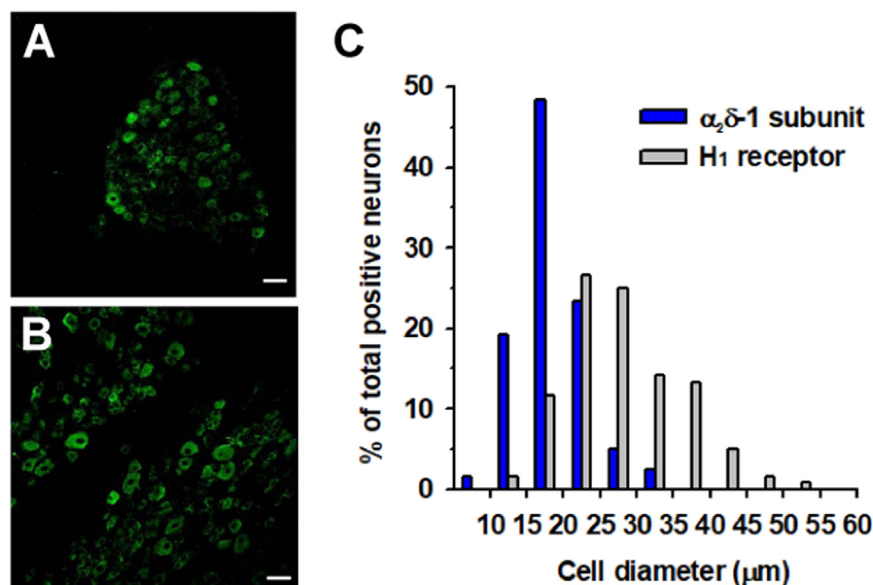


Fig. 4. Distribution of the expression of the calcium channel $\alpha_2\delta-1$ subunit and the histamine H_1 receptor in mouse dorsal root ganglion (DRG). Typical examples of immunoreactivity to the $\alpha_2\delta-1$ subunit (A) and H_1 receptor (B). The scale bar corresponds to 50 μm . (C) Size distribution of $\alpha_2\delta-1$ subunit- and H_1 receptor-immunoreactive neurons in the mouse DRG (n = 4).

various peripheral stimuli (Coggeshall, 2005). Intradermal injection of mosquito allergen, PAR_2 -activating peptide, or histamine induces c-fos expression in cells in the superficial dorsal horn (Nakano et al., 2008a). c-Fos-positive cells are mainly distributed in the region dorsal to the isolectin B₄ (IB₄)-labeled region after injections of mosquito allergen and PAR_2 -activating peptide (Nakano et al., 2008a). In contrast, they were within the IB₄-labeled region (inner aspect of lamina II) following histamine injection (Nakano et al., 2008a). These results suggest that the injection of mosquito allergen or histamine into the skin activates different primary afferents. Recently, gastrin-releasing peptide (GRP) was identified as a transmitter of the itch signal in the spinal dorsal horn (Sun and Chen, 2007). The GRP is distributed in the lamina I region, outside the IB₄-labeled region (Sun and Chen, 2007), and co-localizes with TRPV1 (Lagerström et al., 2010). Although compound 48/80 elicits itch-related responses through histamine (Sugimoto et al., 1998), PAR_2 -activating peptide and chloroquine induce antihistamine-resistant itch-related responses (Shimada et al., 2006; Zhu et al., 2009). GRP receptor mutant mice show itch-related responses induced by histamine, but not PAR_2 -activating peptide and chloroquine (Sun and Chen, 2007). Voltage-dependent calcium channels are

heteromultimeric complexes composed of several different subunits including α_1 , β , $\alpha_2\delta$, and γ (Catterall, 2000; Hofmann et al., 1999). The $\alpha_2\delta$ subunit has three main subtypes: $\alpha_2\delta-1$, -2 , and -3 (Ellis et al., 1988; Gao et al., 2000; Klugbauer et al., 1999). Although gabapentin binds to both the $\alpha_2\delta-1$ and -2 subunits (Fink et al., 2000; Gong et al., 2001), it has a greater binding affinity for the $\alpha_2\delta-1$ subunit (Gong et al., 2001), suggesting that it may mainly act through this subunit. The present study showed that the distributions of $\alpha_2\delta-1$ subunit and H_1 receptor immunoreactivity differed in the mouse DRG. These findings help to explain the differences between the antipruritic effects of gabapentin against mosquito allergy- and histamine-induced responses.

The $\alpha_2\delta-1$ subunit is expressed in DRG neurons (Cole et al., 2005), suggesting that it is expressed in primary afferents. The $\alpha_2\delta-1$ subunit immunoreactivity co-localized with that of TRPV1. A previous study showed that the depletion of TRPV1-expressing neurons after neonatal capsaicin treatment abolished itch-related responses induced by mosquito allergy, but not histamine (Nakano et al., 2008b). The present results also suggest that systemically administered gabapentin acted on the $\alpha_2\delta-1$ subunit in TRPV1-expressing primary afferents.

Gabapentin is also reported to activate the central descending

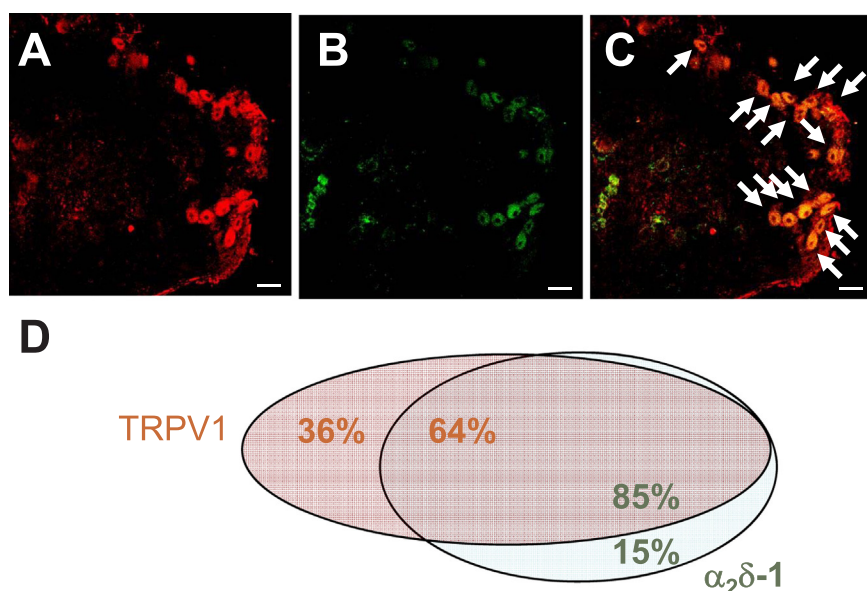


Fig. 5. Co-localization of calcium channel $\alpha_2\delta-1$ subunit and TRPV1 immunoreactivity in the mouse dorsal root ganglion (DRG). Typical examples of immunoreactivity to TRPV1 (A) and the $\alpha_2\delta-1$ subunit (B) are shown, and the merged images (C). The arrows indicate co-localization of immunoreactivity to TRPV1 and the $\alpha_2\delta-1$ subunit. The scale bar corresponds to 50 μm . (D) Sixty-four percent (78/122) of the TRPV1-positive-DRG neurons showed $\alpha_2\delta-1$ subunit immunoreactivity. Eighty-five percent (78/92) of the $\alpha_2\delta-1$ subunit-positive DRG neurons showed TRPV1 immunoreactivity.

noradrenergic system, regulating pain via effects on spinal α_2 -adrenoceptors (Tanabe et al., 2005). We have shown that intrathecal injection of the α_2 -adrenoceptor agonist, clonidine, suppresses pruritogen-induced itch-related responses in mice (Gotoh et al., 2010). In addition, intrathecal injection of the serotonin and noradrenaline reuptake inhibitor, milnacipran, also inhibits itch-related responses (Andoh et al., 2013). Furthermore, the depletion of noradrenaline, but not serotonin, in the spinal cord regulates mosquito allergy-induced itch-associated responses (Gotoh et al., 2011). Taken together with the above findings, this suggests that gabapentin may also have central effects on itch.

5. Conclusion

We conclude that gabapentin regulates the allergic itch induced by mosquito allergens by acting on the $\alpha_2\delta$ -1 subunit of voltage-dependent calcium channels in peripheral TRPV1-positive sensory neurons. Therefore, topical gabapentin may be useful for the treatment of allergic itch. In addition, since H_1 receptor antagonists can be ineffective for the amelioration of severe pruritic diseases such as atopic dermatitis, gabapentin may be useful for the treatment of these conditions.

Acknowledgments

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