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Gabapentin and Pregabalin Inhibit the Itch-Associated Response Induced by the Repeated Application of Oxazolone in Mice

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Abstract. We investigated the effects of gabapentin and pregabalin on the itch-associated response in a mouse model of chronic dermatitis induced by the repeated application of 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone). Challenging the mice with oxazolone-induced chronic dermatitis with the oxazolone evoked severe and transient scratching behavior until 1 h after the application of oxazolone. Thereafter, a more mild and continuous scratching behavior was also observed for at least 8 h. Both severe and continuous scratching behaviors were suppressed by systemic injection of gabapentin and pregabalin. This effect of these compounds was correlated with its affinity for the $\alpha_2\delta$ subunit of voltage-gated Ca^{2+} channels. Intrathecal injection, but not peripheral treatment, with gabapentin inhibited the scratching behavior in this model. Gabapentin failed to suppress the scratching behavior induced by the intradermal injection of compound 48/80 in normal mice. The expression of the $\alpha_2\delta$-1 subunit in dorsal root ganglion (DRG) from mice following repeated application of oxazolone was significantly higher than that from normal mice. These results suggest that gabapentin and pregabalin show an anti-pruritic activity through $\alpha_2\delta$-subunit binding, and the up-regulation of the $\alpha_2\delta$-1 subunit in DRG may therefore play an important role in its anti-pruritic activity.

Keywords: gabapentin, pregabalin, pruritus, $\alpha_2\delta$-1 subunit, oxazolone

Introduction

Pruritus is defined as a sensation that provokes the urge to scratch, and it is one of the most common clinical symptoms associated with dermatitis and several other diseases (1, 2). In patients with atopic dermatitis, itch-associated scratching damages the skin and increases the inflammation, which in turn further increases the itching (3, 4). Therefore, the most effective strategy for preventing this aggravation of the skin lesion and improving the quality of life in patients with atopic dermatitis is a reduction of itching and scratching (5). Although H_1 histamine–receptor antagonists are often the drugs of choice for the treatment of itching, pruritus due to atopic dermatitis responds poorly to these agents (6 – 9). Therefore, it is necessary to develop effective medications against the itching in patients with atopic dermatitis and other itch-inducing diseases.

It has been reported that repeated applications of a hapten, such as oxazolone, onto the skin of mice induce chronic dermatitis that resembles that of patients with atopic dermatitis (10, 11). We recently reported that mice that received repeated applications of oxazolone showed an antigen-induced severe scratching behavior, followed by chronic dermatitis-induced continuous scratching behavior (12). We have also shown that these mice respond similarly to human patients in terms of the therapeutic activity of anti-itch drugs. This suggests that the mechanism(s) responsible for producing the scratching in mice after repeated application with oxazolone resembles that of the itching in patients with atopic dermatitis (12).

Gabapentin and pregabalin have shown efficacy in the treatment of some forms of neuropathic pain and postsurgical pain in humans (13 – 15). These drugs also show anti-nociceptive action in animal models of neuropathic pain or postoperative pain (16 – 18). Although gabapentin and pregabalin are structurally related to the inhibitory

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neurotransmitter γ-aminobutyric acid (GABA), radioligand binding studies have shown that these compounds have negligible affinity for the GABA receptor and many other receptors known to mediate pain (19). In addition, neither compound alters the brain concentrations of GABA in rats (20). Gabapentin and pregabalin are reported to bind with a high affinity to the $\alpha_2\delta$ auxiliary subunit of voltage-gated Ca^{2+} channels (VGCCs) (21). The anti-nociceptive activity of pregabalin and several structurally analogs is directly related to their affinity for the $\alpha_2\delta$ subunit (17, 22), thus suggesting that the anti-nociceptive action of gabapentin and pregabalin is mediated through binding to the $\alpha_2\delta$ subunit of the channels.

Gabapentin has been reported to alleviate itching in patients with several diseases who respond poorly to H_1 histamine–receptor antagonists, thus suggesting that gabapentin and pregabalin have anti-pruritic activities (23 – 25). Since the mechanisms of itch transmission have many, if not all, similarities to those of pain transmission (26), it is reasonable that gabapentin is effective in the treatment of pruritus. However, there have not been any reports about the anti-pruritic action of gabapentin in animal models, and the anti-pruritic mechanisms and site of action of gabapentin are still unknown. In this study, we investigated whether gabapentin and pregabalin show an anti-pruritic action in mice following the repeated application of oxazolone, and thereby determined the anti-pruritic mechanisms and site of action of gabapentin.

Materials and Methods

Animals

Male 5-week-old BALB/c mice and male 6-week-old Sprague-Dawley rats (Charles River Japan, Kanagawa) were kept in a specific pathogen-free animal facility that was maintained at a temperature of 19ºC – 25ºC, humidity of 30% – 70%, and a 12-h light/dark cycle; and they were given access to food and water ad libitum. The experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, and the experimental protocol used in this study was approved by the Committee for Animal Experiments at Kyowa Hakko Kirin Co., Ltd. (Shizuoka).

Drugs and materials

4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) and compound 48/80 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gabapentin, pregabalin, and *R*-isobutylgaba were synthesized at Fuji Research Park of Kyowa Hakko Kirin Co., Ltd., and they are commercially available products. Oxazolone was dissolved in acetone as a 0.5% (w/v) solution and used for sensitization and challenge. Compound 48/80 was dissolved in physiological saline at a concentration of 0.6 mg/mL. The monoclonal anti-dihydropyridine receptor ($\alpha_2\delta$-1 subunit) antibody was purchased from Sigma-Aldrich.

[^{3}H]Gabapentin binding assay

Membrane fractions from rat cerebral cortex were prepared according to the method described previously (27). Membranes (12 μg protein) were incubated with 20 nM [^{3}H]gabapentin in 10 mM HEPES buffer (pH 7.4, 0.1 w/v% BSA) in the presence of varying concentrations of the test compound for 1 h at room temperature (RT). The binding assay was terminated by rapid vacuum filtration through glass fiber filter plates presoaked with 0.3% polyethylenimine using a 96-well filtration apparatus. Filters were rapidly rinsed with ice-cold 100 mM NaCl (0.1 w/v% BSA) and the radioactivity bound to the filters was assessed. Specific radioligand binding was defined by subtracting the non-specific binding, as defined by the inclusion of 100 μmol/L gabapentin, from total binding.

Sensitization and challenge

The mice were sensitized by a single epicutaneous application of 10 μL/site of 0.5% oxazolone solution to the shaved rostral back. Seven days later (day 0), 10 μL/site of 0.5% oxazolone solution was applied to the same area that had previously been sensitized to oxazolone at 2- or 3-day intervals (days 0, 2, 4, 7, 9, 11, 14, and 16). The animals were videotaped for 8 h immediately after the day 16 oxazolone challenge.

Drug administration

Gabapentin was dissolved in 0.5w/v% methylcellulose for oral administration, in physiological saline for intrathecal injection, or in 50w/v% ethanol for rostral back application. Pregabalin and *R*-isobutylgaba were dissolved in 0.5w/v% methylcellulose. The intrathecal injections were given at a volume of 5 μL, and the rostral back application was given at a volume of 50 μL. Both the oral administration and intraperitoneal injections were administered at a volume of 10 mL/kg.

Observation of scratching

The scratching behavior was observed according to the method described previously (28). Briefly, mice were individually placed in sections of the observation chamber (7.5 × 8 × 15 cm) to acclimate for about 1 h. After the challenge with oxazolone or intradermal injection of compound 48/80 (50 μL), the mice were quickly returned to the observation chamber, and the mouse behaviors were videotaped automatically in an unattended environment. Video playback made it possible to count the

scratching behaviors toward either the oxazolone-treated or compound 48/80–injected site. The mice generally scratched several times with the hind paws for about 1 s, and a series of these movements was counted as one bout of scratching.

Immunoblotting

The DRG at the C4-6 levels were removed and extracted with lysis buffer (50 mM Tris-HCl buffer, pH 7.5 containing 0.5w/v% Triton X-100, 150 mM NaCl, 1 mM EDTA) containing protease inhibitors. Equal amounts of total protein (20 μg) from each sample were loaded onto a 4% – 12% NuPage Tris/acetate gel (Invitrogen Co., Carlsbad, CA, USA), and proteins were separated by SDS-PAGE. Proteins were transferred to a poly(vinylidene difluoride) membrane (Invitrogen) and, after blocking (1 mM Tris-HCl buffer, pH 7.4 containing 0.05 w/v% Triton X-100, 150 mM NaCl, 2 w/v% skim milk), were probed with the monoclonal anti-dihydropyridine receptor ($\alpha_2\delta$-1 subunit) antibody (1:200) overnight at 4°C. The protein–antibody complexes were then labeled with a horseradish peroxidase–conjugated secondary antibody (1:2000; GE Healthcare UK, Ltd., Buckinghamshire, UK) for 1 h at RT and detected using the Super Signal West Pico Chemiluminescence Substrate (Thermo Scientific, Rockford, IL, USA). The immunoblot bands were visualized and quantified with Lumino Image Analyzer LAS-4000 (Fuji Photo Film Co., Tokyo).

Immunohistochemistry

The mice were deeply anesthetized with an intraperitoneal injection of pentobarbital (80 mg/kg) and perfused transcardially with 0.01 mM phosphate–buffered saline (PBS) followed by perfusion with 4% paraformaldehyde. The DRG at the C5 level was removed and then postfixed in 4% paraformaldehyde overnight at 4°C. Samples were transferred to a 15% sucrose solution overnight at 4°C and then were sectioned to a thickness of 10 μm using a cryostat. The sections thereafter underwent heat-induced antigen retrieval (10 mM citrate buffer, pH 6.0, 0.05 w/v% Tween 20, 95°C for 10 min) before blocking with 10% normal goat serum in the presence of 0.1 w/v% Triton X-100 in PBS for 1 h. To detect $\alpha_2\delta$-1, sections were incubated with the monoclonal anti-dihydropyridine receptor ($\alpha_2\delta$-1 subunit) antibody (1:100) in 50% blocking buffer for 40 h at 4°C. After extensive washing with PBS containing 0.1% Triton X-100, sections were incubated with biotinylated secondary antibody (1:500; Molecular Probes, Eugene, OR, USA) for 2 h at RT and then incubated with streptavidin-Alexa Fluor 488 (1:500, Molecular Probes) for 1 h. After extensive washing, the samples were mounted, and immunofluorescent preparations were examined with a fluorescence microscope (BIOZERO BZ-8000; Keyence Corp., Osaka).

Statistical analyses

The data were presented as the means and S.E.M. Student's *t*-test was used for the analysis of any differences between the two groups. Multiple comparisons between treatment groups were assessed by Dunnett's test. Values of $P < 0.05$ were considered to be statistically significant. All statistical calculations were performed using the Statistical Analysis System (SAS Institute, Cary, NC, USA) software package.

Results

When the oxazolone-sensitized mice were challenged with acetone on day 0, no marked scratching behavior was observed (Fig. 1A). The skin site given repeated challenge with oxazolone showed chronic dermatitis with erythema, erosion, and lichenification (data not

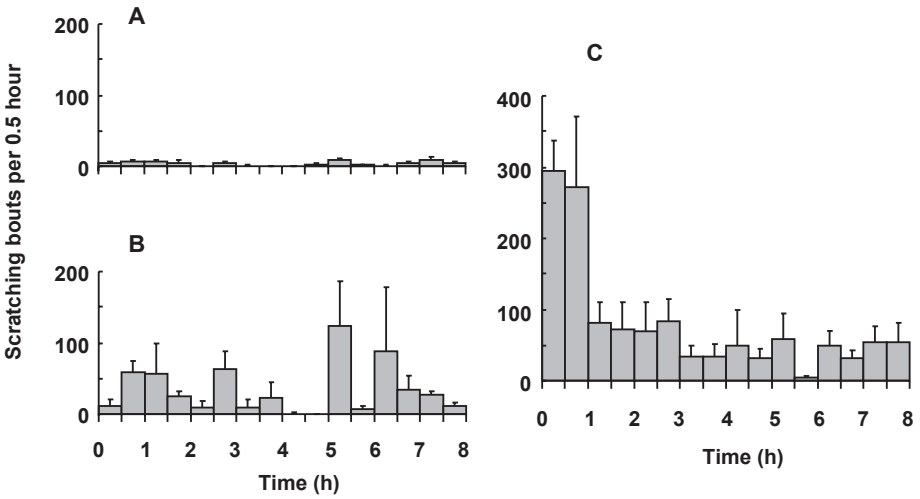


Fig. 1. Scratching response in mice repeatedly exposed to oxazolone. The mice were sensitized on their rostral back with oxazolone for 7 days before the hapten challenge, which was repeated three times per week for 16 days. The time-course of scratching was measured after application of acetone (A) on day 0 and acetone (B) or oxazolone (C) on day 16. Each column and vertical bar represents the mean + S.E.M. of values obtained from 3 – 5 animals.

shown). Mice that received oxazolone until day 14 and acetone only on day 16 showed mild and continuous scratching behavior (Fig. 1B). The final challenge with oxazolone on day 16 to mice with chronic dermatitis induced severe scratching behavior just after the oxazolone application, which decreased rapidly for the first hour after the application (Fig. 1C). Thereafter, a mild scratching behavior was continued for 8 h, similar to the level observed 1 h after the oxazolone application (Fig. 1C). In the subsequent study, we investigated the effects of gabapentin and pregabalin on the severe scratching behavior (0 – 1 h) and mild and continuous scratching between 4 – 6 h after oxazolone application.

Affinity of gabapentin, pregabalin, and R-isobutylgaba for the $\alpha_2\delta$ subunit of VGCC

Table 1 shows the IC_{50} values of gabapentin, pregabalin, and *R*-isobutylgaba for the $\alpha_2\delta$ subunit of VGCCs obtained by using [^{3}H]gabapentin binding to the membrane protein of the rat cerebral cortex, which contains high levels of the $\alpha_2\delta$ subunit (22). Gabapentin, pregabalin (*S*-isobutylgaba), and *R*-isobutylgaba showed dose-dependent inhibition of [^{3}H]gabapentin binding to the $\alpha_2\delta$ subunit. The IC_{50} values of gabapentin, pregabalin, and *R*-siobutylgaba were 82, 57, and 640 nM, respectively.

Effects of systemic gabapentin and pregabalin on scratching induced by repeated challenge with oxazolone

The oral administration of gabapentin (30 – 300 mg/kg) produced a significant ($P < 0.05$) dose-dependent inhibition of both the severe (Fig. 2A) and continuous (Fig. 2B) scratching behavior. Similar to gabapentin, oral administration of pregabalin (10 – 100 mg/kg) produced a significant ($P < 0.05$) dose-dependent inhibition of severe (Fig. 3A) and continuous (Fig. 3B) scratching behavior.

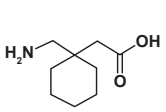
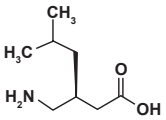
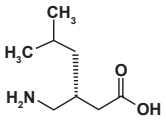
Stereospecificity of the anti-scratching action of pregabalin

The intraperitoneal injection of pregabalin (*S*-isobutylgaba) at a dose of 30 mg/kg produced a significant inhibition of severe scratching behavior ($P < 0.01$). On the other hand, intraperitoneal injection of *R*-isobutylgaba showed no significant inhibition up to 30 mg/kg (Fig. 4).

Site of anti-scratching action of gabapentin

To clarify the site of the anti-scratching action of gabapentin, we investigated the effects of intrathecal in-

Table 1. Chemical structures and IC_{50} values of gabapentin, pregabalin (*S*-isobutylgaba) and *R*-isobutylgaba

	Gabapentin	Pregabalin (*S*-Isobutylgaba)	*R*-Isobutylgaba
Structure			
IC_{50} (nmol/L)	82	57	640

The IC_{50} value for the $\alpha_2\delta$ subunit was estimated by [^{3}H]gabapentin binding to the membrane protein of rat cerebral cortex.

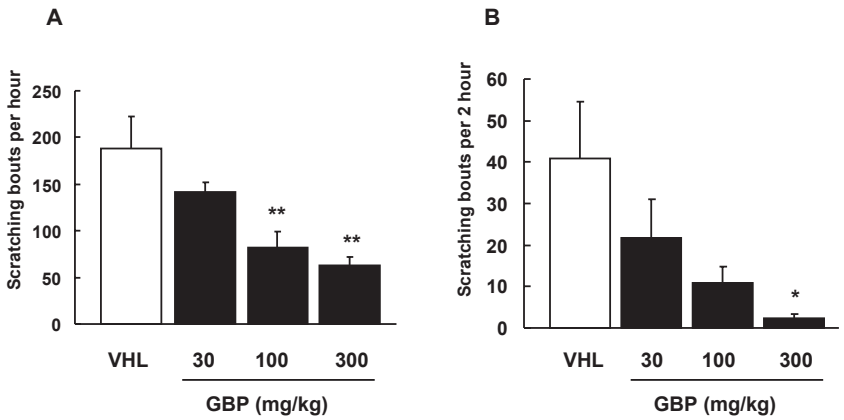


Fig. 2. Effect of gabapentin on mouse scratching behavior induced by repeated application of oxazolone. The vehicle (VHL) or gabapentin (GBP, 30 – 300 mg/kg) was orally administered 60 min before (A) or 3 h after (B) the last oxazolone application on day 16. Scratching was counted for 60 min (A) or for 2 h between 4 to 6 h after the oxazolone challenge (B). Each column represents the mean + S.E.M. of values obtained from 6 animals. *$P < 0.05$, **$P < 0.01$, compared with the VHL group (Dunnett's test).

jection or rostral back application of gabapentin on severe scratching behavior induced by oxazolone application. Intrathecal injection of gabapentin (10 – 100 μg/site) produced a significant ($P < 0.01$) (Fig. 5A), dose-dependent inhibition of the scratching behavior. On the other hand, the epicutaneous application of gabapentin at up to 600 μg/site did not inhibit the scratching behavior (Fig. 5B).

Effect of gabapentin on scratching behavior induced by compound 48/80

We next evaluated the influence of gabapentin on compound 48/80–induced scratching behavior in normal mice. Intradermal injection of compound 48/80 (30 μg/site) elicited scratching behavior, which was not inhibited by pretreatment with up to 300 mg/kg gabapentin (Fig. 6).

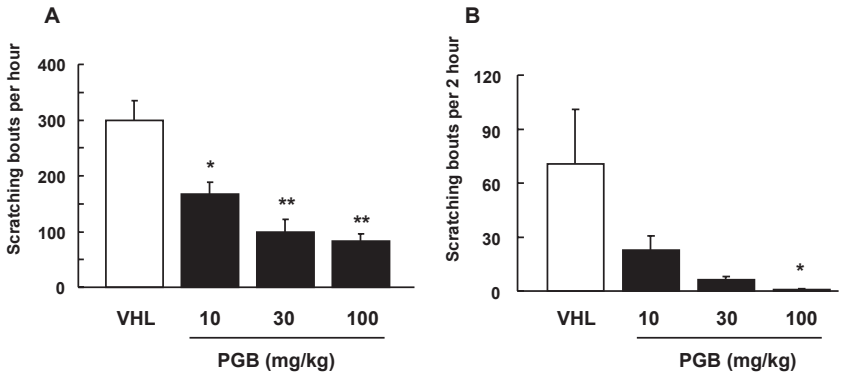


Fig. 3. Effect of pregabalin on mouse scratching behavior induced by repeated application of oxazolone. The vehicle (VHL) or pregabalin (PGB, 10 – 100 mg/kg) was orally administered 60 min before (A) or 3 h after (B) the last oxazolone application on day 16. Scratching was counted for 60 min (A) or for 2 h between 4 to 6 h after the oxazolone challenge (B). Each column represents the mean + S.E.M. of values obtained from 6 animals. $*P < 0.05$, $**P < 0.01$ compared with the VHL group (Dunnett's test).

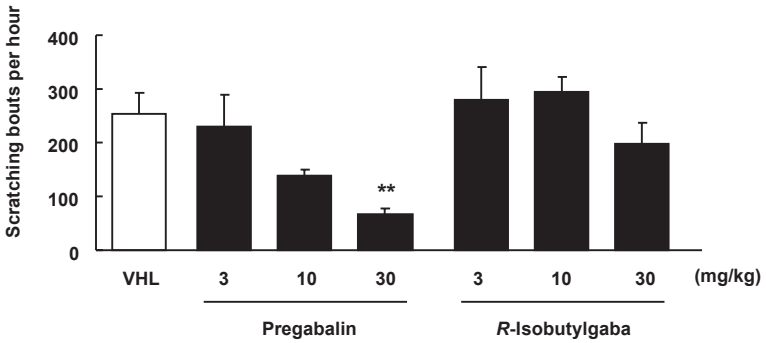


Fig. 4. Effects of pregabalin and *R*-isobutylgaba on mouse scratching behavior induced by repeated application of oxazolone. The vehicle (VHL), pregabalin (3 – 30 mg/kg), or *R*-isobutylgaba (3 – 30 mg/kg) was intraperitoneally injected 30 min before the last oxazolone application on day 16. Scratching was counted for 60 min after the oxazolone challenge. Each column represents the mean + S.E.M. of values obtained from 6 or 12 animals. $**P < 0.01$, compared with the VHL group (Dunnett's test).

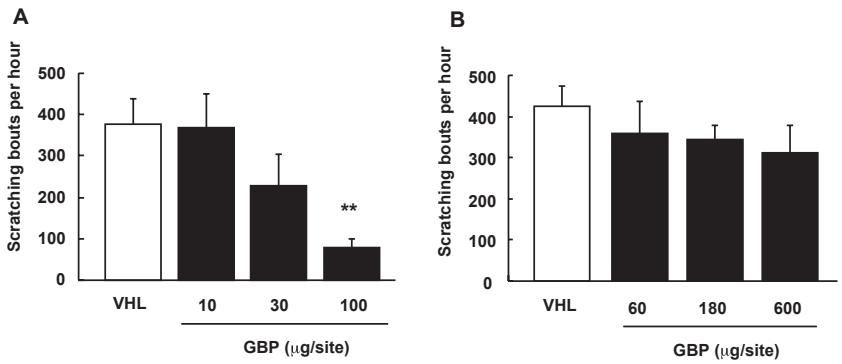


Fig. 5. Effects of intrathecal injection and epicutaneous application of gabapentin on mouse scratching behavior induced by repeated application of oxazolone. The vehicle (VHL, saline, 5 μL) or gabapentin (GBP, 10 – 100 μg/5 μL) was intrathecally injected 60 min before the last oxazolone application on day 16 (A). The vehicle (VHL, ethanol-water, 50 μL) or gabapentin (GBP, 60, 180, 600 μg/50 μL) was epicutaneously applied 60 min before the last oxazolone application on day 16 (B). Scratching was counted 60 min after the oxazolone challenge. Each column represents the mean + S.E.M. of values obtained from 6 animals. $**P < 0.01$, compared with the VHL group (Dunnett's test).

Expression of the $\alpha_2\delta$-1 subunit in DRG

We examined the expression of the $\alpha_2\delta$-1 subunit in cervical DRG in normal mice and mice exposed to repeated application of oxazolone by immunoblot analyses (Fig. 7: A, B). The protein level of the $\alpha_2\delta$-1 subunit in the C4-6 DRG from mice following repeated application of oxazolone was significantly higher than that in normal mice ($P < 0.05$). We further characterized the localization of the $\alpha_2\delta$-1 subunit in the C5 DRG by immunofluorecence. In normal mice, only weak $\alpha_2\delta$-1 immunoreactivity was found in the small to medium, but not in the large DRG neurons (Fig. 7C). Consistent with the immunoblot data, the intensity of $\alpha_2\delta$-1 immunoreactivity was increased in the small to medium DRG neurons from mice following the repeated application of oxazolone (Fig. 7D). There was also no $\alpha_2\delta$-1 immunoreactivity in the large DRG neurons from mice exposed to repeated application of oxazolone (Fig. 7D). In contrast to the cervical site, the level of $\alpha_2\delta$-1 subunit in the DRG at the lumbar site in mice with repeated application of oxazolone was similar to that of normal mice (data not shown).

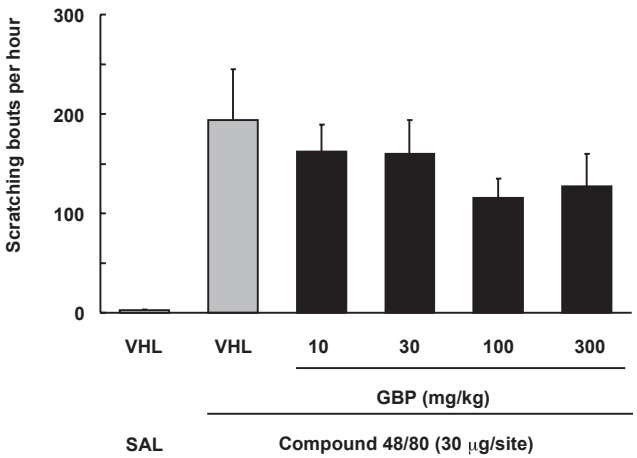


Fig. 6. Effect of gabapentin on compound 48/80–induced scratching behavior. Compound 48/80 (30 μg/50 μL) or saline (SAL, 50 μL) was injected into the rostral back of mice. The scratching at the compound 48/80–injection site by hind limbs was counted for 60 min following compound 48/80 injection. The vehicle (VHL) or gabapentin (GBP, 10 – 300 mg/kg) was orally administered 60 min before the compound 48/80 injection. Each column represents the mean + S.E.M. of values obtained from 8 animals.

Discussion

The results of the present study show that gabapentin and pregabalin suppress scratching behavior induced by repeated application of oxazolone in mice. In our previous study, we demonstrated that the scratching behavior in this model was suppressed by an opioid receptor antagonist, suggesting that this behavior is an itch-associated response (12). Gabapentin and pregabalin have previ-

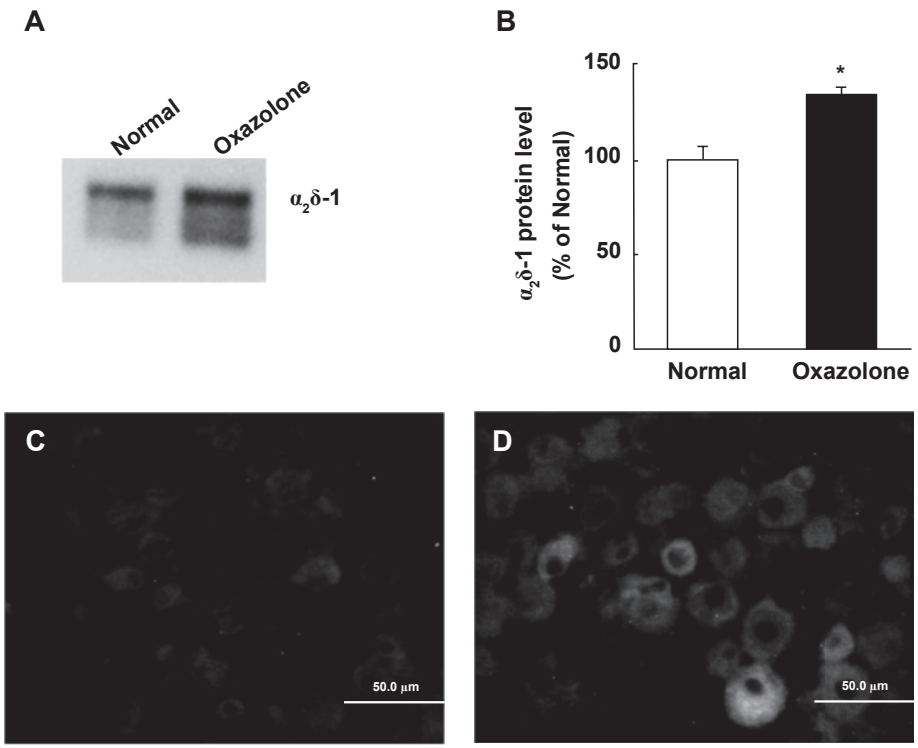


Fig. 7. Expression of the $\alpha_2\delta$-1 subunit in dorsal root ganglion (DRG). Mice were sensitized on the rostral back with oxazolone for 7 days before the challenge, which was repeated three times per week for 14 days, and the C4-6 DRGs were prepared on day 16. A: Representative Western blot data showing $\alpha_2\delta$-1 subunit expression in C4-6 DRG from normal mice or mice with repeated application of oxazolone. B: The percentage of the respective value of the normal mice. Each column and vertical bar represents the mean + S.E.M. of values obtained from 3 animals. *$P < 0.05$, compared with the normal group (Student's *t*-test). C, D: Immunofluorescent micrographs of the $\alpha_2\delta$-1 subunit in the C5 DRG from normal mice (C) and mice following repeated application of oxazolone (D). Scale bar = 50 μm.

ously been reported to have an anti-nociceptive action in neuropathic pain models (16 – 18). The anti-nociceptive action of gabapentin and pregabalin has been reported to be mediated by binding to the $\alpha_2\delta$ subunit of VGCCs (17, 22). In the present study, the dose range of the anti-pruritic action of gabapentin and pregabalin corresponded to its anti-nociceptive action (19, 29). The affinity for the $\alpha_2\delta$ subunit of pregabalin was stronger than that of gabapentin and *R*-isobutylgaba. These results were in agreement with the previous studies (27, 30) and correlated with its anti-pruritic action. With these findings taken into account, the present results suggest that the anti-pruritic activities of pregabalin and gabapentin are mediated through binding to the $\alpha_2\delta$ subunit of VGCCs.

The intrathecal injection, but not peripheral application, of gabapentin was effective against the itch-associated response in mice with oxazolone-induced chronic dermatitis. The dose range of the intrathecal gabapentin-induced anti-pruritic effect corresponded to that of the anti-nociceptive effect in pain models (29, 31, 32). Moreover, high levels of the $\alpha_2\delta$ protein and specific binding of [^{3}H]gabapentin were detected in the superficial layers of the dorsal horn (33 – 35), suggesting that the spinal cord is the primary site of the anti-pruritic action of $\alpha_2\delta$ ligands.

The subsequent cloning and expression of the isoforms of $\alpha_2\delta$ subunit have revealed that gabapentin and pregabalin bind with high affinity to both the $\alpha_2\delta$-1 and $\alpha_2\delta$-2 subtypes, but they demonstrated negligible affinity for the $\alpha_2\delta$-3 or $\alpha_2\delta$-4 subtypes (36 – 40). Transgenic mice expressing the mutant $\alpha_2\delta$-1 subunit (R217A mutant mice) have much lower gabapentin and pregabalin binding levels (41) and they completely lack the anti-nociceptive action of these drugs (42), indicating that binding to the $\alpha_2\delta$-1 subunit is necessary for the anti-nociceptive action of gabapentin and pregabalin. The binding affinity for gabapentin has been estimated to be 2.5-fold higher for the $\alpha_2\delta$-1 subunit than the $\alpha_2\delta$-2 subunit (40), thus raising the possibility that the anti-pruritic action of gabapentin and pregabalin is due to the binding to the $\alpha_2\delta$-1 subunit.

In contrast to the oxazolone-induced chronic dermatitis model, a dose of 300 mg/kg of gabapentin did not affect the acute itch-associated response induced by intradermal injection of compound 48/80 to normal mice. These results suggest that the anti-scratching action of gabapentin is not due to its sedative action. Similar findings were also reported regarding the anti-nociceptive action of gabapentin. Gabapentin has been shown to have an anti-nociceptive effect in rats with neuropathic pain, but not in transient pain in normal rats (18). The up-regulation of $\alpha_2\delta$-1 in the DRG has also been reported to be important for the anti-nociceptive action of $\alpha_2\delta$ ligands (43). In the present study, the expression of the $\alpha_2\delta$-1 subunit in the DRG from mice exposed to repeated application of oxazolone was significantly higher than that in normal mice. It is therefore possible that the up-regulation of the $\alpha_2\delta$-1 subunit in the DRG is important for the anti-pruritic activity of gabapentin.

Although the regulatory mechanisms of $\alpha_2\delta$-1 subunit expression are still controversial, the up-regulation of $\alpha_2\delta$-1 subunit was found in DRG at the cervical site, but not the lumbar site. This result suggests that local pathological changes, such as dermatitis, may regulate $\alpha_2\delta$-1 subunit expression in the DRG. Further studies will be necessary to clarify the regulatory mechanism of the $\alpha_2\delta$-1 subunit expression in DRG.

The $\alpha_2\delta$ subunit is considered to mediate some of the functions of VGCC, such as voltage dependence and/or kinetics (44). Gabapentin has been reported to inhibit Ca^{2+} currents in DRGs of mice overexpressing the $\alpha_2\delta$-1 subunit (45) and capsaicin-induced neurotransmitter release in spinal cord slices from an inflammatory pain model (46). Moreover, gabapentin has also been reported to inhibit spinal excitatory amino acid release induced by intraplanter injection of formalin in a neuropathic pain model (47). Therefore, the anti-pruritic activity of gabapentin and pregabalin may be due to their inhibition of neurotransmitter release in the spinal cord as a result of their binding to the $\alpha_2\delta$-1 subunit up-regulated in the presynaptic terminal of primary afferent nerves.

The expression of gastrin-releasing peptide (GRP) was found in small- and medium-sized DRG, and GRP-positive primary afferent nerves have been reported to be important for itch transduction (48). Interestingly, our study demonstrated that up-regulation of the $\alpha_2\delta$-1 subunit was also found in small- and medium-sized DRG. Further studies will be necessary to clarify the functional interactions between $\alpha_2\delta$ ligands and GRP-positive primary afferent nerves.

In conclusion, gabapentin and pregabalin show an anti-pruritic activity through binding to the $\alpha_2\delta$ subunit of VGCC. The anti-pruritic action of the $\alpha_2\delta$ ligands is mainly mediated by actions on the spinal cord, and it may be involved in the up-regulation of the $\alpha_2\delta$-1 subunit in primary afferent nerves. Gabapentin and pregabalin may therefore represent an effective treatment for patients suffering from chronic itching, such as that which develops due to atopic dermatitis.

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