

Plastic Change of N-type Ca Channel Expression after Preconditioning Is Responsible for Prostaglandin E₂-induced Long-lasting Allodynia

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Background: Although considerable evidence indicates neuronal Ca channels play significant roles in pain perception, their possible importance in hypersensitization after acute inflammation has not been investigated.

Methods: Using carrageenan for inducing hypersensitization, the authors investigated the analgesic effects of intrathecally administered N- and P/Q-type channel blockers, ω -conotoxin GVIA and ω -agatoxin IVA, respectively, and also examined the level of N-type channel expression.

Results: Acute inflammation, produced by carrageenan injection in a rat hind paw, caused mechanical hypersensitivity that resolved within several days. Injection of prostaglandin E₂ into the same hind paw after resolution caused a markedly prolonged mechanical allodynia lasting more than 4 h. Similar but less potent prolonged allodynia was also induced in the contralateral hind paws. Intrathecal administration of ω -conotoxin GVIA (0.03–0.3 μ g) produced dose-dependent inhibition of the allodynia in both control and carrageenan-preconditioned rats. However, the potency of ω -conotoxin GVIA was significantly lower in carrageenan-preconditioned paws than in those in the contralateral and saline-preconditioned paws. In contrast, ω -agatoxin IVA (0.01–0.1 μ g) did not reduce the allodynia. Significant up-regulation of N-type channel expression was observed in both dorsal root ganglia and the spinal cord ipsilateral to the carrageenan-preconditioned hind paw.

Conclusions: The results suggest an aggravating role of the N-type channel in pain sensation and a selective plastic change of this channel expression that could underlie the mechanism of hypersensitization after acute inflammation.

SENSITIZATION of the central and peripheral nervous system is thought to be responsible for abnormal sensation, such as allodynia and hyperalgesia. Although considerable efforts have been made in elucidating the physiologic bases of pathologic pain, the cellular changes underlying the sensitization after inflammation of peripheral tissues remains unclear.^{1,2} It has been suggested that the plastic change of nociceptive neurotransmission and/or modulatory systems during the acute inflammatory pain state may be important mechanisms in the development of chronic inflammatory pain, because these pain states follow an episode of acute inflammation.³ In the experimental recurrent inflammation mod-

els that may represent human painful joint disorders, it is suggested that the response of the second inflammation is enhanced by the sensitization of the nervous system after resolution of the first inflammation.^{4,5} Therefore, it would be clinically beneficial to investigate the mechanisms of sensitization associated with acute inflammation.

Several types of voltage-dependent Ca channels are known to be expressed in nociceptive neurons.⁶ Especially, N- and P/Q-type channels are highly expressed in primary sensory neurons^{7,8} and the spinal cord.⁹ These channels have been demonstrated to be responsible for the release of nociceptive neurotransmitters, such as glutamate, calcitonin gene-related peptide, and substance P, from primary sensory neurons.^{10,11} Behavioral and *in vivo* electrophysiologic experiments have revealed that N- and P/Q-type channel blockers have analgesic effects on several kinds of pain.⁶ In the models of acute inflammatory pain, for instance, spinal administration of ω -conotoxin GVIA (GVIA), an N-type channel blocker, reduced the spinal neuronal responses to innocuous or noxious pressure applied to either a knee that was inflamed by kaolin and carrageenan (primary hyperalgesia and allodynia) or a noninflamed ankle ipsilateral to the inflamed knee (secondary hyperalgesia and allodynia) in anesthetized rats.¹² Spinal administration of a P/Q-type channel blocker, ω -agatoxin IVA (AgaIVA), also depressed the primary hyperalgesia and allodynia without effects on the secondary hyperalgesia and allodynia.¹³ Thus, it is expected that voltage-dependent Ca channel blockers may also have some inhibitory effects on the hypersensitization.

In this study, we tested the hypothesis that voltage-dependent Ca channels contribute to the hypersensitization after acute inflammation. First, we assessed the presence of long-lasting allodynia as an increased sensitivity to inflammatory mediators. Next, we investigated the effect of intrathecal administration of N- and P/Q-type channel blockers on this allodynia. Finally, we evaluated the possible plastic change of N-type channel expression in dorsal root ganglia (DRGs) and the spinal cord of rats in this sensitized state. In addition, we used the heterozygous alleles of N-type channel knockout (Ca_v2.2 +/–) mice¹⁴ to investigate the change of spinal expression of the N-type channel to supplement the experiment in rats.

Materials and Methods

All experiments were conducted under the ethical guidelines for the study of experimental pain in con-

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scious animals,¹⁵ and the protocol of the pain behavioral studies described here was approved by the Animal Care Committee of Tokyo Medical and Dental University.

Animals

We used male Sprague-Dawley rats weighing 250–300 g (Japan Clea, Sizuoka, Japan) for behavioral and immunoblot analysis and heterozygous alleles of N-type channel knock-out ($Ca_v2.2 +/−$) mice¹⁴ weighing 28–35 g for 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-Gal) staining. The animals were housed in a temperature- and humidity-controlled environment with a 12-h light-dark cycle and free access to food and water.

Experimental Model

The current study used a recently introduced experimental system with some modification. In the original study, long-lasting hypersensitivity to proinflammatory mediators (100 ng/5 μ l prostaglandin E_2 [PGE_2]) after the resolution of carrageenan (1%; 5 μ l)-induced acute inflammation was evaluated by the Randall-Selitto test for the nociceptive flexion reflex.¹⁶ In our preliminary study, however, it was difficult to detect reproducible mechanical hypersensitivity under a similar condition. Therefore, we used greater amounts of carrageenan (2%; 100 μ l) and PGE_2 (400 ng/20 μ l) and evaluated a long-lasting mechanical hypersensitivity using von Frey hairs (Stoelting Company, Wood Dale, IL). Carrageenan is a classic agent causing experimental inflammation, and the induced inflammatory pain is considered relevant to clinically important inflammatory pain states.^{17,18} PGE_2 is one of the best-characterized proinflammatory mediators known to be present in increased concentration at sites of inflammation and is shown to induce hyperalgesia and allodynia by a direct action on primary afferent nociceptors.^{1,2,19}

Behavioral Analyses

All behavioral experiments were performed in a sound-proof room during the light cycle (7:00 AM to 7:00 PM). Rats were acclimatized to the experimental room for at least 1 h before the experiments.

Tactile allodynia was evaluated with calibrated von Frey hairs by measuring the tactile stimulus producing a 50% likelihood of a withdrawal response (50% gram threshold), which was calculated by using the up-down paradigm of Dixon as described in detail before.²⁰ A stimulus strength of 15.1 g was adopted as the upper limit of testing.

Pharmacology and Drug Administration

All subcutaneous and intrathecal injections were performed using a 30-gauge needle during a few minutes of anesthetization with halothane, 2% (Takeda Chemical Industries, Ltd., Osaka, Japan). In this study, rats and the $Ca_v2.2 +/−$ mice received a solution, 2%, of λ -carrageenan

(Sigma Chemical Company, St. Louis, MO) in saline in the plantar surface of the hind paw (100 μ l for rats and 20 μ l for mice). The injection side was selected randomly. Control experiments were conducted by injection of the same volume of physiologic saline solution. Seven days after carrageenan or saline injection, rats received either GVIA (0.03, 0.1, or 0.3 μ g) or AgaIVA (0.01, 0.03, or 0.1 μ g) intrathecally in a total volume of 10 μ l 30 min before PGE_2 injection (400 ng/20 μ l) into the pretreated plantar surface of the hind paw. Each animal received no more than a single dose of the Ca channel blockers. Saline (10 μ l) was administered intrathecally in the control experiments. Although the effects of GVIA were known to be relatively slow in onset,²¹ this interval was sufficient to establish maximal inhibitory effects of GVIA in our preliminary study.

AgaIVA and GVIA (Peptide Institute, Inc., Osaka, Japan) and PGE_2 (Cayman Chemical Company, Ann Arbor, MI) were made up as concentrated stock solutions in MilliQ water (Nihon Millipore, Tokyo, Japan) or 10% ethanol in saline for PGE_2 , divided into aliquots, and stored at -20°C . Aliquots were thawed and diluted to the desired concentrations in saline immediately before use. The doses of these drugs were determined according to previous studies^{22–25} and our preliminary study.

Immunoblot Analysis

Rats were deeply anesthetized with intraperitoneal sodium pentobarbital (45 mg/kg) at 7 days after carrageenan or saline injections, and L4–L6 DRGs (ipsilateral to the injections) or the spinal cord was dissected. After dissection, rats were killed by further injection of excess doses of sodium pentobarbital (100 mg/kg). Pooled DRGs from at least eight rats were homogenized in 700 μ l lysis buffer, pH 8.0, containing 150 mM NaCl, 1 mM EDTA, and protease inhibitors (0.2 mM phenylmethylsulfonyl fluoride, 1 μ g/ml aprotinin, 1 μ g/ml pepstatin A, 1 μ g/ml leupeptin, 0.1 mg/ml benzamidine). The 100,000g membrane fraction was dissolved in sample buffer (10 mM Tris/HCl, pH 6.8, containing 0.005% Coomassie brilliant blue-G, 1.5% sodium dodecyl sulfate, 2 M urea, and 10 mM dithiothreitol), and the proteins (150 μ g per lane) were resolved on a 5% polyacrylamide gel. Protein concentrations were determined with a protein assay kit (Bio-Rad Laboratories, Hercules, CA). The proteins were transferred onto a polyvinylidene difluoride membrane (Immobilon P; Millipore, Billerica, MA), and the blot was probed with a rabbit polyclonal anti- α_{1B} antibody (Exalpha Biologicals, Watertown, MA) by using the ECL system (Amersham Bioscience, Piscataway, NJ). The N-type channel protein levels from seven independent experiments using four different pooled samples were quantified using the analysis software NIH image 1.62 (National Institutes of Health, Bethesda, MD). After immunoblotting, the membranes were stained with SYPRO Orange (Molecular Probes, Eugene, OR) and

scanned with a FluorImager 595 (Molecular Dynamics, Sunnyvale, CA), and the total transferred proteins were quantified with IMAGE QUANT software (Molecular Dynamics) to normalize the N-type channel protein band intensities.

For each set of experiments, the normalized band intensity of the saline control sample was set as 100, and then the ratio of the normalized band intensity of the carrageenan-treated sample to the saline control sample was calculated. For negative controls, antibody was excluded or incubated with α_{1B} antigen peptide (Alomone Laboratories, Jerusalem, Israel).

X-Gal Staining

Seven days after carrageenan injection, $Ca_v2.2$ +/- mice were anesthetized with sodium pentobarbital and subjected to fixation by transcardial perfusion with paraformaldehyde, 4%, in phosphate-buffered saline. X-Gal staining was performed on spinal cord sections as reported previously.²⁶ Briefly, after perfusion fixation, the spinal cord was dissected, rinsed with phosphate-buffered saline, and then stained with X-Gal solution (Sigma Chemical Company). Because the gene for *Escherichia coli* β -galactosidase with a nuclear localization signal was inserted in frame in the N-type channel gene *cacna 1b*, the β -gal activity monitored by X-Gal staining is considered to represent the native expression of the gene encoding the N-type channel.¹⁴ Nuclear localization is useful for excluding the contribution of the channel protein, which exists on the terminals of primary sensory neurons. Thus, this method is more advantageous than immunoblot analysis using dissected spinal cord samples, which inevitably include the central terminal component of primary sensory neurons. The levels of staining intensity in the spinal dorsal horn were quantified with NIH image and calculated as the ratio of the carrageenan-treated side to the contralateral side.

Statistical Analysis

Experimental data in the figures are mean \pm SEM. The immunoblot and X-Gal staining data were tested according to the Student *t* test. For behavioral analyses, we used the Mann-Whitney U test for comparisons between two groups and the Steel-Dwass test for multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

PGE₂ Induced Long-lasting Allodynia after Carrageenan Preconditioning

We observed that a 100- μ l carrageenan (2%) challenge resulted in swelling, erythema, and reduced threshold of paw withdrawal to mechanical stimuli with von Frey filaments in rats. These alterations began 30–60 min after injection, reaching a maximum in 2–4 h, and re-

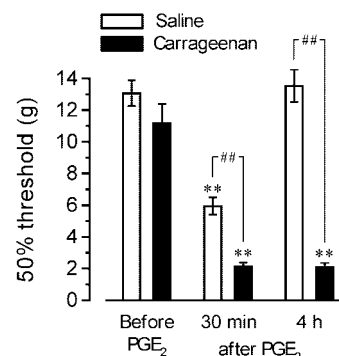


Fig. 1. Mechanical allodynia at 30 min and 4 h after prostaglandin E₂ (PGE₂) injection in rats preconditioned with carrageenan or saline 7 days ago (n = 6 each). ** $P < 0.01$ (Mann-Whitney U test), compared with their respective baseline control (before PGE₂ injection). ## Significant difference between saline-treated group and carrageenan-treated group at $P < 0.01$ (Mann-Whitney U test).

solved within 3 to 4 days. Consistent with findings of numerous previous reports, this routinely used dose of carrageenan^{27,28} produced only short-term hyperalgesia and allodynia from which the rats could fully recover.

Intradermal injection of PGE₂ at the same site where carrageenan had been injected 7 days earlier resulted in a prolonged mechanical allodynia lasting more than 4 h (fig. 1). In contrast, PGE₂ produced only transient allodynia lasting less than 1 h (acute allodynia) in saline-injected control rats (fig. 1). Furthermore, the mean values of 50% thresholds of the early (30 min) and late (4 h) phases of PGE₂-induced allodynia in carrageenan-preconditioned rats were significantly lower than those for their respective control derived from saline-preconditioned rats (fig. 1). PGE₂ injection into the carrageenan-preconditioned paw also evoked long-lasting allodynia in the contralateral hind paw ("mirror-image" pain), although the mean values of 50% thresholds were less marked (fig. 2). The 50% threshold values between early and late phases of the PGE₂-evoked allodynia were not different on ipsilateral and contralateral sides, respectively. There was no allodynia in the contralateral hind paw of saline-preconditioned rats.

Intrathecal Injection of GVIA Reduced the PGE₂-induced Long-lasting Allodynia

In saline-preconditioned rats, the PGE₂-induced acute allodynia was significantly attenuated by GVIA at the lowest dose used in this study (0.03 μ g) and almost completely blocked at 0.1 μ g (fig. 2A). In carrageenan-preconditioned rats, however, the early and late phases of PGE₂-induced allodynia were not significantly affected by 0.03 μ g GVIA, and greater than 0.1 μ g GVIA was required to achieve significant inhibition of both allodynia phases (fig. 2A and B).

The early and late phases of PGE₂-induced allodynia

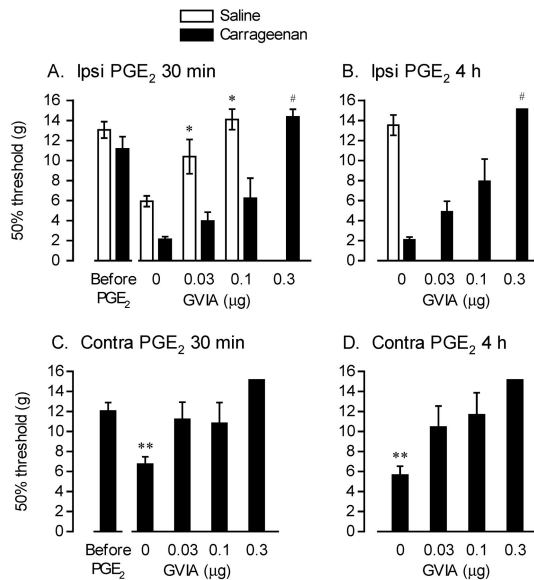


Fig. 2. Effects of intrathecal ω -conotoxin GVIA (GVIA) injection on the early (30 min; *A,C*) and late (4 h; *B,D*) phases of the prostaglandin E_2 (PGE_2)–induced mechanical allodynia in rats preconditioned with carrageenan or saline 7 days ago. Data for both ipsilateral (Ipsi) (*A,B*) and contralateral (Contra) (*C,D*) hind paws are shown ($n = 6-8$ each). In saline-preconditioned rats, the effects of GVIA were examined only on the early phase, and the highest dose of GVIA (0.3 μ g) was not tested. For control experiments, saline was injected intrathecally (GVIA, 0 μ g). * $P < 0.05$ and # $P < 0.02$, compared with their respective saline-administered (GVIA, 0 μ g) groups (Steel–Dwass multiple comparison test). ** $P < 0.01$, compared with baseline control (Mann–Whitney U test).

observed in the contralateral hind paws were almost completely blocked by lower doses of GVIA (fig. 2C and D). At the GVIA doses used in this study, rats behaved apparently normal, and we did not observe any aversive responses reported for this toxin.²²

Intrathecal Injection of AgaIVA Had No Effects on the PGE₂-induced Allodynia

To test whether the P/Q-type channel was also involved in this long-lasting allodynia, we investigated the effects of intrathecal injection of AgaIVA on this PGE_2 -induced long-lasting allodynia. As shown in figure 3A and B, AgaIVA did not attenuate PGE_2 -induced prolonged ipsilateral allodynia at both time points. AgaIVA also had no effects on the contralateral allodynia at 0.01 and 0.03 μ g (fig. 3C and D). At 0.1 μ g, however, AgaIVA significantly enhanced the early, but not the late, phase of the contralateral allodynia (fig. 3C and D). PGE_2 -induced acute allodynia in saline-preconditioned rats was also significantly potentiated at 0.1 μ g (fig. 3A). We did not increase the dose of AgaIVA to greater than 0.1 μ g, because higher doses induced some anomalous neurologic behaviors (sudden intense irritation and agitation behaviors against environmental stimuli including von Frey stimulation).²²

N-type Channel Expression Was Enhanced after Carrageenan Inflammation

We studied possible changes in the level of the N-type channel in the DRG and spinal dorsal horn after carrageenan inflammation. By quantitative Western blotting, we found that the normalized expression level of N-type channel protein in the DRGs from carrageenan-preconditioned rats was significantly higher than that of saline controls (fig. 4). Although we tried hard to quantify the N-type channel protein expression in L4–L6 spinal cord sections from these animals, in many cases, we could not detect the channel protein.

It has been suggested previously that both rats and mice have a similar time course for carrageenan-induced acute inflammation and hyperalgesia.²⁹ We confirmed that both wild-type and $Ca_v2.2 +/−$ mice have the acute inflammatory pain behavior similar to that in rats (unpublished data, K. Yokoyama, M.D., T. Kurihara, Ph.D., and T. Tanabe, Ph.D., Tokyo, Japan [June 4, 2003]). Thus, we investigated the expression level of the N-type channel in the spinal cord of $Ca_v2.2 +/−$ mice, preconditioned by carrageenan 7 days ago, as the β -galactosidase activity monitored by X-Gal staining. X-Gal staining revealed a broad expression of the N-type channel throughout the spinal gray matter, particularly in the dorsal horn (fig. 5A), which is generally consistent with findings of a previously reported immunohistochemical study.⁹ The expression level of the channel protein ip-

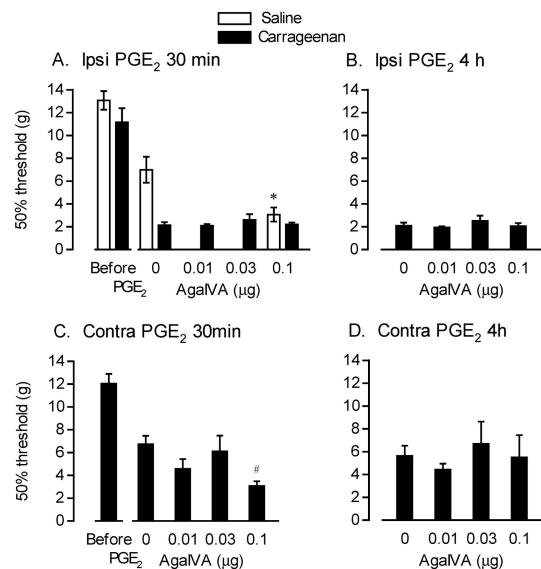


Fig. 3. Effects of intrathecal ω -agatoxin IVA (AgaIVA) injection on the early (30 min; *A,C*) and late (4 h; *B,D*) phases of the prostaglandin E_2 (PGE_2)–induced mechanical allodynia in rats preconditioned with carrageenan or saline 7 days ago ($n = 6-8$ each). Data for both ipsilateral (Ipsi) (*A,B*) and contralateral (Contra) (*C,D*) hind paws are shown. Only the highest dose of AgaIVA (0.1 μ g) was given to the saline-conditioned rats. * $P < 0.05$, compared with their saline-administered (AgaIVA, 0 μ g) groups (Mann–Whitney U test). # $P < 0.05$, compared with the saline-administered group (Steel–Dwss multiple comparison test).

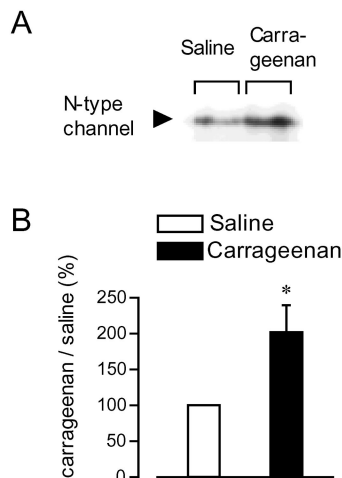


Fig. 4. Carrageenan preconditioning increases N-type channel protein expression in the dorsal root ganglia (DRGs). (A) A representative Western blot for the N-type channel in the DRGs from rats preconditioned with carrageenan or saline 7 days ago. (B) Summarized data from seven independent immunoblot experiments. Note that the expression level of N-type channel protein in the DRGs from carrageenan-injected rats was significantly higher than that of saline controls. * $P < 0.05$ (Student t test).

ilateral to the carrageenan-preconditioned paw was significantly higher than that of the contralateral side (fig. 5B).

Discussion

To our knowledge, this is the first behavioral and biochemical study addressing the plastic nature of N-type channel expression in a latent hypersensitization state after acute inflammation. Our results are in line with the hypothesis that the up-regulation of the N-type channel in DRGs and the spinal dorsal horn is a cause of the long-lasting hypersensitivity to an inflammatory mediator.

The up-regulation of channel proteins in DRGs or the spinal cord after experimental short-term inflammation has not been reported before. However, the up-regulation of P/Q-type, but not N- or L-type, channel protein has been shown in the trigeminal ganglion after tooth pulp inflammation.³⁰ In the peripheral nerve injury/neuropathic pain models, the up-regulation of N-type channel protein in the spinal cord after chronic constrictive nerve injury has been reported³¹; on the other hand, no change of N-type channel expression in the spinal cord after spinal nerve injury³² and injured DRGs at the mRNA level³²⁻³⁴ has been demonstrated. A previous electrophysiologic study showed that densities of high-voltage-activated Ca^{2+} currents in DRG neurons were reduced by the peripheral nerve injuries.^{8,35,36} In the current study, we could not detect N-type channel expression constantly in the rat spinal cord by Western blotting. This might be partly due to a much lower basal expression of the channel protein in the spinal cord than in the

DRG.³² The study of N-type channel expression in the $Ca_v2.2$ +/- mice spinal cord, although the possibility of species differences could not be completely eliminated, further suggested the plastic nature and importance of this channel in a latent hypersensitization state.

Another important finding of this study was that the carrageenan-conditioning stimulus could induce PGE_2 -evoked prolonged allodynia not only in the conditioned hind paw but also in the contralateral (noninflamed) hind paw. This result is consistent with previous findings of recurrent inflammation^{4,5} and neuropathic pain models.^{37,38} A recent study suggested the role of spinal glia and proinflammatory cytokines in "mirror-image (contralateral)" neuropathic pain.³⁹ Although the precise mechanism causing pain on the contralateral side remains to be explored, our results may suggest that the spinal N-type channel is involved in "mirror-image" pain.

It has been reported that the activation of protein kinase C ϵ in the sensitized peripheral neurons might be involved in this long-lasting hypersensitivity.¹⁶ Another recent report suggested a possible functional link between protein kinase C ϵ activation and N-type channel up-regulation.⁴⁰ There is evidence indicating that protein kinase C ϵ is one of the major protein kinase C isozymes expressed in sensory neurons and is important for nociceptor regulation.^{41,42} Therefore, an increase or decrease of some factors in inflamed hind paw and spinal

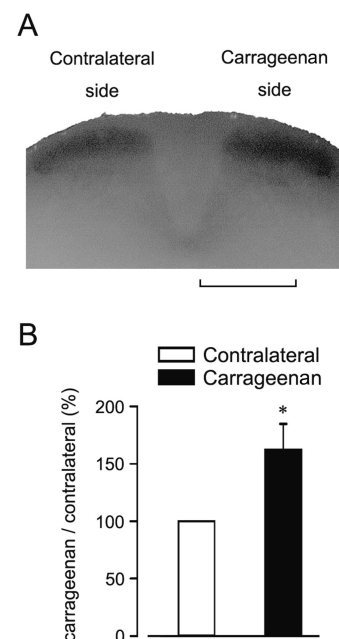


Fig. 5. Carrageenan preconditioning increases N-type channel protein expression in the spinal cord. (A) Representative 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-Gal) staining for the N-type channel in the spinal cord from the heterozygous alleles of N-type channel knockout ($Ca_v2.2$ +/-) mice preconditioned with carrageenan 7 days ago. Scale bar = 0.5 mm. (B) Summarized data for seven mice. Note that the expression level of N-type channel protein on the ipsilateral side to the carrageenan-injected paw was significantly higher than that of the contralateral side. * $P < 0.05$ (Student t test).

cord during acute inflammation would eventually enhance the activity of protein kinase C ϵ , leading to the up-regulation of the N-type channel.

Recently, several N-type channel blockers have been developed as promising analgesics for a variety of pain conditions.⁴³ In particular, ziconotide (a synthetic form of ω -conotoxin MVIIA) has been evaluated in a number of clinical trials (mainly for neuropathic and cancer pain) *via* intrathecal administration. Although ziconotide is generally tolerated, it has been reported to produce significant adverse effects such as bradycardia, nausea, ataxia, and so on.⁴⁴ Recently, CVID, a more selective N-type channel blocker, was shown to have a wider therapeutic window than ω -conotoxin MVIIA in the treatment of a chronic inflammatory pain⁴⁵ (adjuvant-induced mechanical hyperalgesia). Our results suggest that the efficacy of N-type channel blockers might be reduced in the chronic inflammatory pain conditions preceded by the acute inflammation. In this context, we showed recently that the induction level of VDCC $\alpha_2\delta$ subunit expression might determine the sensitivity of gabapentin analgesia among the different neuropathic pain models.³⁴

In contrast to the N-type channel blocker, the P/Q-type channel blocker had no inhibitory actions on any type of the PGE₂-induced allodynia. At the highest dose in this study, AgaIVA rather exerted stimulatory actions on the contralateral allodynia in carrageenan-conditioned rats and the acute allodynia in saline-conditioned rats. The doses used in this study (0.01–0.1 μ g: roughly corresponding to 2–20 pmol) appear to be within the commonly used range. Malmberg and Yaksh²² showed that less than 10 pmol of intrathecal AgaIVA had potent inhibitory effects of formalin-induced nociceptive behavior. Sluka^{23,24} demonstrated that intraspinal preadministration of AgaIVA within the similar range reduced the secondary hyperalgesic and allodynic behavior induced by intradermal capsaicin or acute joint inflammation. Thus, the P/Q-type channel plays a role in spinal pain transmission but does not seem to be involved in the allodynia induced by PGE₂ administration. Although the reason for a lack of antiallodynic action of AgaIVA is not known at present, the stimulatory effect is partly consistent with the observations suggesting that the P/Q-type channel is preferentially involved in inhibitory neurotransmission in the spinal dorsal horn in the normal state.^{6,13}

In summary, PGE₂ produced prolonged mechanical allodynia in both hind paws (more prominent on the ipsilateral side) after apparent resolution of unilateral carrageenan-induced acute inflammation. On the other hand, PGE₂ induced only short-lasting mechanical allodynia in saline-preconditioned paws of control rats. An N-type channel blocker, GVIA, but not a P/Q-type channel blocker, AgaIVA, inhibited PGE₂-induced allodynia. High doses of GVIA were necessary in carrageenan-pre-

conditioned rats, which might be caused by the up-regulation of N-type channel expression in DRGs and the spinal cord ipsilateral to the carrageenan pretreatment. Thus, clarifying the mechanism of up-regulation of N-type channel protein would be important as a future study.

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