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# Paclitaxel Increases High Voltage–Dependent Calcium Channel Current in Dorsal Root Ganglion Neurons of the Rat

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**Abstract.** Peripheral neuropathic pain is a serious side effect of paclitaxel treatment. However, the mechanism of this paclitaxel-induced neuropathic pain is unknown. In this study, we investigated the effects of paclitaxel on the voltage-dependent calcium channel (VDCC) current in rat dorsal root ganglion (DRG) neurons using the whole-cell patch clamp technique. Behavioral assessment using von Frey filament stimuli showed that 2 and 4 mg/kg paclitaxel treatment induced mechanical allodynia/hyperalgesia. Paclitaxel-induced mechanical hyperalgesia was significantly inhibited by gabapentin (100 mg/kg). Using the patch clamp method, we observed that paclitaxel (4 mg/kg) treatment significantly increased the VDCC current in small- and medium-diameter DRG neurons. Moreover, paclitaxel-induced increase in the VDCC current in medium-diameter DRG neurons was completely inhibited by 10 and 100 $\mu$M gabapentin. Similar effects in small-diameter DRG neurons were only seen with 100 $\mu$M gabapentin. Western blotting revealed that paclitaxel increased protein levels of the VDCC subunit $\alpha_2\delta$-1 ($Ca_v\alpha_2\delta$-1) in DRG neurons. Immunohistochemistry showed that paclitaxel treatment increased $Ca_v\alpha_2\delta$-1 protein expression in DRG neurons. Thus, paclitaxel treatment increases the VDCC current in small- and medium-diameter DRG neurons and upregulates $Ca_v\alpha_2\delta$-1. The antihyperalgesic action of gabapentin may be due to inhibition of paclitaxel-induced increases in the VDCC current in DRG neurons.

***Keywords***: paclitaxel, voltage-dependent calcium channel current, dorsal root ganglion, gabapentin, peripheral neuropathy

## Introduction

The chemotherapeutic drug paclitaxel is commonly used for the treatment of solid tumors such as ovarian and breast cancer. Paclitaxel frequently induces peripheral neuropathies such as pain in the distal extremities, forming a glove-and-stocking–like pattern (1). The symptoms of paclitaxel-induced neuropathy are mostly sensory and peripheral in nature, consisting of mechanical allodynia/hyperalgesia, tingling, and numbness (2 – 4).

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Transmission of peripheral pain signals involves the concerted action of several types of ion channels, including $Na^+$ channels, various $K^+$ channels, and voltage-dependent calcium channels (VDCCs) in the dorsal root ganglia (DRG); these channels modulate cellular excitability in nociceptive pathways (5). Neuropathic pain arising from damage to peripheral nerves is associated with allodynia and hyperalgesia (6) and is associated with adaptive changes in ion channel expression in nociceptive pathways, including sensory neurons in the DRG (7). VDCCs have emerged as potential ion channel targets for the treatment of neuropathic pain (8, 9). These findings suggest that VDCCs play a role in the transmission of pain by allowing calcium ion influx into DRG neurons. VDCCs are composed of a main pore-forming

$\alpha_1$-subunit, together with auxiliary $\alpha_2\delta$-, $\beta$-, and $\gamma$-subunits. There is evidence that ethosuximide, a relatively selective T-type $Ca^{2+}$-channel blocker, and gabapentin (GBP), an antagonist of $Ca^{2+}$ channels containing the $Ca_v\alpha_2\delta$-1 and $Ca_v\alpha_2\delta$-2 subunits, significantly reduce paclitaxel-evoked neuropathic pain (10). The binding of GBP to the $\alpha_2\delta$-1 subunit may be responsible for its efficacy in the treatment of neuropathic pain (11 – 13). GBP has much higher affinity for the $Ca_v\alpha_2\delta$-1 subunit than the $Ca_v\alpha_2\delta$-2 subunit (14). In addition, the $Ca_v\alpha_2\delta$-1 subunit upregulates total and surface expression of $Ca_v3$ channels as well as significantly increases T-type current density (15). Upregulation of $Ca_v\alpha_2\delta$-1 protein and mRNA in DRGs occurs in models of peripheral nerve injury (16, 17). This upregulation may be correlated with the onset of paclitaxel-induced peripheral neuropathic pain. However, the participation of VDCCs in paclitaxel-induced neuropathic pain remains unclear. Thus, using a rat model of paclitaxel-induced painful peripheral neuropathy, we investigated the effects of paclitaxel on VDCC current recorded from sensory DRG neurons, the mediators of pain perception, and whether GBP alters the VDCC current during paclitaxel treatment.

## Materials and Methods

*Experimental animals*

Male Wistar rats weighing 250 – 300 g were used in the present study. All rats were housed individually under automatically controlled environmental conditions, using a 12-h light-dark cycle (lights on from 08:00 to 20:00) with free access to food and water. All animals were quarantined in centralized animal facilities for at least 7 days upon arrival. Each animal was used only once. Experiments were carried out according to the guidelines for animal care and use published by the National Institutes of Health and the committee of Showa Pharmaceutical University.

*Drug administration*

Paclitaxel [2 or 4 mg/kg per mL, prepared with saline from Taxol, Bristol-Myers-Squibb 6 mg/mL paclitaxel (Paris, France) in Cremophor EL vehicle] or vehicle (Cremophor EL-polyethoxylated castor oil and ethanol, diluted with two parts saline to one part Cremophor EL) was administered intraperitoneally (i.p.) on four alternate days (days 0, 2, 4, and 6; cumulative dose, 8 or 16 mg/kg).

*Mechanical stimulation*

Observers kept unaware of the experimental conditions performed the tests for mechanical hyperalgesia at the same time on days 0, 14, and 21 after paclitaxel treatment. In brief, rats were placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate to the environment for 15 min. The tactile sensitivity of the plantar surface of the hind paw was measured from the withdrawal responses to mechanical stimulation with von Frey filaments. von Frey filaments with bending forces of 2.0 and 5.0 g were applied to the mid-plantar surface of the hind paw five times, with each application held for 3 s (maximum). A 1-min rest was allowed between tests on alternate hind paws and 2 – 3 min between subsequent tests on the same hind paw. A positive response was recorded if the paw was withdrawn during the application of the von Frey filament or immediately after its removal. Withdrawal responses to the von Frey filaments from both hind paws were counted and then expressed as an overall percentage response. Normal rats show very low withdrawal from the 2-g stimulus; an increased response after paclitaxel treatment is thus indicative of mechano-allodynia. Normal rats withdraw from 5-g stimulation about 20% of the time; an increased response to these hairs after paclitaxel treatment is thus indicative of mechano-hyperalgesia as described previously (18).

*Western blot analysis*

Paclitaxel (2 or 4 mg/kg) was injected i.p. on four alternate days (days 0, 2, 4, and 6). The rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) on day 7 after the start of paclitaxel treatment, and rat DRGs ($L_{4–6}$) were then removed for western blot analysis. DRG proteins (30 $\mu$g) were separated through a 7.5% SDS-polyacrylamide gel and then transferred onto nitrocellulose membranes. For western blotting, anti-VDCC ($Ca_v$) $\alpha_2\delta$-1 antibody (Sigma-Aldrich, St. Louis, MO, USA; 1:200) was used, and anti-$\beta$-actin antibody (Sigma-Aldrich, 1:1000) was used as the loading control. Horseradish peroxidase–labeled rabbit antibody (1:2000) was used as the secondary antibody. Specific bands were detected using enhanced chemiluminescence plus the TM Western Blotting Detection Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's protocol. The intensities of immunoreactive bands were analyzed with MultiGage Ver. 3 software (Fuji Film, Tokyo).

*Immunohistochemistry*

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused transcardially with 20 mL potassium-free phosphate-buffered saline ($K^+$-free PBS, pH 7.4) followed by 50 mL 4% paraformaldehyde solution. The DRGs ($L_{4–6}$) were removed, post-fixed for 3 h, and cryoprotected overnight in 25% sucrose solution. The DRGs were stored at −80°C until use. DRGs were cut at 15-$\mu$m thickness, thaw-mounted on silane-coated

glass slides, and air-dried overnight at room temperature. DRG sections were incubated with excess blocking buffer containing 2% skim milk in 0.1% Triton X-100 in $K^+$-free PBS and subsequently reacted overnight at 4°C with anti-$Ca_v\alpha_2\delta$-1 antibodies (Sigma-Aldrich, 1:200) in 2% bovine serum albumin / 0.1% Triton X-100 in $K^+$-free PBS. The sections were then incubated in fluorescein isothiocyanate-conjugated anti-rabbit IgG (Sigma-Aldrich, 1:200) for 2 h at room temperature. All sections were treated with Permafluor (Thermo Shandon, Pittsburgh, PA, USA) and coverslipped and evaluated by microscopy.

The immunostained sections were mounted on slides, covered with microslips, and observed with an Olympus laser-scanning confocal microscope (FLUOVIEW BW50; Olympus, Tokyo) at a wavelength of 495 nm. Neurons with visible nuclei were considered for calculation. A total of five sections (90 $\mu$m apart) were randomly selected from each DRG. Optical density (OD) of the stained sites was determined with the public domain Image J program using a computer. The OD of $Ca_v\alpha_2\delta$-1–positive cells was calculated for day 7 after paclitaxel or vehicle treatment. The proportion of $Ca_v\alpha_2\delta$-1–positive cells was calculated according to the size of the cell body. At least 300 neurons from each DRG of each rat were measured.

*Culture methods*

The rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) on day 7 after the start of paclitaxel treatment, and rat DRG neurons were isolated from the DRG ($L_{4–6}$). Briefly, lumbar ganglia were excised and freed from their connective tissue sheaths, and then DRG neurons were incubated in Hank's F12 solution containing 0.1% collagenase (type I, Sigma-Aldrich) for 90 min at 37°C, followed by incubation in HBSS solution containing 0.125% trypsin (Sigma-Aldrich) for 10 min at 37°C. Cells were triturated with a constricted Pasteur pipette. Dispersed DRG neurons were plated onto coverslips coated with poly-L-lysine and cultured at 37°C in a water-saturated atmosphere of 5% $CO_2$ – 95% $O_2$ in Hank's F12 solution containing 10% fetal bovine serum albumin, 100 U/mL penicillin, and 200 mg/mL streptomycin.

*Electrophysiology recording*

The VDCC current of small- and medium-diameter DRG neurons was recorded at room temperature using the whole-cell mode of the patch clamp using a Multiclamp 700A unit (Axon Instruments, Sunnyvale, CA, USA). The cell capacitance ($C_m$) was calculated by integrating the area under capacitive transients as described previously (19) or read directly from the amplifier. Fire-polished patch pipettes were formed from borosilicate glass capillaries (Narishige, Tokyo) using a P-87 puller (Narishige). Recording electrodes had resistances of 5 – 8 M$\Omega$ after heat-polishing. Data were analyzed using the pCLAMP 8.2 software (Axon Instruments). Calcium currents were sampled at 10 kHz after low-pass filtering at 2 kHz (−3 db); otherwise, they were sampled at 2 kHz after low-pass filtering at 1 kHz. The external solution contained 5 mM $BaCl_2$, 155 mM TEA, 5 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES, and it was adjusted to pH 7.4 with 20% TEA-OH. The pipette solution contained 120 mM CsCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES, and it was adjusted to pH 7.4 with Trismabase. Glass coverslips to which the cells were attached were removed from the incubator and placed into a small-volume recording chamber (approximately 250 $\mu$L). The DRG neurons were incubated with GBP for 60 min prior to VDCC current measurements. GBP was also present in the external recording solution. All cells were subsequently examined within 10 to 60 min. All experiments were done at room temperature.

*Statistical analyses*

All data are expressed as the mean ± S.E.M. Cremophor vehicle treatment versus paclitaxel treatment groups were statistically analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. Immunohistochemical staining data are shown as the percentage of OD obtained from each cremophor vehicle–treated rat and paclitaxel-treated rat. The F-test and unpaired *t*-test were used in this experiment. Statistical significance was accepted at $P < 0.05$.

## Results

*The effects of paclitaxel on mechanical allodynia/hyperalgesia*

As expected, mechano-allodynia and mechano-hyperalgesia of the paw were observed upon both 2 and 4 mg/kg paclitaxel treatment with the von Frey test at 7, 14, and 21 days after the start of paclitaxel treatment compared to the cremophor vehicle (Fig. 1). In addition, body weight did not differ between the groups prior to treatment with either paclitaxel or the cremophor vehicle (data not shown). Normal weight gain was observed in groups receiving either paclitaxel or the cremophor vehicle.

*Identification of neuronal size*

Using previously described criteria (7, 20), DRG neurons were first assigned as either small-diameter DRG neurons ($C_m$: < 42 pF) or medium-diameter DRG neurons ($C_m$: 42 – 72 pF). Using optical measurements of

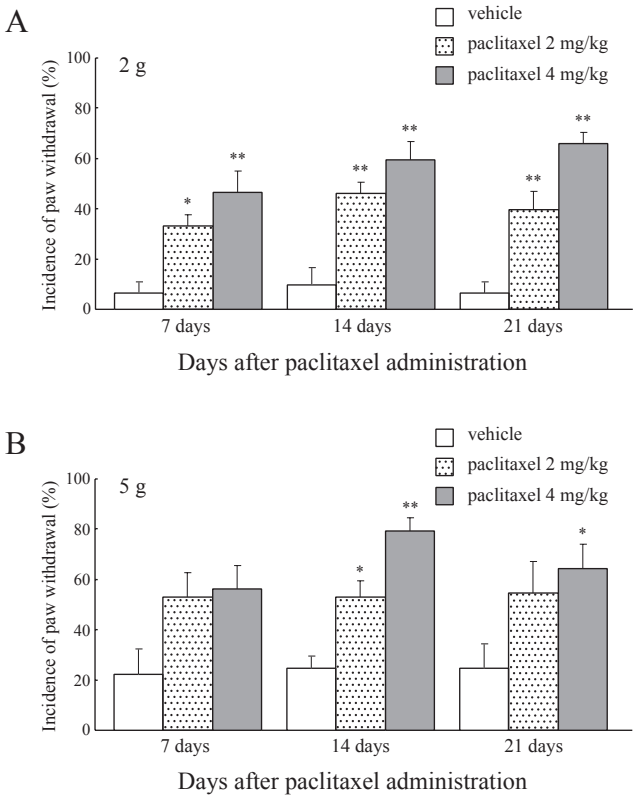


**Fig. 1.** Behavioral responses of mechano-allodynia and mechano-hyperalgesia induced by paclitaxel treatment (2 and 4 mg/kg, i.p.; administered on days 0, 2, 4, and 6). Graph shows the mean ± S.E.M. of the response frequency to mechanical stimulation by 2-g (A) and 5-g (B) von Frey filament. n = 8 – 10 for cremophor vehicle treatment, n = 10 – 12 for paclitaxel treatment. Symbols indicate a significant difference from cremophor vehicle (*$P < 0.05$, **$P < 0.01$).

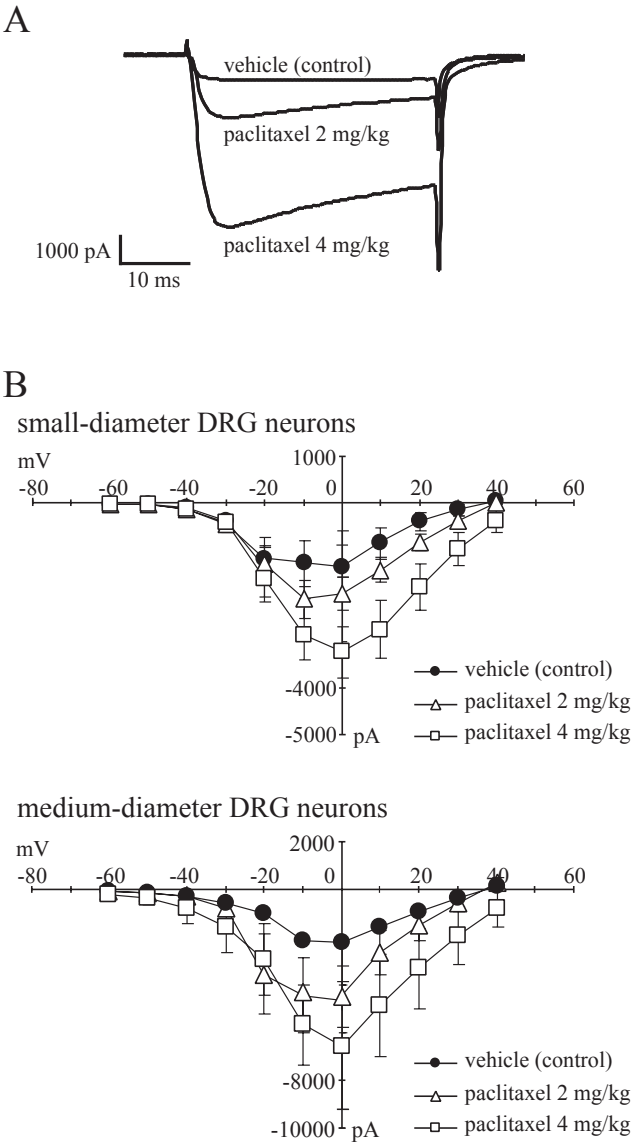


**Fig. 2.** Effects of paclitaxel treatment on VDCC current in rat DRG neurons. A) Inward VDCC current of medium-diameter DRG neurons was evoked by depolarization from a holding potential of −80 mV to a test potential of +10 mV in the cremophor vehicle and in the paclitaxel treatment (2 and 4 mg/kg) rats. The current trace in the paclitaxel treatment was obtained 7 days after the start of paclitaxel administration. B) Current-voltage relationship of the VDCC current of small- and medium-diameter DRG neurons. The peak VDCC current density in the cremophor vehicle treatment (control: closed circles) and in the paclitaxel treatment (2 mg/kg, open triangles, n = 6; 4 mg/kg, open squares, n = 6) rats was plotted against each test pulse potential. Each point on the voltage curve represents the mean ± S.E.M.

mean diameter, DRG neurons were classified into two groups based on size: approximately 23 – 30 and 31 – 40 $\mu$m in diameter. We focused on small and medium neurons, with a mean $C_m$ of 27.1 ± 2.7 pF (n = 6) for small-diameter DRG neurons and 59.8 ± 3.7 pF (n = 6) for medium-diameter DRG neurons.

*The effect of paclitaxel treatment on VDCC current in DRG neurons*

We used the whole-cell recording technique on small- and medium-diameter DRG neurons to examine the effects of 2 and 4 mg/kg, i.p. paclitaxel treatment on VDCC currents 7 days after the start of paclitaxel treatment. The effects of paclitaxel on VDCC currents evoked by depolarization from a holding potential of −80 mV to a testing potential of 0 mV on medium-diameter DRG neurons are shown in Fig. 2A. The current–voltage relationship of the VDCC currents of small- and medium-diameter DRG neurons is shown in Fig. 2B. In this figure, the voltage dependence on enhancement is demonstrated by the current–voltage curve for the paclitaxel treatment (2 and 4 mg/kg). In cells demonstrating peak current at 0 mV, paclitaxel enhancement started at around −20 mV and reached a maximum at 0 mV, after which enhancement persisted with further depolarization potentials. The data

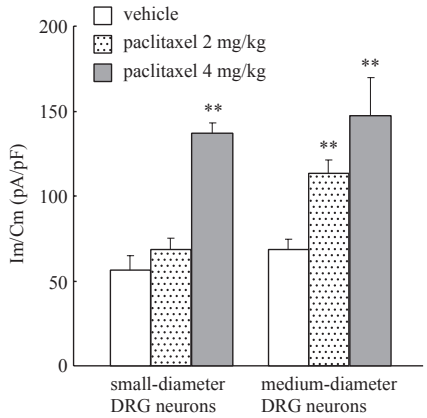


**Fig. 3.** Effects of paclitaxel treatment on VDCC current of small- and medium-diameter rat DRG neurons. Histograms represent the VDCC current density (pA/pF) at 0 mV observed in the cremophor vehicle and the paclitaxel treatment (2 and 4 mg/kg, n = 6) rats. The VDCC current was evoked by depolarization from a holding potential of −80 mV to a test potential of +10 mV. The means ± S.E.M. of VDCC currents in small- and medium-diameter DRG neurons are indicated by the columns and vertical bars. Symbols indicate a significant difference from cremophor vehicle (**$P < 0.01$).

in Fig. 3 show that in cremophor vehicle–treated rats, the VDCC currents in small- and medium-diameter DRG neurons elicited by a +10 mV test pulse were 56.8 ± 9.1 and 70.1 ± 6.5 pA/pF, respectively. In paclitaxel-treated rats (4 mg/kg), the VDCC currents of small- and medium-diameter DRG neurons were significantly higher at 139.8 ± 6.1 and 150.1 ± 22.5 pA/pF, respectively. Moreover, VDCC currents of medium-diameter DRG neurons were significantly higher at 108.6 ± 8.6 pA/pF with 2 mg/kg paclitaxel treatment. However, the VDCC currents of small-diameter DRG neurons were not affected by 2 mg/kg paclitaxel treatment. Thus, the VDCC currents of medium-diameter DRG neurons were significantly increased by 2 and 4 mg/kg paclitaxel treatment.

*The effect of paclitaxel on the mechanical allodynia/hyperalgesia and VDCC current in DRG neurons was inhibited by GBP*

The effects of GBP (100 mg/kg, i.p.) on paclitaxel (4 mg/kg)-induced mechanical allodynia/hyperalgesia are shown in Fig. 4A. Administration of GBP (100 mg/kg, i.p.) 30 min before the von Frey test significantly inhibited mechanical hyperalgesia on day 7 after paclitaxel treatment compared to the cremophor vehicle. Thus, GBP reversed paclitaxel-induced mechanical hyperalgesia.

To test the effect of GBP on VDCC currents, small- and medium-diameter DRG neurons were exposed to 10 and 100 $\mu$M GBP. This concentration is within the range

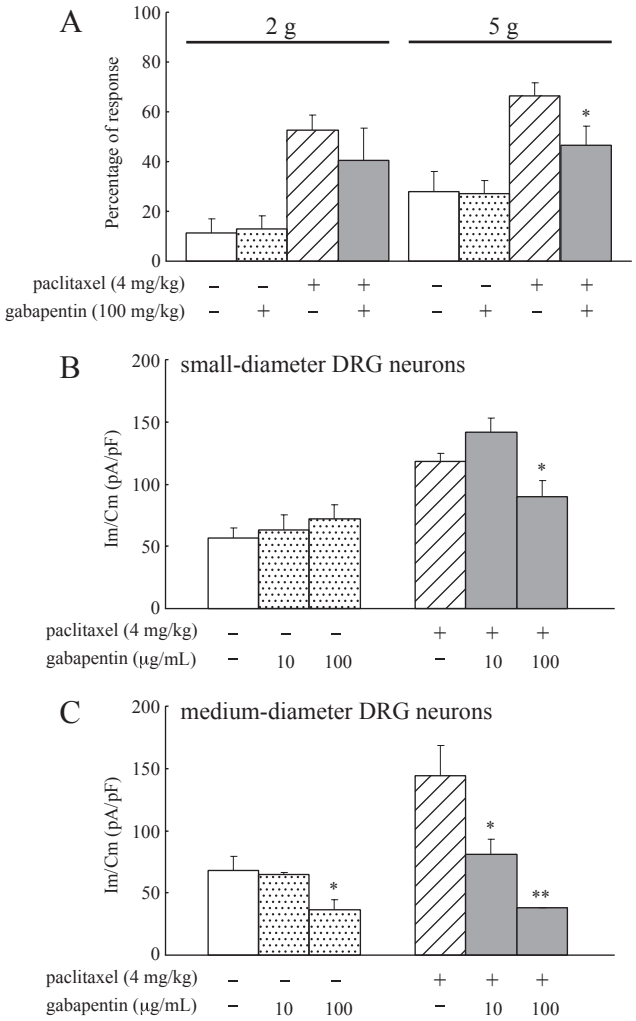


**Fig. 4.** Effect of gabapentin treatment on the mechanical allodynia/hyperalgesia and paclitaxel-induced VDCC current in DRG neurons. A) Behavioral responses to gabapentin (100 mg/kg, i.p.) for mechano-allodynia and mechano-hyperalgesia induced by paclitaxel treatment (4 mg/kg) at day 7. Graph shows the mean ± S.E.M. of the response frequency to mechanical stimulation by 2-g and 5-g von Frey filaments. n = 4 per group. B, C) Histograms represent the peak VDCC current density (pA/pF) in small- and medium-diameter DRG neurons observed in the gabapentin (10 and 100 $\mu$M, n = 5) and the paclitaxel treatment (4 mg/kg, n = 6) rats. B) Gabapentin alone (10 and 100 $\mu$M) did not affect the current in small-diameter DRG neurons (left). Paclitaxel-induced enhancement of VDCC currents in the small-diameter DRG neurons was inhibited by 60-min pretreatment with gabapentin (100 $\mu$M) (right). C) Gabapentin alone (10 and 100 $\mu$M) significantly decreased the current in medium-diameter DRG neurons (left). Paclitaxel-induced enhancement of VDCC currents in the medium-diameter DRG neurons was significantly inhibited by 60-min pretreatment with gabapentin (10 and 100 $\mu$M) (right). The means ± S.E.M. of VDCC currents in small- and medium-diameter DRG neurons are indicated by the columns and vertical bars. Symbols indicate a significant difference (*$P < 0.05$, **$P < 0.01$, vs. cremophor vehicle or paclitaxel single treatment group).

(10 – 100 $\mu$M) found in the serum of human patients and is therefore clinically relevant (21, 22). The effects of 10 and 100 $\mu$M GBP on paclitaxel (4 mg/kg, i.p.)-induced enhancement of VDCC currents in small- and medium-diameter DRG neurons are shown in Fig. 4, B and C. Acute application of 10 and 100 $\mu$M GBP did not affect the paclitaxel-induced enhancement of VDCC currents (data not shown). In contrast, after a 60-min incubation with 100 $\mu$M GBP alone, VDCC currents in medium-diameter DRG neurons were significantly decreased (Fig. 4C, left), but GBP alone (100 $\mu$M) did not affect current in small-diameter DRG neurons (Fig. 4B, left). After a 60-min incubation with GBP, paclitaxel-induced enhancement of VDCC currents in the small-diameter DRG neurons was significantly decreased by 100 $\mu$M GBP but not 10 $\mu$M GBP (Fig. 4B, right). However, the paclitaxel-induced enhancement of VDCC currents was significantly inhibited by 10 and 100 $\mu$M GBP in the medium-diameter DRG neurons (Fig. 4C, right). VDCC currents were decreased by 44.3% ± 8.5% by 10 $\mu$M GBP and by 73.6% ± 0.1% by 100 $\mu$M GBP.

*The effect of paclitaxel treatment on $Ca_v\alpha_2\delta$-1 protein in DRG neurons*

We removed DRGs at day 7 after the start of 2 and 4 mg/kg paclitaxel treatment, and $Ca_v\alpha_2\delta$-1 protein expression was quantified by western blot analysis. As shown in Fig. 5, treatment with 2 and 4 mg/kg paclitaxel significantly increased $Ca_v\alpha_2\delta$-1 protein expression in DRGs at day 7 (2 mg/kg: 52.8% ± 14.7%, 4 mg/kg: 63.2% ± 20.1%). A significant increase in $Ca_v\alpha_2\delta$-1 protein expression was found in paclitaxel-treated rats compared with cremophor vehicle–treated rats.

*Immunohistochemistry*

Immunohistochemistry revealed that most of the $Ca_v\alpha_2\delta$-1 protein in the DRG neurons was mainly localized to small- or medium-diameter cells (Fig. 6A). Using computerized OD image analysis, we found that 34.4% ± 6.5% of DRG neurons were positively labeled for $Ca_v\alpha_2\delta$-1 protein in rats treated with cremophor vehicle. Paclitaxel (4 mg/kg, i.p.) treatment induced a significant increase in the percentage of DRG neurons expressing $Ca_v\alpha_2\delta$-1 protein at day 7 (Fig. 6B). Thus, paclitaxel (4 mg/kg)-induced enhancement of $Ca_v\alpha_2\delta$-1 protein expression resulted from upregulation of expression in DRG neurons.

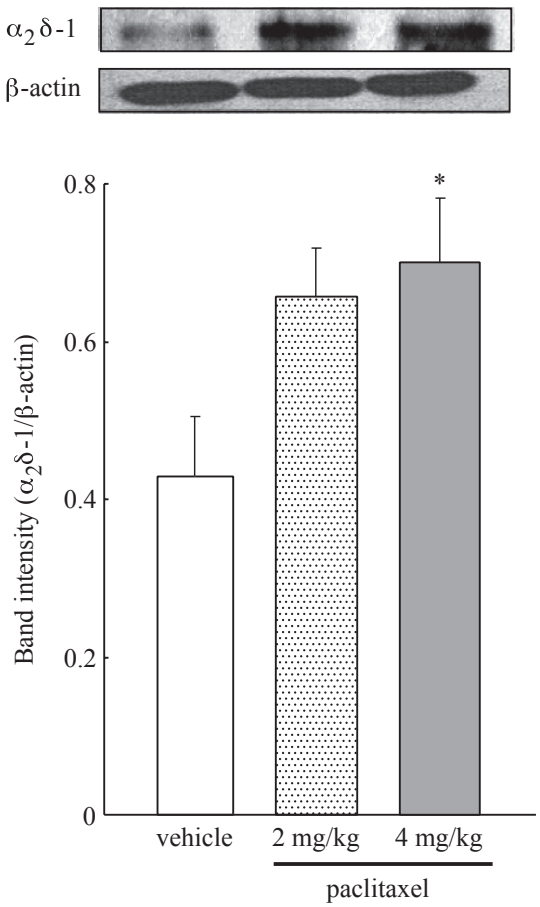


**Fig. 5.** Expression of $Ca_v\alpha_2\delta$-1 protein in DRGs ($L_{4-6}$) after paclitaxel treatment. Western blot analysis of $Ca_v\alpha_2\delta$-1 and $\beta$-actin protein in DRGs 7 days after the start of paclitaxel (2 and 4 mg/kg, i.p.) treatment. Photograph shows representative data. Histograms represent the $Ca_v\alpha_2\delta$-1 protein observed in the cremophor vehicle and the paclitaxel treatment (2 and 4 mg/kg, n = 6) rats. $Ca_v\alpha_2\delta$-1 protein was normalized to $\beta$-actin. The means ± S.E.M. of $Ca_v\alpha_2\delta$-1 proteins are indicated by the columns and vertical bars. *$P < 0.05$ (vs. cremophor vehicle).

## Discussion

In the present study, mechano-allodynia was observed 7 and 14 days after the start of 2 or 4 mg/kg paclitaxel treatment given on four alternate days from day 0 to day 6 inclusive. In previous experimental studies, single or multiple administration of paclitaxel induced mechanical allodynia/hyperalgesia, thermal hyperalgesia, and A-fiber-specific hypersensitization (23, 24). The results of the present study agree with these previously published findings.

In a primary sense, voltage-gated calcium channels as a family represent one of the most important regulators of $Ca^{2+}$ concentration in neurons and hence have an important role in neuronal function, including physiologic nociception and neuropathic pain (8, 9). In the present study, it is interesting that paclitaxel treatment induced enhancement of VDCC current in small- and medium-diameter DRG neurons. Small- and medium-diameter DRG neurons are well known to exhibit electrophysio-

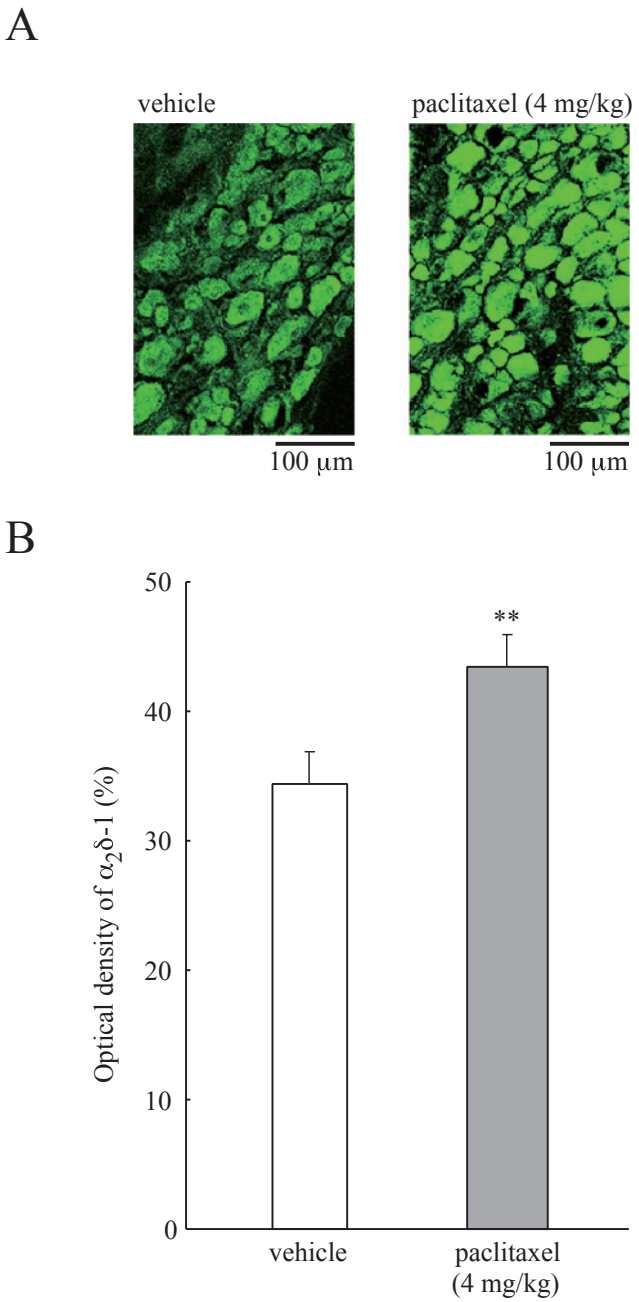


**Fig. 6.** Immunohistochemistry of $Ca_v\alpha_2\delta$-1 protein expression in DRG ($L_{4-6}$) neurons after paclitaxel treatment. A) DRG slices (15-$\mu$m-thick) 7 days after the start of the cremophor vehicle (control, n = 3) or paclitaxel (4 mg/kg, i.p., n = 3) treatment were stained with antibodies against $Ca_v\alpha_2\delta$-1 protein. B) Optical density (OD) of $Ca_v\alpha_2\delta$-1–stained DRG neurons 7 days after the start of cremophor vehicle or paclitaxel (4 mg/kg, i.p.) administration. The means ± S.E.M. of the OD for $Ca_v\alpha_2\delta$-1 protein expression are indicated by the columns and vertical bars. $^{**}P < 0.01$ (vs. cremophor vehicle).

logical characteristics consistent with C and A$\delta$ fibers, respectively, which convey different sensory modalities. Moreover, Cata et al. have shown increased levels of excitability in spinal cord dorsal horn neurons in rats with paclitaxel-evoked neuropathic pain (25). The VDCC N-type $Ca^{2+}$ channels are densely distributed in DRG neurons and spinal dorsal horn terminals associated with small-diameter C-type fibers and medium-sized A$\delta$ fibers, both of which are involved in neuropathic pain (9, 26, 27). Miyano et al. recorded a paclitaxel-induced increase in intracellular $Ca^{2+}$ concentration in cultured rat DRG neurons, suggesting that paclitaxel activates VDCCs (28). Therefore, our findings suggest that enhancement of VDCC current in small- and medium-diameter DRG neurons contributes to paclitaxel-induced mechanical allodynia/hyperalgesia and may be related to modulation of synaptic neurotransmission in pain processing pathways. We postulate that paclitaxel treatment induces peripheral neuropathies by enhancing VDCC current in small- and medium-diameter DRG neurons.

In the present study, we demonstrated that paclitaxel treatment (2 and 4 mg/kg) significantly increased the expression of $Ca_v\alpha_2\delta$-1 protein in the rat DRG, and immunohistochemistry revealed that this $Ca_v\alpha_2\delta$-1 expression was increased in DRG neurons. The data suggest that the upregulation of $Ca_v\alpha_2\delta$-1 in DRG neurons may contribute to the paclitaxel treatment–induced enhancement of VDCC current. This upregulation may be due to the onset of paclitaxel-induced mechanical allodynia. $Ca_v\alpha_2\delta$-1 mRNA and $Ca_v\alpha_2\delta$-1 protein are also increased in mouse DRG neurons following paclitaxel treatment. In addition, immunohistochemical analysis showed that $Ca_v\alpha_2\delta$-1 protein is also increased in medium/large-diameter DRG neurons following paclitaxel treatment (24). On the other hand, administration of paclitaxel (2 mg/kg, i.p.) significantly increases $Ca_v\alpha_2\delta$-1 protein in the rat spinal cord but not in the DRG (18). Single administration of paclitaxel (5 mg/kg) does not affect the expression of the $Ca_v\alpha_2\delta$-1 subunit mRNA in mouse DRG (29). Our results in DRG neurons are similar to those of Matsumoto et al. who found that paclitaxel treatment increases expression of $Ca_v\alpha_2\delta$-1 protein in the DRG (24). However, it is hard to explain the discrepancy between our results regarding $Ca_v\alpha_2\delta$-1 protein in the DRG and those by Xiao et al. who found that paclitaxel does not affect $Ca_v\alpha_2\delta$-1 protein in the DRG (18).

GBP, an analgesic and anti-epileptic agent, specifically binds to the $Ca_v\alpha_2\delta$-1 subunit of the VDCCs (11). The efficacy of GBP for the alleviation of neuropathic pain occurs via binding to the $Ca_v\alpha_2\delta$-1 subunit (30). In the present study, administration of GBP inhibited paclitaxel-induced mechanical hyperalgesia but not mechanical allodynia. An additional interesting finding is that 60-min incubation with 100 $\mu$M GBP significantly reduced VDCC current amplitude in medium-diameter DRG neurons but not in small-diameter DRG neurons. Several

studies have shown a small reduction in $Ca^{2+}$ currents when GBP is applied acutely, but others did not observe such an acute inhibitory effect on either heterologously expressed channels or endogenous $Ca^{2+}$ currents in DRG neurons in vitro (31 – 35). Furthermore, Alden and Garcia have shown that the effect of GBP depends on the state of the channel, and they suggest that it preferentially binds to inactivated $Ca^{2+}$ channels (36). In vivo, GBP produces a substantial inhibition of calcium currents when applied chronically but not acutely, and this effect occurs by reducing the expression of $Ca_v\alpha_2\delta$-1 subunits at the plasma membrane (34). Thus, the difference in expression of $Ca_v\alpha_2\delta$ subunits between cell types and the heterologous expression may represent the effect of GBP on $Ca^{2+}$ currents. Our findings may explain the efficacy of GBP as an analgesic, because calcium channels in medium-diameter DRG neurons but not small-diameter DRG neurons are selectively affected by GBP, which implicates the $Ca_v\alpha_2\delta$-1 subunit in modulation of VDCCs. Moreover, when $Ca^{2+}$ channels are predominantly activated by prolonged exposure to paclitaxel, which results in high levels of $Ca_v\alpha_2\delta$-1 subunits in the VDCCs of small- and medium-diameter DRG neurons, chemicals such as GBP may have an analgesic effect in paclitaxel-induced neuropathic pain. Moreover, the mechanism of action of GBP-induced analgesia may differ in different types of chemotherapy-induced pain, and the causal role of an increase in VDCC currents in small- and medium-diameter DRG neurons, such as with paclitaxel, may differ in other chemotherapy-induced neuropathic pain syndromes. The present study indicates that paclitaxel treatment may alter the sensitivity of the $Ca_v\alpha_2\delta$-1 subunit of VDCCs in small- and medium-diameter DRG neurons. Further studies are necessary to define the role of paclitaxel and GBP on calcium channels that mediate primary afferent sensory neurons.

In conclusion, we show that paclitaxel treatment increases the VDCC current in small- and medium-diameter DRG neurons. Moreover, GBP inhibits paclitaxel-induced mechanical hyperalgesia and enhancement of VDCC current in DRG neurons.

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