Pharmacological Differences Between Static and Dynamic Alloodynia in Mice With Herpetic or Postherpetic Pain

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Abstract. In the present study, we investigated whether dynamic and static alloodynia would be developed in the affected dermatome in murine models of herpetic pain and postherpetic neuralgia and pharmacologically characterized the alloodynia. Inoculation with herpes simplex virus type-1 on the femur induced skin lesions in the dermatome including the plantar region of the hind paw from day 5 to day 21 after inoculation. Dynamic alloodynia became apparent in the hind paw from day 3 to at least day 42. Static alloodynia was not obvious during the stage of herpetic pain and gradually increased after the lesion healing. Mexiletine hydrochloride (30 mg/kg, p.o.) and ketamine hydrochloride (50 mg/kg, i.p.) produced a moderate attenuation of static but not dynamic alloodynia. Diclofenac sodium (50 mg/kg, i.p.) did not affect both static and dynamic alloodynia. Gabapentin (30 mg/kg, p.o.) markedly inhibited both static and dynamic alloodynia. Developmental and pharmacological differences between static and dynamic alloodynia suggest that independent mechanisms are responsible for dynamic and static alloodynia. This murine model may be useful for the study of the mechanisms of dynamic alloodynia of herpetic pain or postherpetic neuralgia and the development of new analgesics effective against the dynamic alloodynia.

Keywords: dynamic and static alloodynia, herpes simplex virus type-1, herpetic pain, postherpetic neuralgia, dorsal root ganglion

Introduction

Herpes zoster characterized by clustered vesicles and severe pain is caused by the reactivation of human herpesvirus 3 (varicella-zoster virus) in the sensory ganglion in human subjects (1). In some herpes zoster patients, pain persists long after healing of the skin lesions, which is postherpetic neuralgia (1). Once established, postherpetic neuralgia is particularly difficult to treat and is often resistant to conventional analgesics (2). Patients with postherpetic neuralgia report various types of pain, including a continuous burning or aching pain and a periodic piercing pain (1). In addition, a large proportion, nearly 90% (3), describes tactile alloodynia, which is a painful sensation elicited by normally innocuous light mechanical stimulation, in the affected and adjacent dermatomes (4).

It has been reported that two distinct types of tactile alloodynia are detected in patients suffering from neuropathic pain including postherpetic neuralgia (5). They have been termed static and dynamic alloodynia according to the type of stimulation; static alloodynia is induced by applying light static pressure to the skin, whereas the dynamic type is elicited by lightly stroking the skin with a soft brush (6). In many patients, tactile, especially dynamic, alloodynia is so severe that the quality of life is decreased and dynamic alloodynia is a hallmark symptom of herpes zoster and postherpetic
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neuralgia. Static and dynamic allodynia have been claimed to be mediated by unmyelinated and myelinated primary afferents, respectively, in humans with neuropathic pain (6). Similarly, static and dynamic allodynia have been shown to be mediated by capsaicin-sensitive and insensitive nerve fibers, respectively, in rats with neuropathy induced by sciatic nerve injury (7, 8). These findings suggest that these two kinds of allodynia represent discrete pathophysiological entities. Static allodynia has been used as a behavioral measure of pain in various animal models of neuropathic pain (9, 10). It has been shown to be mediated by many mediators such as adenosine 5'-triphosphate, dynorphin, lysophosphatidic acid, nitric oxide, and substance P (11 – 15). However, a few studies have evaluated dynamic allodynia and mediators involved in dynamic allodynia remain unknown.

We have previously established mouse models of herpetic pain and postherpetic neuralgia (16, 17). When mice are given transdermal inoculation with human herpesvirus 1 (herpes simplex virus type-1, HSV-1), they show herpes zoster–like skin lesions throughout the inoculated dermatome and static allodynia in the adjacent dermatome in which there are no vesicles (16). However, static allodynia was not apparent in the affected dermatome (A. Sasaki et al., unpublished observation). In the present study, therefore, we investigated whether dynamic allodynia would develop in the affected dermatome in mouse models of herpetic pain and postherpetic neuralgia and pharmacologically characterized dynamic allodynia.

Materials and Methods

Animals

Female C57BL/6J mice (Japan SLC, Shizuoka) were used; they were six-weeks-old at the start of experiments. Housing (six per cage) and behavioral experiments were done under controlled temperature (22 ± 1°C) and humidity (55 ± 10%). The observation room was lit from 7:00 AM to 7:00 PM and during the behavioral test. Food and water were freely available. Experiments were conducted with the approval of the Animal Care Committee at University of Toyama. Behavioral pain test was done according to the guidelines for investigations of experimental pain in animals published by the International Association for the Study of Pain (18).

HSV-1 inoculation

The mice were inoculated with HSV-1, as described previously (16), except for inoculation site. Briefly, 10 μl of a suspension of HSV-1 (7401H strain, 1 × 10^6 plaque-forming units) was administered topically on the scarified skin of the femur of the right hind paw. The contralateral hind paw was without inoculation. At the progress stage (until day 8 after inoculation), skin lesions were scored as follows: 0 = no lesions; 2 = one or two vesicles on the back; 4 = many vesicles on the back, the surrounding inoculated area, or both; 6 = mild herpes zoster–like lesions; 8 = apparent zoster-like lesions, paw inflammation, or both; 10 = severe zoster-like lesions. At the recovery stage (from day 10 after inoculation), skin lesions were scored as follows: 10 = severe herpes zoster–like lesions; 5 = the presence of scabs flaking off from cutaneous lesions; 0 = complete recovery of the lesions (17).

Assessment of static and dynamic allodynia

The animals were acclimated to an observation cage with a wire mesh bottom for at least 30 min before the behavioral test. Static allodynia was assessed by punctate stimulation of the plantar region of the hind paw with von Frey filament (North Coast Medical Inc., Morgan Hill, CA, USA), as described previously (16, 17); a filament with a bending force of 1.6 mN was pressed perpendicularly against the plantar surface of the hind paw and held for 1 – 3 s with it slightly buckled. Dynamic allodynia was assessed by light stroking of the plantar surface of the hind paw from the toe to the heel with an art paint brush [Artetje (round) Camlon-Pro 720™ #4/0; Chugoku Art Material Inc., Okayama], the hairs of which were trimmed with 10 hairs left (Fig. 1). Stimulation of the hind paw of the naive mouse with von Frey filament of 1.6 mN or less strength elicits practically no behavioral responses. On the other hand,
the naive mice occasionally move the stimulated paw aside following stroking stimulation with the paint brush; they often glance at but not lick the paw. When the affected hind paw is stimulated with von Frey filament or the paint brush, the mice often lift the stimulated paw toward the abdomen. Based on these behavioral observations, responses to the stimulus were ranked as follows: 0, no response or moving the stimulated paw aside; 1, lifting of the stimulated paw toward the abdomen; 2, flinching or licking of the stimulated paw. The stimulation was applied six times to the hind paw at intervals of several seconds and the average served as pain-related score. For the experiments on the effects of medicines at the stage of postherpetic pain, mice that showed 0.5 or higher pain-related score were considered to have tactile allodynia (19).

**Immunohistochemistry**

Under deep anesthesia with intraperitoneal (i.p.) injection of sodium pentobarbital (70 mg/kg), the mice were transcardially perfused with 15 ml phosphate-buffered saline (PBS, pH 7.4) and subsequently, with 15 ml of 4% paraformaldehyde in PBS. The dorsal root ganglion (DRG) at the L5 level on the inoculated side were removed and post-fixed in the same fixative at 4°C for 4 h. The DRG was then transferred to 30% sucrose in PBS at 4°C overnight. They were cut on a freezing microtome (Leica CM 3050S IV; Leica, Nussloch, Germany) at a 16-μm thickness. They were incubated in blocking solution (1.5% fetal bovine serum and 0.2% Triton X-100 in PBS) for 30 min at room temperature and then with fluorescein isothiocianate-conjugated polyclonal rabbit anti-HSV-1 antibody (1:100; DAKO Japan Co., Ltd., Kyoto) and biotinylated mouse monoclonal antibody against NeuN, a neuron-specific DNA-binding nuclear protein, (1:100; Chemicon, Temecula, CA, USA) overnight at 4°C in a humid chamber. After washing with PBS, they were incubated with avidin-Alexa 593 (1:1000; Molecular Probes, Eugene, Oregon, USA) for 2 h at room temperature and coverslipped with glycerol-PBS containing 2.5% triethylenediamine. Fluorescence signals were observed using a Bio-Rad Radiance 2000 Confocal system (Bio-Rad Microscopy Division, Cambridge, MA, USA).

**Agents**

Mexiletine hydrochloride was purchased form Sigma-Aldrich (St. Louis, MO, USA). Ketamine hydrochloride and dicyfenac sodium were purchased from Research Biochemical International (Natick, MA, USA). Gabapentin was synthesized by HT. All agents were dissolved in 0.5% carboxymethyl cellulose (Wako Pure Chemical Industries, Ltd., Osaka) in physiological saline. Ketamine hydrochloride and dicyfenac sodium were administered i.p. Mexiletine hydrochloride and gabapentin were administered orally (p.o.). Administration was done in a volume of 0.1 ml/10 g of body weight. Effects of the agents on herpetic and postherpetic allodynia were tested on day 7 and day 30 – 40 after inoculation, respectively.

**Data analyses**

The means of data are presented together with S.E.M. Data on the time course of allodynia and anti-allodynic effects were analyzed with Friedman repeated measures analysis of variance on ranks and then with Dunnett’s test. P<0.05 was considered significant.

**Results**

**Herpes zoster–like skin lesions**

No vesicles appeared until day 4 after inoculation and a few vesicles erupted on the back on the inoculated side on day 5. Thereafter, the vesicles spread within a dermatome on the inoculated side (Fig. 2A). Some vesicles were also observed in the plantar region of the hind paw on day 7 after inoculation. Skin lesions peaked around day 7 and subsided by day 21 (Fig. 2).

![Fig. 2.](image-url)
The DRG at the L5 level was removed from mice on day 5 after inoculation, a day when HSV-1 proliferation peaked (16, 20). There were many HSV-1 antigen-positive neurons in the DRG (Fig. 3); positive neurons were $28.2 \pm 9.7\%$ ($n = 3$) of total neurons. The neurons that were strongly positive for HSV-1 antigen were mostly negative for NeuN (Fig. 3).

Static and dynamic alldynia in the affected dermatome

Dynamic and static alldynia of individual mice are shown in Fig. 4, A and B, respectively. HSV-1 inoculation induced dynamic alldynia in the plantar region of the hind paw, an affected dermatome; it became apparent in some mice on day 3 after inoculation, was obvious in all inoculated mice on day 7, and slightly increased from day 7 to day 21 (Fig. 4: A, C). All mice showed dynamic alldynia even after the complete cure of the skin lesions (day 21 – 42 after inoculation) (Fig. 4: A, C, D). On the other hand, static alldynia was observed in three (43%) of seven mice on day 7 after inoculation (Fig. 4: B – D). It gradually increased and mild static alldynia was observed in four (57%) of seven mice on day 35 after inoculation (Fig. 4: C, D).

Effects of medicines on dynamic alldynia in mice with herpetic pain

The effects of analgesic adjuvants and analgesic on gross behaviors were tested in naive mice. Mexiletine hydrochloride (30 mg/kg, p.o.) induced mild sedation from 15 to 30 min, and a higher dose of 60 mg/kg induced prostrate posture and passivity from 15 to 60 min. Therefore, the effect of 30 mg/kg on alldynia was tested in the following experiments. Ketamine hydrochloride induced a mild ataxia at 15 min at an i.p. dose of 25 mg/kg and induced severe ataxia from 15 to
A 30 min at a higher dose of 50 mg/kg. Apparent alterations in gross behaviors and motor dysfunction were not observed after the administration of gabapentin (10 and 30 mg/kg, p.o.) and diclofenac sodium (25 and 50 mg/kg, i.p.). We tested the effects of the agents on allodynia 60 and 120 min after administration in the following experiments.

Since dynamic, but not static, allodynia was marked in the region of herpes zoster-like lesions, the effects of the above agents on dynamic allodynia were examined; we tested their efficacy on day 7 after inoculation, a day when dynamic allodynia reached almost maximum and skin lesions peaked. Gabapentin (10 and 30 mg/kg, p.o.) produced the dose-dependent inhibition of dynamic allodynia; the 30 mg/kg dose of gabapentin produced about 85% inhibition (Fig. 5A). Moxifloxacin hydrochloride (30 mg/kg, p.o.), ketamine hydrochloride (25 and 50 mg/kg, i.p.), and diclofenac sodium (25 and 50 mg/kg, i.p.) were without effects on the dynamic allodynia (Fig. 5).

**Effects of medicines on dynamic and static allodynia in mice with postherpetic pain**

In this series of experiments the mice that showed allodynia day 30–40 after inoculation were used. Dynamic allodynia was significantly inhibited by gabapentin (30 mg/kg, p.o.), but moxifloxacin hydrochloride (30 mg/kg, p.o.), ketamine hydrochloride (50 mg/kg, i.p.), and diclofenac sodium (50 mg/kg, i.p.) were without effects (Fig. 6A). Static allodynia was inhibited by gabapentin (30 mg/kg, p.o.), moxifloxacin hydrochloride (30 mg/kg, p.o.), and ketamine hydrochloride (50 mg/kg, i.p.), but diclofenac sodium (50 mg/kg, i.p.) was without effect (Fig. 6B). Gabapentin inhibited more markedly static allodynia than dynamic allodynia (Fig. 6).

**Discussion**

In humans, dynamic and static allodynia are present in the affected dermatome of patients suffering from acute herpes zoster and postherpetic neuralgia (5, 21). In mice, dynamic allodynia was clearly observed in the affected dermatome and persisted long after the healing of the skin lesions. Dynamic allodynia appeared before the eruption of vesicles. This observation is consistent with clinical reports that 70%–80% of herpes zoster patients have a prodrome of dermatomal pain beginning several days before the vesicle eruption (22, 23). Static allodynia was not obvious in the affected dermatome during the acute herpetic pain and gradually increased.
after the lesion healing. It has been shown that static allodynia becomes apparent in the adjacent dermatome on the day of eruption (16, 17). About 20% of the DRG neurons were positive for HSV antigen in these experiments (20), and about 28% of the neurons were positive in the present study. The similarity suggests that the difference in the time course of static allodynia is due to the difference between the affected and adjacent dermatomes but not to the extent of infection.

HSV-1 antigen-positive neurons were negative for NeuN, a neuron-specific marker, suggesting that the expression of NeuN is markedly suppressed in sensory neurons in which HSV-1 proliferated. Although HSV-1 infection induces the expression of cyclooxygenase-2 in the sensory neurons, the induction is not observed in infection.Treatment prevents anterograde spread of HSV along the primary afferents (27). Considering that static allodynia is mediated mainly by unmyelinated and capsaicin-sensitive primary afferents (6 – 8), the functional suppression by HSV-1 of these primary afferents innervating the affected dermatome may be a cause of difference in the time-course of static allodynia between affected and adjacent dermatomes.

Dynamic allodynia was more prominent and frequent than static allodynia in HSV-1-inoculated mice. It should be noted that the onset and time-course of dynamic and static allodynia were different from each other. This developmental difference between dynamic and static allodynia is consistent with the idea that these two kinds of mechanical allodynia represent discrete pathophysiological entities.

Mexiletine hydrochloride (30 mg/kg, p.o.), an orally active local anesthetic, produced a moderate attenuation of static allodynia at the postherpetic stage, but it did not inhibit dynamic allodynia at the herpetic and postherpetic stages. The differential blockade of conduction by local anesthetics in nerve fibers of different diameter has been reported. For example, small myelinated Aδ-fibers are more susceptible to local anesthetic block than larger myelinated Aβ-fibers (28).

With these findings taken into account, the present results suggest that static and dynamic allodynia are induced by the stimulation of small myelinated Aδ- and large myelinated Aβ-fibers, respectively.

Ketamine hydrochloride (50 mg/kg, i.p.), an NMDA glutamate–receptor antagonist, produced a moderate attenuation of static allodynia, but it did not suppress dynamic allodynia at herpetic and postherpetic stages. Stimulation of NMDA glutamate receptors in the spinal dorsal horn activates neuronal nitric oxide synthase to produce nitric oxide (29). In this context, the activity of neuronal nitric oxide synthase increases in the dorsal horn of mice with postherpetic allodynia, which is inhibited by 7-nitroindazole, an inhibitor of neuronal nitric oxide synthase (15). Thus, NMDA receptors may be involved in static allodynia at the postherpetic stage through the increase of nitric oxide production. In contrast, ketamine did not inhibit dynamic allodynia, which raises the possibility that neither the NMDA receptor nor neuronal nitric oxide synthase is not involved in the dynamic allodynia.

Gabapentin at a dose of 30 mg/kg almost completely inhibited static allodynia at the postherpetic stage. Gabapentin at this dose produced almost complete and partial inhibition of dynamic allodynia at herpetic and postherpetic stages, respectively. It may be more effective against static allodynia than dynamic allodynia in mice with postherpetic pain. In humans, although relatively high doses (900 – 3,600 mg/day) are needed, gabapentin is effective against postherpetic neuralgia without severe adverse effects (30). Gabapentin may inhibit pain mainly through action on the spinal dorsal horn (31). Although the analgesic mechanisms of gabapentin have not been fully elucidated, the involvement of α2δ subunit of voltage-dependent calcium channel in the sensory neuron has been suggested (32). Therefore, it may be interesting to examine whether alterations in the expression of the channel subunit in the primary sensory neurons are associated with the development of dynamic and static allodynia.

Diclofenac sodium (50 mg/kg), a nonsteroidal anti-inflammatory drug, affected neither static nor dynamic allodynia in mice with postherpetic pain. It was previously shown that static allodynia at the postherpetic stage was insensitive to diclofenac sodium in mice (17). These results suggest that prostaglandins are not involved in both static and dynamic allodynia in mice with postherpetic pain. Diclofenac sodium (25 and 50 mg/kg, i.p.) did not affect dynamic allodynia in mice with herpetic pain. The similar doses of diclofenac markedly inhibited static allodynia of non-affected dermatome in mice with herpetic pain (20). The static allodynia is also markedly inhibited by deficiency of the EP3 prostanoid receptor (24). Thus, unlike static allodynia, the increased production of prostaglandins may not be involved in dynamic allodynia in mice with
herpetic and postherpetic pain.

At the stage of postherpetic pain, static allodynia of the affected and adjacent dermatomes was pharmacologically similar to each other; both were suppressed by gabapentin and mexiletin, but not by diclofenac (ref. 17 and the present study). In contrast, dynamic and static allodynia of the affected dermatome at the stage of postherpetic pain were pharmacologically different from each other. Mexiletine and ketamine inhibited static, but not dynamic, allodynia in mice with postherpetic pain. The opioid morphine and the anti-depressant amitriptyline were reported to suppress static, but not dynamic, allodynia in rats with streptozocin-induced diabetes (33). Gabapentin inhibited both static and dynamic allodynia in mice with postherpetic pain, but the inhibition was more marked in static allodynia than in dynamic allodynia. Similarly, this anticonvulsant inhibits static and dynamic allodynia in rats with streptozocin-induced diabetes, but higher doses are needed to inhibit dynamic allodynia (33). The precise reason for the pharmacological differences between dynamic and static allodynia is not obvious, but such differences may be partly due to the involvement of different primary afferents in dynamic and static allodynia (6–8). Considering that dynamic allodynia is a hallmark symptom of herpes zoster and postherpetic neuralgia and that dynamic allodynia is more resistant to the analgesics and analgesic adjuvants than is static allodynia, it is necessary to search compounds effective against dynamic allodynia.

In summary, dynamic allodynia is more prominent and frequent than static allodynia in the affected dermatome of mice with herpetic or postherpetic pain. Developmental and pharmacological differences between static and dynamic allodynia suggest that independent mechanisms are responsible for dynamic and static allodynia. This murine model may be useful for the study of the mechanisms of dynamic allodynia of herpetic pain or postherpetic neuralgia and the development of the new analgesics effective against the dynamic allodynia.

References
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