Roles of $\alpha$-Adrenoceptors and Sympathetic Nerve in Acute Herpetic Pain Induced by Herpes Simplex Virus Inoculation in Mice

Atsushi Sasaki$^1$, Ichiro Takasaki$^1$, Tsugunobu Andoh$^1$, Hiroshi Nojima$^1$, Kimiyasu Shiraki$^2$, and Yasushi Kuraishi$^1$*

$^1$Department of Applied Pharmacology, Faculty of Pharmaceutical Sciences, and $^2$Department of Virology, Faculty of Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

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Abstract. Percutaneous inoculation with herpes simplex virus type-1 brings about herpes zoster-like skin lesions, tactile allodynia, and mechanical hyperalgesia in mice. This study was conducted to determine whether the sympathetic nervous system and $\alpha$-adrenoceptors would be involved in these pain-related responses and whether the $\alpha_2$-adrenoceptor agonist clonidine would suppress these responses. The adrenergic neuron blocker guanethidine and the non-selective $\alpha$-adrenoceptor antagonist phentolamine did not affect the pain-related responses, although these agents suppressed the pain-related responses induced by partial ligation of the sciatic nerve. The pain-related responses induced by herpetic inoculation was suppressed by intraperitoneal and intrathecal injections, but not by intraplantar and intracerebroventricular injections, of clonidine. The suppressive effect of an intraperitoneal injection of clonidine (0.1 mg/kg) was antagonized by intrathecal injections of phentolamine and the $\alpha_2$-adrenoceptor antagonist yohimbine, but not the $\alpha_1$-adrenoceptor antagonist prazosin. The results suggest that sympathetic nerves and $\alpha$-adrenoceptors are not involved in the pain-related responses induced by herpetic infection. Clonidine suppresses the responses probably through the action on $\alpha_2$-adrenoceptors in the dorsal horn.

Keywords: acute herpetic pain, clonidine analgesia, $\alpha_2$-adrenoceptor, guanethidine, sympathetic nerve

Introduction

Herpes zoster is caused by reactivation of dormant varicella-zoster virus in the dorsal root ganglion and usually accompanied by severe pain (1). Herpetic pain includes continuous aching and burning sensations and often allodynia, which is precipitated by touching or moving the involved area (2). The pathophysiological mechanisms of herpetic pain remain poorly understood. Acute herpetic pain is treated with combination therapy, including antiviral agents, antidepressants, corticosteroids, opioids, and nonsteroidal and steroidal anti-inflammatory drugs (3). However, severe herpetic pain is not well alleviated by these medicines and sympathetic nerve block is also tried (4). Under normal conditions, efferent sympathetic nerve activity is functionally separate from that of primary afferent neurons (5). On the other hand, in the presence of nerve injury and tissue inflammation, chemical and anatomical coupling may mediate pain by acting on $\alpha_2$-adrenoceptors on the sympathetic terminals to release prostaglandins (6) or on $\alpha_2$-adrenoceptors on the primary afferents to evoke abnormal activation (5). When the sensory nerve is injured, pain is precipitated or exacerbated by the increased activity of sympathetic nerve and by an injection of adrenaline or noradrenaline (7, 8). Such clinical condition is referred to as sympathetically maintained pain. Sympathetic blocks have been traditionally used for acute herpetic pain. Despite many reports describing success, the role of sympathetic nerves in acute herpetic pain remains unclear.

Clonidine and other $\alpha_2$-adrenoceptor agonists have been used in clinical practice for the treatment of pain, especially postoperative pain, neuropathic pain, and...
sympathetically maintained pain (9, 10). The spinal cord, brain, and periphery have been claimed to be the site of analgesic action. With regard to the spinal cord, continuous epidural infusion of clonidine reduces pain intensity of cancer patients with neuropathic pain (9). In animal experiments, intrathecal and epidural injections of \( \alpha_2 \)-adrenoceptor agonists produce antinociception (11, 12). One possible mechanism is the inhibition of sympathetic preganglionic neurons in the lateral column of the spinal cord (12). Another possible mechanism is the inhibition of sympathetic postganglionic neurons (11, 12). One possible mechanism is the inhibition of sympathetic terminals to release prostaglandins (6) or directly on the primary afferents to evoke abnormal activation (19, 20). In contrast, topical application of clonidine relieves sympathetically maintained pain and painful diabetic neuropathy (21, 22). Intra-articular injection of clonidine produces analgesia in patients submitted to arthroscopic knee surgery (23) and antinociception in animals (24). Thus, the analgesic potency and site of action of clonidine may depend on the type of pain.

We have recently found that inoculation with herpes simplex virus type-1 (HSV-1) causes herpes zoster-like skin lesions and pain-related responses in mice (25). When inoculated with HSV-1 of the hind paw, the skin lesions developed in a corresponding dermatome after 4-day latent period. The mice show pain-related responses (allodynia and hyperalgesia) to mechanical stimulation, which may be due to the proliferation of the virus in the dorsal root ganglia (25, 26). The responses are suppressed by a systemic injection of the aspirin-like drug diclofenac, the opioid morphine, or the anti-convulsant gabapentin (26, 27). However, the effect of clonidine on and the role of sympathetic activity in the acute herpetic pain remain unknown. Therefore, we conducted the present experiments to determine whether the sympathetic nervous system and \( \alpha_2 \)-adrenoceptors would be involved in the pain-related responses induced by herpetic infection and whether clonidine would suppress the responses.

### Materials and Methods

#### Animals

Female BALB/c mice (Japan SLC, Shizuoka) were used; they were 6 weeks old and weighing 18 – 20 g at the start of experiments. They were housed six per cage under controlled temperature (22 ± 1°C) and humidity (55 ± 10%). The room was lighted from 7:00 AM to 7:00 PM and during the behavioral test. Food and water were freely available. HSV-1 inoculation and behavioral experiments were done in the infection room of the Molecular Genetics Research Center, Toyama Medical and Pharmaceutical University. Procedures for animal experiments were approved by the Committee for Animal Experiments at Toyama Medical and Pharmaceutical University.

#### Virus inoculation

The mice were inoculated with HSV-1 as described previously (25). Briefly, 10 \( \mu l \) of suspension containing HSV-1 (7401H strain, 1 \( \times 10^6 \) plaque-forming units) was applied to the scarified skin of the right shin (5 \( \times 5 \) mm). The contralateral hind paw was without inoculation.

#### Sciatic nerve ligation

Under ether anesthesia, 1/3 to 1/2 dorsal portion of the sciatic nerve was ligated with a 7-0 silk suture (28). The sciatic nerve on the contralateral side was exposed without ligation.

#### Drugs

Clonidine hydrochloride, phentolamine hydrochloride, prazosine hydrochloride, guanethidine monosulfate, and yohimbine hydrochloride, all purchased from Sigma (St. Louis, MO, USA), were dissolved in physiological saline. Intrathecal (i.t.) and intracerebroventricular (i.c.v.) injections were given in a volume of 5 \( \mu l \) and intraplantar (i.pl.) injection was given in a volume of 20 \( \mu l \). Intraperitoneal (i.p.) injection was given in a volume of 0.1 ml/10 g. Antagonists were given by i.t. injection 15 min before clonidine injection. These agents were administered on day 6 post-inoculation, with the exception of two experiments in which guanethidine and phentolamine were administered 7 days after nerve ligation.

#### Pain tests

Pain tests were performed according to the guidelines published in a Guest Editorial in Pain on ethical standards for investigations of experimental pain in animals (29). Tactile allodynia and mechanical hyperalgesia of the hind paw were assessed as described (25). The mice...
were placed individually in a plastic cage (11 × 18 × 15 cm) with a wire mesh bottom. After at least 15-min acclimation period, von Frey filaments with bending forces of 0.17 g (innocuous stimulation) or 1.20 g (noxious stimulation) were pressed perpendicularly against the plantar skin and held for 3 – 5 s with it slightly buckled. The responses to these stimuli were ranked as follows: 0, no response; 1, move away from von Frey filament; 2, immediate flinching or licking of the hind paw. The stimulation of the same intensity was applied six times to each hind paw at intervals of several seconds and the average served as the pain-related score. Thermal paw withdrawal latencies were determined by exposing the hind paw to a focused beam of radiant heat using an Ugo Basile tail flick unit (cut-off latency was 30 s). Baseline latencies were determined by averaging three measurements, and infrared intensity of radiant heat was set to a 1 that baseline latency was about 13 s in the normal condition.

Locomotor activity and motor coordination
Mice were placed individually in a wheel cage (25 cm in diameter and 6-cm-wide), and spontaneous locomotor activity was measured as the number of wheel revolutions for 60 min after clonidine injection.

Motor function was tested using an accelerating rotarod for mice (model 7650; Ugo Basile, Comerio, Italy). The apparatus consists of a base platform and a rotating rod of 3-cm diameter with a non-skid surface. The rod of 50 cm in length is divided into five equal sections by six disks. Five mice were tested simultaneously. The treadmill was set to accelerate from 4 to 40 rev/min in a period of 5 min. The integrity of motor coordination was assessed as the performance time on the rod measured from acceleration start until fall from the drum.

Statistical analyses
Unless otherwise mentioned, the means of data are presented together with the S.E.M. Data on the time course of analgesic effects were analyzed with Friedman repeated measures analysis of variance on ranks and then with Dunnett’s test. Other data were analyzed with Dunnett’s test for numeric values and the Wilcoxon signed rank test or Kruskal-Wallis analysis of variance on ranks followed by Dunnett’s test for rank data. A P<0.05 value was considered significant.

Results
HSV-1-induced allodynia and hyperalgesia
HSV-1 inoculation on the hind paw produced tactile allodynia (response to 0.17-g von Frey filament) and mechanical hyperalgesia (increased response to 1.20-g von Frey filament), but not thermal hyperalgesia (reduction in the latency of withdrawal response to heat stimulation) (Fig. 1). HSV-1 inoculation did not affect the responses of the contralateral hind paw to mechanical and thermal stimuli (data not shown). In the following experiments, the effects of agents on tactile allodynia and mechanical hyperalgesia were tested on day 6 post-inoculation.

Effects of systemic clonidine on tactile allodynia and mechanical hyperalgesia
An i.p. injection of clonidine (0.03 – 0.3 mg/kg) produced the dose-dependent inhibition of tactile allodynia and mechanical hyperalgesia; the higher doses of 0.1 and 0.3 mg/kg decreased the responses to 1.20-g von Frey filament to under the normal level (Fig. 2: A and B). The highest dose of 0.3 mg/kg also significantly suppressed the responses of the contralateral hind paw, and a dose of 0.1 mg/kg also significantly decreased the response (Fig. 2D).

An i.p. injection of clonidine (0.03 – 0.3 mg/kg) did not affect spontaneous locomotor activity during a 60-min period after injection (Fig. 3). When the mice were tested for rotarod ability 30 min after clonidine injection, low doses of clonidine (0.03 and 0.1 mg/kg) did not affect the ability, whereas the highest dose (0.3 mg/kg) reduced it (Fig. 3B). Similar results were also given by 15-min pretreatment with clonidine.
An i.t. injection of clonidine (0.03 – 0.3 μg/animal) produced a dose-dependent inhibition of both tactile allodynia and mechanical hyperalgesia without effects on the contralateral hind paw (Fig. 4: A and B). The effect of the highest dose (0.3 μg/animal) peaked 15 – 30 min after injection and subsided by 60 min. In contrast, i.pl. injections of clonidine (0.03 – 0.3 μg/animal) into the affected hind paw did not inhibit tactile allodynia and mechanical hyperalgesia (Fig. 4: C and D). I.c.v. injections of clonidine (0.03 – 0.3 μg/animal) also did not affect tactile allodynia and mechanical hyperalgesia (Fig. 4: E and F).

Site of action of clonidine

An i.t. injection of clonidine (0.03 – 0.3 μg/animal) produced a dose-dependent inhibition of both tactile allodynia and mechanical hyperalgesia without effects on the contralateral hind paw (Fig. 4: A and B). The effect of the highest dose (0.3 μg/animal) peaked 15 – 30 min after injection and subsided by 60 min. In contrast, i.pl. injections of clonidine (0.03 – 0.3 μg/animal) into the affected hind paw did not inhibit tactile allodynia and mechanical hyperalgesia (Fig. 4: C and D). I.c.v. injections of clonidine (0.03 – 0.3 μg/animal) also did not affect tactile allodynia and mechanical hyperalgesia (Fig. 4: E and F).

Effects of i.t. pretreatment with α-adrenoceptor antagonists on the antiallodynic and antihyperalgesic effects of systemic clonidine

In order to confirm the spinal action of systemic clonidine and to clarify α-adrenoceptor subtypes involved, several α-adrenoceptor antagonists were injected intrathecally 15 min before clonidine (0.1 mg/kg, i.p.). An i.t. pretreatment with the α-adrenoceptor antagonist prazosin or the α₂-adrenoceptor antagonist yohimbine, but not the α₁-adrenoceptor antagonist phentolamine, blocked dose dependently the antiallodynic and antihyperalgesic effects of systemic clonidine (Fig. 5). The highest dose of phentolamine or yohimbine (10 μg/animal) completely blocked the effect of clonidine (Fig. 5).
Acute Herpetic Pain and \( \alpha \)-Adrenoceptor Effects of guanethidine and phentolamine on allodynia and hyperalgesia induced by HSV-1 inoculation and sciatic nerve injury

Partial sciatic nerve ligatition produced tactile allodynia and mechanical hyperalgesia in mice, without effects on the contralateral hind paw. These pain-related responses became apparent since a few hours after ligation and persisted at least until day 14 (data not shown).

The effects of guanethidine and phentolamine were tested on day 7 after ligation. An i.p. injection of guanethidine (60 mg/kg) produced a marked inhibition of tactile allodynia and almost complete inhibition of mechanical hyperalgesia (Fig. 6: A and B). The lower dose of 30 mg/kg produced a slight inhibition of allodynia (Fig. 6A). On the other hand, tactile allodynia and mechanical hyperalgesia induced by HSV-1 inoculation were not affected by guanethidine at the same doses tested (Fig. 6: C and D). Guanethidine at doses tested did not affect the pain-related responses of the hind paw contralateral to either nerve ligation or HSV-1 inoculation (Fig. 6).

An i.p. injection of phentolamine (10, 30 mg/kg) produced a significant inhibition of tactile allodynia and a decreased tendency of mechanical hyperalgesia induced by the nerve ligation (Fig. 7: A and B). On the other hand, tactile allodynia and mechanical hyperalgesia induced by HSV-1 inoculation were not affected.
by phentolamine at the same doses tested (Fig. 7: C and D). The pain-related responses of the hind paw contralateral to either nerve ligation or HSV-1 inoculation were not affected by phentolamine at the doses tested (Fig. 7).
Discussion

The mice inoculated with HSV-1 exhibited tactile allodynia and mechanical hyperalgesia since day 5 after inoculation, which is consistent with the previous report (25). The tactile allodynia and mechanical hyperalgesia were not affected by the adrenergic neuron blocker guanethidine and the $\alpha$-adrenoceptor antagonist phentolamine, suggesting that the sympathetic nervous system does not play a key role in the pain-related responses induced by HSV-1 inoculation. In contrast, tactile allodynia and mechanical hyperalgesia after the partial ligation of the sciatic nerve were inhibited by guanethidine and phentolamine. The results are consistent with the reports of other groups (30, 31) and support the idea that the sympathetic nervous system is involved in pain induced by surgical injury to the sensory neurons. Thus, the role of the sympathetic nervous system in herpetic pain, if there is any, may be different from that in the surgically induced neuropathic pain.

Another marked difference between HSV-1 inoculation and surgical injury is the pain-related response to thermal stimulation. In the present experiments, the mice did not show thermal hyperalgesia in the inoculated hind paw. On the other hand, surgical injury to sensory nerve produces thermal hyperalgesia (30, 32). PGI$_2$ and IP receptors are involved in thermal hyperalgesia induced by nerve injury (33). However, HSV-1 inoculation increases the production of PGE$_2$, but not PGI$_2$, in the affected dorsal root ganglia (34). No increase in PGI$_2$ may be the reason why thermal hyperalgesia was not induced by HSV-1 inoculation.

Acute herpetic pain-related responses were inhibited by systemic injection of clonidine. The highest dose tested (0.3 mg/kg) partially but significantly inhibited the rotarod performance. On the other hand, a lower dose (0.1 mg/kg) inhibited allodynia and hyperalgesia without effects on rotarod performance and locomotor activity, suggesting that clonidine inhibits acute herpetic pain without sedation and motor incoordination. I.t. injections of clonidine produced dose-dependent antiallodynic and antihyperalgesic effects, and i.p.l. and i.c.v. injections were without effects. The results suggest that the inhibition by clonidine of acute herpetic pain is mainly mediated by the action in the spinal cord, but not in the periphery and brain. This idea is supported by the results that antiallodynic and antihyperalgesic effects of systemic clonidine were almost completely antagonized by i.t. injections of the $\alpha$-adrenoceptor antagonists phentolamine and yohimbine. There are reports that i.t. injection of clonidine produces antinoceptive effects in normal animals (35, 36), suggesting that clonidine can inhibit physiological pain. However, in the present experiments, i.t. clonidine suppressed the pain-related responses of the affected hind paw without effects on the responses of normal hind paw to noxious stimulation. Thus, it is suggested that i.t. injection of clonidine at relatively low doses can relieve herpetic pain without effects on physiological pain. As mentioned in the Introduction, there are two possible mechanisms for the spinal analgesic action of clonidine: the inhibition of sympathetic preganglionic neurons and inhibition of the transmission of pain signals in the dorsal horn. The latter is more likely than the former, because the sympathetic nervous system may not play a key role in the pain-related responses induced by HSV-1 inoculation.

The antiallodynic and antihyperalgesic effects of clonidine were inhibited by i.t. injections of the non-selective $\alpha$-adrenoceptor antagonist phentolamine and the selective $\alpha_2$-adrenoceptor antagonist yohimbine, but not by the selective $\alpha_1$-adrenoceptor antagonist prazosin. The results suggest the importance of $\alpha_2$-adrenoceptors in the spinal cord. There are at least three different subtypes (2A, 2B, and 2C) of $\alpha_2$-adrenoceptors (37). Prazosin blocks $\alpha_{2A}$- and $\alpha_{2C}$-adrenoceptors as well as $\alpha_1$-adrenoceptors (38). Thus, the lack of effect of prazosin suggests the involvement of $\alpha_{2A}$-adrenoceptors.

Sympathetic blocks have been traditionally used for acute herpes zoster pain as well as PHN, although the efficacy remains controversial. The efficacy might be dependent on the pathological conditions, such as the age of patients, the severity of pain and rash, and disease duration. The present results do not support the efficacy of sympathetic block for the acute herpetic pain. However, we do not rule out the possibility of the involvement of the sympathetic nervous system on the late phase of herpetic pain and postherpetic neuralgia. In this respect, we have recently found that about a half of the mice with acute herpetic pain show pain-related responses long after the complete cure of the cutaneous lesions (39). The involvement of sympathetic nerves on the delayed postherpetic pain in mice will be examined.

In summary, percutaneous inoculation with HSV-1 brought about tactile alldynia and mechanical hyperalgesia, but not thermal hyperalgesia, in mice. The sympathetic nervous system and $\alpha$-adrenoceptors may not be involved in the tactile allodynia and mechanical hyperalgesia. Clonidine suppresses these pain-related responses through the action on $\alpha_2$-adrenoceptors in the spinal cord. $\alpha_2$-Adrenoceptor agonists may be effective against acute herpetic pain. The suppression may be due to the inhibition of pain transmission in the dorsal horn rather than the sympathetic preganglionic neurons.
References


