DIFFERENT ROLES OF NITRIC OXIDE SYNTHASE-1 AND -2 BETWEEN HERPETIC AND POSTHERPETIC ALLODYNA IN MICE

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Abstract—We investigated using the mice role of nitric oxide synthase (NOS) in the spinal dorsal horn in herpetic and postherpetic pain, especially allodynia, which was induced by transdermal inoculation of the hind paw with herpes simplex virus type-1 (HSV-1). The virus inoculation induced NOS2 expression in the lumbar dorsal horn of mice with herpetic alldynia, but not postherpetic alldynia. There were no substantial alternations in the expression level of NOS1 at the herpetic and postherpetic stages. Herpetic alldynia was significantly inhibited by i.p. administration of the selective NOS2 inhibitor S-methylisothiourea, but not the selective NOS1 inhibitor 7-nitroindazole. NOS2 expression was observed around HSV-1 antigen-immunoreactive cells. On the other hand, postherpetic alldynia was significantly inhibited by i.p. administration of 7-nitroindazole, but not S-methylisothiourea. The activity of reduced nicotinamide adenine dinucleotide phosphate diaphorase, an index of NOS1 activity, significantly increased in the laminae I and II of the lumbar dorsal horn of mice with postherpetic alldynia, but not mice without postherpetic alldynia. The expression level of NOS1 mRNA in the dorsal root ganglia was similar between mice with and without postherpetic alldynia. The results suggest that herpetic and postherpetic alldynia is mediated by nitric oxide in the dorsal horn and that NOS2 and NOS1 are responsible for herpetic and postherpetic alldynia, respectively. It may be worth testing the effects of NOS2 and NOS1 inhibitors on herpetic pain and postherpetic neuralgia in human subjects, respectively.

Key words: herpes zoster, postherpetic neuralgia, nitric oxide synthase isoenzymes, dorsal horn, primary sensory nerve.

Herspes zoster characterized by clustered vesicles and severe pain is caused by the reactivation of human herpes virus 3, varicella-zoster virus, in the sensory ganglion in human subjects (Loeser, 1986). Patients with herpes zoster complain of severe spontaneous pain and allodynia, pain due to a non-noxious stimulus. Early treatment with antiviral agents, such as acyclovir and vidarabine, shortens the duration of skin lesions and complications related to herpes zoster (Gnann, 1994). However, these medicines do not promptly relieve acute herpetic pain (Lancaster et al., 1995). In addition, although nonsteroidal anti-inflammatory drugs such as diclofenac, antidepressants such as amitriptyline and sympathetic nerve block are used for the management of herpetic pain, these treatments do not always relieve severe pain (Loeser, 1986; Dworkin and Portenoy, 1996). In some herpes zoster patients, pain persists long after healing of the skin lesions, which is postherpetic neuralgia (Loeser, 1986). Patients with postherpetic neuralgia report various types of pain, including a continuous burning or aching pain, a periodic piercing pain, and tactile alldynia (Loeser, 1986). Once established, postherpetic neuralgia is particularly difficult to treat and is often resistant to conventional analgesics (Argoff et al., 2004). The mechanisms of the induction and maintenance of herpetic pain and postherpetic neuralgia are still unclear.

We previously established mouse models of herpetic pain and postherpetic pain using human herpesvirus 1 (herpes simplex virus type-1, HSV-1) (Takasaki et al., 2000, 2002). When mice are given transdermal HSV-1 inoculation on the hind paw, they show herpes zoster-like skin lesions throughout the inoculated dermatome and pain-related behaviors (Takasaki et al., 2000). Pain-associated behaviors (herpetic pain) and vesicles become apparent 5 days after inoculation and skin lesions heal by day 15 after inoculation (Takasaki et al., 2000, 2002). In some mice, pain-associated behaviors subside by day 20 after inoculation and in the rest pain-associated behaviors (postherpetic pain) last long after the skin lesions completely heal (Takasaki et al., 2002).

Nitric oxide (NO) is produced mainly by nitric oxide synthase-1 (NOS1, neuronal NOS) in normal nervous system (Downen et al., 1999; Millan, 1999), but viral invasion induces NOS2 (inducible NOS) expression (Fujii et al., 1999; Christian et al., 1996; Dugas et al., 2001). Induction of NOS2 expression produces beneficial antiviral effects (Reiss and Komatsu, 1998). In addition, NO plays impor-
tand complex roles in nociceptive modulation. There is considerable evidence that NO is involved in the generating of spinal cord hyperexcitability and hyperalgesia in some animal models of pain (Saller et al., 1998; Yoon et al., 1998; Osborne and Codrerre, 1999). However, the involvement and role of NO in herpetic and postherpetic pain is not clear. In the present study, we examined the roles of NO and NOS isoenzymes in the spinal dorsal horn in herpetic and postherpetic pain, especially allodynia in mice.

**EXPERIMENTAL PROCEDURES**

**Animals**

Female C57BL/6j mice (Japan SLC, Shizuoka, Japan) were used; they were 6 weeks old at the start of experiments. Housing (six per cage) and behavioral experiments were done under controlled temperature (22±1 °C), humidity (55±10%) and lighting (lighted 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 (Zimmermann, 1983). All efforts were made to minimize the number of animals used and their suffering.

**HSV-1 inoculation**

Mice were inoculated with HSV-1 as described previously (Takasaki et al., 2000). Briefly, HSV-1 (7401H strain; 1×10^6 plaque-forming units in 10 µl) was inoculated on the shin skin of the right hind paw after scarification with 27-gauge needles. The contralateral hind paw was without inoculation. At the development stage of skin lesions (until day 8 after inoculation), they 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 of skin lesions (from day 10 after inoculation), they 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 (Takasaki et al., 2002).

**Assessment of allodynia**

Tactile allodynia of the hind paw was assessed as described (Takasaki et al., 2000). After at least 30-min acclimation period, von Frey filament with a bending force of 1.6 Nm (0.16 g) was pressed perpendicularly against the plantar skin and held for 1–3 s with it slightly buckled. The responses to the stimulus were ranked as follows: 0, no response; 1, lifting of the hind paw; 2, 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 pain-related score. Since most of normal mice tested do not respond to von Frey filament of 1.6 Nm strength, mice that show 0.5 or higher pain-related scores were considered to have allodynia (Kuraishi et al., 2004).

**Agents**

The selective NOS2 inhibitor S-methylisothiourea sulfate (SMT) (Szabo et al., 1994) and the selective NOS1 inhibitor 7-nitroindazole (7-NI) (Moore et al., 1993; Murakami et al., 2002) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). SMT was dissolved in physiological saline. 7-NI was dissolved in a mixture of 10% dimethyl sulfoxide and 30% propylene glycol in distilled water. Both drugs were administered intraperitoneally in a volume of 0.1 ml/10 g body weight. The effects of the drugs on herpetic and postherpetic allodynia were tested on day 6 and days 35–40, respectively, after HSV-1 inoculation.

**Immunohistochemistry**

Under deep anesthesia with sodium pentobarbital (70 mg/kg, i.p.), the mice were perfused transcardially with phosphate-buffered saline (PBS, pH 7.4) and subsequently with 4% paraformaldehyde in PBS. The lumbar enlargement was removed and post-fixed in the same fixative at 4 °C for 4 h. The tissues were then transferred to 30% sucrose in PBS at 4 °C overnight for cryoprotection. They were cut on a freezing microtome (Leica CM 3050S IV, Nussloch, Germany) at a 40-µm thickness. After being preincubated in blocking solution (1.5% fetal bovine serum and 0.2% Triton X-100 in PBS) for 30 min at room temperature, sections were incubated with a rabbit anti-NOS2 antibody (1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4 °C for 3 days. Subsequently the sections were incubated for 2 h at room temperature with either of the following reagents: 1) biotinylated anti-rabbit IgG antibody (1:200; DAKO Japan Co. Ltd., Kyoto, Japan) and avidin-Cy3 or FITC (1:500; Vector Laboratories, Inc., Burlingame, CA); or 2) Cy3-conjugated anti-rabbit IgG antibody (1:200; DAKO Japan Co. Ltd.).

For double immunostaining, sections were reacted with the anti-NOS2 antibody as mentioned above. Sections were then further reacted with FITC-conjugated polyclonal rabbit anti-HSV-1 antibody (1:100; DAKO Japan Co. Ltd.) at 4 °C overnight. Fluorescence signals were observed using Bio-Rad Radiance 2000 confocal system (Bio-Rad Microscopy Division, Cambridge, MA, USA); sequential confocal images were collected at 2-µm steps and were used to construct the fluorescence image of the whole specimens.

**Western blot analysis**

The expression of NOS1 and NOS2 protein in the dorsal horn of lumbar enlargement was analyzed by Western blot. Tissues were homogenized with 100 µl of lysis buffer (137 mM NaCl, 20 mM Tris–HCl (pH 7.5), 1% Nonidet P40, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 1 µg/ml leupeptin, 10% glycerol) and centrifuged at 5000 r.p.m. for 15 min at 4 °C. The protein extracts (50 µg) were separated by 7.5% sodium dodecylsulfate–polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride membrane. After blocking with 5% skim milk solution for 1 h, the membrane was reacted with a rabbit anti-NOS1 antibody (1:1000; Santa Cruz Biotechnology, Inc.), a rabbit anti-NOS2 antibody (1:5000; Sigma Chemical Co.) at 4 °C overnight. After washing with Tris-buffe red saline containing Tween (TBS-T; 100 mM NaCl, 10 mM Tris–HCl (pH 7.5), and 0.1% Tween 20), the membrane was reacted with horse-radish peroxidase–conjugated anti-rabbit IgG antibody for NOS2 or anti-mouse IgG antibody for β-actin at a dilution of 1:5000 for 2 h at room temperature. After washing with TBS-T, the membrane was reacted with chemiluminescence reagents (Amersham Bioscience, Piscataway, NJ, USA) and signals were detected using X-ray film. The density of the band was analyzed using NIH Image program and values were normalized to β-actin.

**Zymohistochemistry**

The activity of reduced nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase is a reliable index of NOS1 activity (Laing et al., 1994) and the distribution of NADPH diaphorase serves as an index of the distribution of NOS1 activity (Mabuchi et
RESULTS

Development of herpetic and postherpetic allodynia

HSV-1 inoculation on the hind paw produced herpes zoster–like skin lesions and induced allodynia in all mice examined. Vesicular lesion erupted on day 5 after inoculation, peaked around day 7 and subsided by day 20 (Fig. 1A). Allodynia also became apparent on day 5 and then gradually increased over a few days (Fig. 1B). In 5 of 15 mice, allodynia gradually subsided from day 8 and completely resolved by day 20 after inoculation (Fig. 1B). However, the rest (10 of 15) showed allodynia long after the complete cure of the skin lesions (from day 20 to at least day 40 after inoculation) (Fig. 1B). The mouse that showed allodynia on day 20 was considered to have postherpetic allodynia.

Effects of NOS inhibitors on herpetic and postherpetic allodynia

I.p. injections of 7-NI at doses of 3 and 10 mg/kg did not affect herpetic allodynia (Fig. 2A), but the same doses produced the dose-dependent inhibition of postherpetic allodynia; the effects peaked 1–2 h after injection and were slightly reduced after 4 h (Fig. 2B). On the other hand, i.p. injections of SMT at doses of 3 and 10 mg/kg produced the dose-dependent inhibition of herpetic allodynia; the effects were dose-dependent and the inhibition was complete at 3 mg/kg. Postherpetic allodynia was not affected by these doses of SMT. The combination of both 7-NI and SMT produced a dose-dependent and complete inhibition of allodynia (Fig. 2D).

Data analysis

The means of data are presented together with S.E.M. Data on the time course of anti-allodynic effects were analyzed with the Friedman repeated-measures analysis of variance on ranks followed by post hoc Dunnett’s test. Statistical differences between two groups were analyzed using Student’s t-test or Mann-Whitney rank sum test, and among three groups were with Kruskal-Wallis one-way analysis of variance on ranks or one-way analysis of variance with post hoc Dunnett’s test. A value of P<0.05 was considered significant.
peaked 1–2 h after injection and were slightly reduced after 4 h (Fig. 2C). Postherpetic allodynia was not affected by SMT at the same doses (Fig. 2D).

**Expression of NOS1 and NOS2 protein in the lumbar dorsal horn**

NOS1 was substantially present in the lumbar dorsal horn of naive mice, and the expression level was not altered in mice with herpetic allodynia and ones with or without postherpetic allodynia (Fig. 3A, B). NOS2 was not detected in the lumbar dorsal horn of naive mice, but it was markedly induced on day 6 after inoculation (Fig. 3C). NOS2 induction subsided on day 40 in mice with or without postherpetic allodynia (Fig. 3C). Fig. 3D shows the time course of NOS2 induction during the acute phase. NOS2 was not detected even on day 4, markedly induced on day 6, and then gradually decreased on days 8 and 10.

**NOS2- and HSV-1 antigen-immunoreactive cells in the lumbar dorsal horn**

In the lumbar dorsal horn ipsilateral to inoculation, HSV-1 antigen-immunoreactive cells were observed by days 5, 6 and 8 after inoculation, but not before days 3 and 10 after inoculation (Fig. 4A). The number of the immunoreactive cells peaked at day 6 after inoculation and markedly decreased on days 8 (Fig. 4A). The size of most NOS2-immunoreactive cells was less than 20 μm. In the spinal cord section prepared from the mouse at day 6 after inoculation, a few clusters of HSV-1 antigen-immunoreactive cells were observed in the dorsal horn and NOS2-immunoreactive cells were localized in the same area (Fig. 4B–D). NOS2-immunoreactive cells were distributed around the HSV-1 antigen-immunoreactive cells, but there were no cells in which HSV-1 antigen and NOS2 immunoreactivities were co-localized (Fig. 4B–D). There were no HSV-1- and NOS2-immunoreactive cells on the contralateral dorsal horn (Fig. 4E–G).

**NADPH diaphorase in the lumbar dorsal horn**

The content of NOS1 in the lumbar dorsal horn was similar between naive mice and mice with postherpetic allodynia and NOS2 was not observed in the lumbar dorsal horn of mice with postherpetic allodynia. Therefore, to determine whether NOS activity would be altered in the mice with postherpetic allodynia, we investigated NOS activity by NADPH diaphorase histochemistry. NADPH diaphorase activities were distributed mainly in the superficial dorsal horn, especially laminae I and II (Fig. 5A–C). The intensity of NADPH diaphorase staining and the number of cells with NADPH diaphorase activity were similar between naive mice and mice without postherpetic allodynia (Fig. 5A, B, D and E). The intensity of NADPH diaphorase staining was significantly increased in mice with postherpetic allodynia (Fig. 5A, C and D). There was an increased tendency of the number of cells with NADPH diaphorase activity in mice with postherpetic allodynia (Fig. 5A, C and E).
Expression of NOS1 mRNA in the dorsal root ganglia

Increased activity of NADPH diaphorase in the laminae I and II of the dorsal horn of mice with postherpetic allodynia suggested that NOS activity was increased mainly in the primary sensory fibers. The result that the level of NOS1 protein in the dorsal horn was not increased in the state of postherpetic allodynia raised two possibilities; one is that the turnover of NOS1 was increased and another is that NOS1 activity was increased without alteration in the turnover. To address these questions, the levels of NOS1 mRNA in the dorsal root ganglia, in which there are the cell bodies of primary sensory fibers, were determined by qRT-PCR. In this study, mechanical stimuli were applied to the hind paw, which are mainly innervated by primary sensory neurons in the L5 and L6 dorsal root ganglia. In our preliminary experiments, L3 dorsal root ganglion was found to be most extensively infected with HSV-1. Therefore, we determined NOS1 mRNA separately at L2–L4 and L5–S1 levels. The expression of NOS1 mRNA in the dorsal root ganglia at the both levels was not

Fig. 3. Effects of HSV-1 inoculation on the expression of NOS1 and NOS2 in the lumbar dorsal horn. (A) Typical examples of Western blot analysis of NOS1, NOS2 and β-actin. (B) Expression level of NOS1 in mice with herpetic allodynia (HA, day 6 after inoculation) and mice with or without postherpetic allodynia (PHA, day 40 after inoculation). (C) Expression level of NOS2 in mice with HA and mice with or without PHA. (D) Time-dependent alteration in the expression level of NOS2 during the HA stage. Naive mice and mice with HA were the same age. The expression levels of NOS1 and NOS2 were normalized to that of β-actin. The data presented are means±S.E.M. (n=4). * P<0.05 as compared with naive (Dunnett's test). Expression of NOS1 mRNA in the dorsal root ganglia

Increased activity of NADPH diaphorase in the laminae I and II of the dorsal horn of mice with postherpetic allodynia suggested that NOS activity was increased mainly in the primary sensory fibers. The result that the level of NOS1 protein in the dorsal horn was not increased in the state of postherpetic allodynia raised two possibilities; one is that the turnover of NOS1 was increased and another is that NOS1 activity was increased without alteration in the

Fig. 4. Expression of NOS2 in the dorsal horn cells negative for HSV-1 antigen. (A) Time-dependent changes in the number of HSV-1 antigen-positive cells in the lumbar dorsal horn on the inoculated side. (B–G) Double immunostaining of HSV-1 and NOS2. The spinal cord was removed from mice on day 6 after inoculation and the lumbar dorsal horns on the inoculated (B–D) and contralateral sides (E–G) were immunostained for HSV-1 antigen (B and E) and NOS2 (C and F). (D, G) Merged images of immunoreactive signals of HSV-1 and NOS2. The data presented are means±S.E.M. (n=6).
altered in mice with or without postherpetic allodynia and also mice with herpetic allodynia (Fig. 6).

**DISCUSSION**

NO is produced from L-arginine by NOS and acts as inter- and intracellular messenger in a variety of cells and tissues (Moncada and Higgs, 1993). NO mediates pathological pain processing in the spinal cord (Meller and Gebhart, 1993). Behavioral evidence of NO involvement in pain mainly comes from studies using intrathecal injection of agents, in which the suppression of NO production with NOS inhibitors blocked hyperalgesia and allodynia (Salter et al., 1996; Dolan and Nolan, 1999; Osborne and Coderre, 1999). In the present study herpetic allodynia was significantly inhibited by the selective NOS2 inhibitor SMT, but not the selective NOS1 inhibitor 7-NI. On the other hand, postherpetic allodynia was significantly inhibited by 7-NI, but not SMT. These results suggest that NO is responsible for herpetic and postherpetic allodynia and that NOS2 and NOS1 are involved in herpetic and postherpetic allodynia, respectively. NOS2 was markedly induced in the dorsal horn at the herpetic stage, supporting the idea that NOS2-derived NO is responsible for herpetic allodynia. On the other hand, NOS2 almost disappeared at the postherpetic stage and NADPH-dependent NOS activity markedly increased in the lumbar dorsal horn of mice with postherpetic allodynia, but not those without postherpetic allodynia. These results support the idea that NOS1-derived NO is responsible for postherpetic allodynia.

In the CNS, NO is thought to be primarily produced by NOS1, which is Ca²⁺/calmodulin-dependent (Downen et al., 1999; Millan, 1999). Ca²⁺-independent NOS2 is present in macrophages and inflammatory cells while not normally expressed in the brain and spinal cord (Guhring et al., 2000; Van Dam et al., 1995). However, central virus infections induce NOS2 expression in the CNS (Fuji et al., 1999; Christian et al., 1996; Dugas et al., 2001). The production of NO by this isoenzyme is 10–100 times more than that of the other constitutive isoenzymes, NOS1 and NOS3 (Akaike and Maeda, 2000). Therefore, although NOS1 is constitutively present at the herpetic stage, NO produced by NOS2 rather than NOS1 may play an important role in the herpetic allodynia. The degree of allodynia was similar between day 6 and day 8–10 after inoculation, but NOS2 expression was much less on day 8–10 than on day 6. Therefore, the role of NOS2 in the allodynia may be more important at the early phase than at the late phase of herpetic pain.
traveling along the dorsal root to the spinal cord (Devinsky et al., 1998), which might be induced by the virus centripetally towards the spinal cord (Schmidbauer et al., 1992) and segmental atrophy of the spinal dorsal horn. The expression of NOS1 protein is responsible for postherpetic allodynia and that the increase of NOS activity is due to an increase in intracellular calcium but not an increase in intracellular calcium after the activation of NMDA receptor, an ionotropic glutamate receptor that is voltage- and ligand-gated and shows high permeability to Ca^{2+} (Meller and Gebhart, 1993). NMDA receptor is important in the synaptic events that lead to spinal sensitization (Besson et al., 1999). Clinically, the NMDA receptor antagonist ketamine was reported to be effective in some patients with postherpetic neuralgia (Eide et al., 1994). However, the relatively common adverse effects such as fatigue, dizziness and disturbances of somatosensory perception limit its usefulness in treating pain (Klepstad et al., 1990; Eide et al., 1994). NOS1 inhibitor may be an alternative analgesic for postherpetic neuralgia.

CONCLUSION

In summary, HSV-1 invasion of the spinal dorsal horn induced the expression of NOS2 in the dorsal horn and the NOS2-mediated overproduction of NO is responsible for

Fig. 6. Effects of HSV-1 inoculation on the expression of NOS1 mRNA in the dorsal root ganglia. Mice were inoculated with HSV-1 and the dorsal root ganglia at the L2–S1 levels on the inoculated side were isolated from mice with herpetic allodynia (HA) on day 6 after inoculation and mice with or without postherpetic allodynia (PHA) on day 40 after inoculation. Age-matched naive mice were used for control. The dorsal root ganglia at the L2–L4 (A) and L5–S1 levels (B) were separately pooled and NOS1 mRNA was determined by qRT-PCR analysis. The expression level of NOS1 mRNA was normalized to GAPDH mRNA in each sample. The data presented are means ± S.E.M. (n = 4).

Pathological studies on patients with herpes zoster pain demonstrated inflammatory changes in the spinal cord (Schmidbauer et al., 1992) and segmental atrophy of the spinal dorsal horn (Watson et al., 1991; Haanpaa et al., 1998), which might be induced by the virus centripetally traveling along the dorsal root to the spinal cord (Devinsky et al., 1991). These findings raise the possibility that the invasion of varicella zoster virus into the spinal cord is a cause of zoster pain. In the present study, HSV-1 antigen-immunoreactive cells were observed in the lumbar dorsal horn on days 5 and 6 after inoculation and the number of the immunoreactive cells peaked on day 6 after inoculation. The temporal pattern of HSV-1 proliferation was similar to that of NOS2 expression. Similar results were reported in an experimental model of HSV-1 encephalitis in rats after intranasal inoculation (Fuji et al., 1999). The temporal and spatial patterns of NOS2 expression coincide with those of viral proliferation, suggesting that NOS2 expression is triggered by HSV-1 invasion and proliferation in the spinal dorsal horn.

The size of most immunoreactive cells was less than 20 μm and in our preliminary experiments, NOS2 immunoreactivity was not observed in cells immunoreactive for neuron-specific nuclear protein, a neuronal marker (Sasaki et al., unpublished observations). Therefore, NOS2-immunoreactive cells may not be neurons. Viral infections of the CNS induce the activation of glial cells and the infiltration of macrophages and neutrophils which have the ability to induce the expression of NOS2 (Fujii et al., 1999; Christian et al., 1996; Andrews et al., 1999; Dugas et al., 2001). At present, the type of NOS2-immunoreactive cells is unclear, but we are trying to identify the type of dorsal horn cells expressing NOS2 at the herpetic stage.

As mentioned above, NOS1, but not NOS2, may be involved in postherpetic allodynia. NOS1 is abundant in the normal dorsal horn and is localized mainly in neurons (Downen et al., 1999; Millan, 1999). The activity of NOS1 generally depends on increase in intracellular calcium rather than on the expression level of the enzyme (Downen et al., 1999; Millan, 1999). In the present study, NADPH-dependent NOS activity markedly increased in the laminae I and II of the lumbar dorsal horn, suggesting the increase of NOS activity in the primary sensory fibers. There were no substantial alternations in the expression level of NOS1 protein in the area of nerve fiber distribution (dorsal horn) and NOS1 mRNA in the area of nerve cell body distribution (dorsal root ganglion). The results were similar to the report that spinal nerve ligation increased NOS1 activity in spinal dorsal horn with NOS1 immunoreactivity unchanged (Mabuchi et al., 2004). Taken together, it is suggested that neuronal NOS1-derived NO is responsible for postherpetic allodynia and that the increase of NOS activity is due to an increase in intracellular calcium but not an increase in synthesis or turnover of the enzyme.

Spinal sensitization contributes to pathological pain, consisting of tissue injury–induced inflammatory pain and nerve injury–induced neuropathic pain (Ji and Woolf, 2001). NO has been hypothesized to facilitate glutamate release (Moncada and Higgs, 1993) leading to an ongoing activity in primary afferents, increased sensitivity of dorsal horn neurons and finally spinal sensitization (Ji and Woolf, 2001). NOS1 is normally a constitutive enzyme whose activity is thought to depend on increase in intracellular calcium after the activation of NMDA receptor, an ionotropic glutamate receptor that is voltage- and ligand-gated and shows high permeability to Ca^{2+} (Meller and Gebhart, 1993). NMDA receptor is important in the synaptic events that lead to spinal sensitization (Besson et al., 1999). Clinically, the NMDA receptor antagonist ketamine was reported to be effective in some patients with postherpetic neuralgia (Eide et al., 1994). However, the relatively common adverse effects such as fatigue, dizziness and disturbances of somatosensory perception limit its usefulness in treating pain (Klepstad et al., 1990; Eide et al., 1994). NOS1 inhibitor may be an alternative analgesic for postherpetic neuralgia.

CONCLUSION

In summary, HSV-1 invasion of the spinal dorsal horn induced the expression of NOS2 in the dorsal horn and the NOS2-mediated overproduction of NO is responsible for
herpetic allodynia. On the other hand, postherpetic alldynia was modulated by spinal NOS1, but not NOS2. It may be worth testing the effects of NOS2 and NOS1 inhibitors on herpetic pain and postherpetic neuralgia in human subjects, respectively.

REFERENCES


