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HEMOKININ-1 MEDIATES PRURICEPTIVE PROCESSING IN THE RAT SPINAL CORD

H. FUNAHASHI,[a] R. NAONO-NAKAYAMA,[b] K. EBIHARA,[a] G. KOGANEMARU,[a] A. KURAMASHI,[a] T. IKEDA,[b] T. NISHIMORI[a] AND Y. ISHIDA[a]*

[a] *Department of Psychiatry, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan*

[b] *Division of Neurobiology, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan*

Abstract—Hemokinin-1 (HK-1) is a new mammalian tachykinin peptide consisting of the amino acid sequence similar to substance P (SP). Although the function of SP, a representative tachykinin peptide, has been well established in the pain system, that of HK-1 has not yet been elucidated. [Leu^{11}]-SP had an antagonistic effect on SP-induced scratching behavior, suggesting that [Leu^{11}]-HK-1 may also attenuate the induction of scratching behavior by HK-1. Thus, the effects of a pretreatment with [Leu^{11}]-HK-1 were evaluated to clarify the function of HK-1. The intrathecal administration of [Leu^{11}]-HK-1 attenuated the induction of scratching by HK-1, but not SP, while [Leu^{11}]-SP reduced the induction of scratching by SP, but not HK-1. These results indicated that [Leu^{11}]-HK-1 may be a more specific antagonist of HK-1-preferred receptors and [Leu^{11}]-SP has an antagonistic effect on the SP-preferred receptor, the neurokinin 1 receptor. In the formalin test for examining noxious response, the intrathecal administration of [Leu^{11}]-SP, but not [Leu^{11}]-HK-1, reduced the number of flinchings and c-Fos-positive cells in the spinal dorsal horn following formalin injection into the plantar region of the hind paw. These results indicated that SP, but not HK-1, is involved in nociceptive processing at the spinal level. To evaluate the involvement of HK-1 and SP in pruritic processing, the effect of [Leu^{11}]-HK-1 and [Leu^{11}]-SP on the induction of scratching behavior and c-Fos expression by serotonin (5-HT) and histamine was evaluated. The increased induction of scratching behavior and c-Fos expression by 5-HT and histamine was markedly attenuated by pretreatment with both [Leu^{11}]-HK-1 and [Leu^{11}]-SP, suggesting that HK-1 and SP may be involved in pruritic processing. These results indicate that HK-1 is involved in pruritic processing and [Leu^{11}]-HK-1 is a valuable tool for clarifying the mechanisms underlying pruritic processing.

Key words: pruritus, serotonin, histamine, [Leu^{11}]-hemokinin-1, spinal cord, rat.

INTRODUCTION

Two preprotachykinin genes, TAC1 and TAC3, are known to encode three mammalian tachykinin peptides, substance P (SP), neurokinin A, and neurokinin B. Hemokinin-1 (HK-1) is another mammalian tachykinin peptide encoded by the new preprotachykinin gene, TAC4, identified in mouse bone marrow cells (Zhang et al., 2000). These tachykinin peptides share a common carboxyl terminal (C-terminal) Phe-Xaa-Gly-Leu-Met-amide motif and more varied amino terminals (N-terminal) (Page, 2004, 2005, 2006).

The function of SP has been well characterized in the pain system of the spinal cord. SP is found in and released from small primary afferents, and is believed to function as a pain transmitter or modulator associated with primary afferent depolarization following noxious peripheral stimuli (Henry, 1976; De Koninck and Henry, 1991). SP and the SP-preferred receptor, the neurokinin 1 (NK1) receptor, are expressed in the superficial layer of the spinal dorsal horn, on which nociceptive primary afferents mainly terminate (Höckfelt et al., 1977; Brown et al., 1995). HK-1 mRNA is also localized in the spinal cord and dorsal root ganglion (Duffy et al., 2003), and is enhanced in the spinal dorsal horn of rats with neuropathic condition and increased in microglia activated by lipopolysaccharide (Matsumura et al., 2008; Sakai et al., 2012).

The similarities in amino acid sequences between SP and HK-1 prompted us to assume that HK-1 may also have functions similar to SP at the level of the spinal cord. Indeed, the binding manner of HK-1 and SP to the NK1 receptor was found to be very similar (Morteau et al., 2001; Kurtz et al., 2002). Furthermore, the intrathecal administration of HK-1 and SP induced scratching behavior, and the increase in the number of scratchings was attenuated by the pretreatment with NK1 receptor antagonists (Endo et al., 2006; Yoshioka et al., 2006; Naono et al., 2007b). These findings suggested that HK-1 as well as SP may be an agonist of the NK1 receptor; however, the functions of HK-1 have not yet been fully understood.

Itch is described as an irritating sensation that triggers a desire to scratch, and SP-induced scratching was attenuated by the administration of NK1 receptor antagonists, suggesting that SP and the NK1 receptor

*Corresponding author. Tel: +81-985-85-2969; fax: +81-985-85-5475.

E-mail address: ishiday@med.miyazaki-u.ac.jp (Y. Ishida).

Abbreviations: 5-HT, serotonin; EKC/D, endokinin C and endokinin D; GRP, gastrin-releasing peptide; HK-1, hemokinin-1; NK1, neurokinin 1; Nppb, natriuretic polypeptide b; SP, substance P.

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may be involved not only in nociceptive processing, but also in pruriceptive processing (Kuraishi et al., 1995; Carstens et al., 2010). An intradermal injection of histamine and serotonin (5-HT, 5-hydroxytriptamine), which are well-known pruritic agents, induced scratching behavior (Yamaguchi et al., 1999; Nojima and Carstens, 2003; Shimada and LaMotte, 2008; Sun et al., 2009; LaMotte et al., 2011) and also enhanced c-Fos expression in the spinal dorsal horn (Yao et al., 1992; Nojima et al., 2003; Nakano et al., 2008). These findings indicated that the induction of scratching behavior and enhancement of c-Fos expression in the spinal cord following the administration of pruritic agents may be valuable tools for identifying pruriceptive processing at the level of the spinal cord.

Recently, it has been demonstrated that intrathecal administration of the N-terminal fragment peptide of HK-1, HK-1 (1–5), attenuated the induction of scratching behavior by intrathecal administration of HK-1 and SP and by intradermal injection of pruritic agents such as 5-HT and histamine, suggesting that HK-1 (1–5) may be a potent inhibitor of pruritus and HK-1 may be involved in pruriceptive processing (Naono-Nakayama et al., 2014). Thus, identifying proper blockers of HK-1 is important for clarifying the mechanisms underlying pruriceptive processing; however, no reliable blocker of HK-1 has so far been reported. A method for finding new blockers derived from tachykinin peptides has recently been proposed. The SP-derived peptide, [Leu^{11}]-SP, in which Met at the C-terminus of SP was replaced by Leu, exhibited a clear antagonistic effect on the induction of scratching by the intrathecal administration of SP, demonstrating that Leu at the C-terminus of this peptide was crucially involved in eliciting the antagonist effect (Naono et al., 2009). When this finding was applied to HK-1, it was suggested that the HK-1-derived peptide, [Leu^{11}]-HK-1, in which Met at the C-terminus of HK-1 was replaced by Leu, may have a similar antagonist effect on HK-1; however, the function of [Leu^{11}]-HK-1 currently remains unknown.

Therefore, to clarify the involvement of HK-1 in pruriceptive processing, the effects of a pretreatment with [Leu^{11}]-HK-1 on the induction of scratching behavior by the intrathecal administration of HK-1 and also on the induction of scratching behavior and c-Fos expression induced by the intradermal administration of histamine or 5-HT were evaluated, while comparing with the effects of the pretreatment with [Leu^{11}]-SP on the induction of scratching and c-Fos by intrathecal administration of SP and by the intradermal injection of histamine, 5-HT, or the induction of flinching and c-Fos by formalin, well-known noxious stimuli.

EXPERIMENTAL PROCEDURES

Experimental design

The Institutional Animal Care and Use Committee of the University of Miyazaki approved the experimental protocol used in the present study, and all efforts were made to minimize the number of animals used (Zimmerman, 1983).

Male Sprague–Dawley rats, weighing 200–250 g, were intrathecally catheterized after an acclimation period of at least 1 week at the Experimental Animal Center of the University of Miyazaki and maintained under a 12/12-h light/dark cycle with food and water freely available. Catheterization was performed as previously described (Yaksh and Rudy, 1976; Naono et al., 2007a). Under anesthesia with sodium pentobarbital (50 mg/kg, i.p.; Abbot Laboratories, Abbot Park, IL, USA), a 7.2-cm or 1.0-cm length from the elongated part of the catheter was threaded caudally into the subarachnoid space through a slit in the atlanto-occipital membrane. Rats showing neurological deficits during the one-week recovery period were excluded from the study.

Peptides and chemicals

Peptides were administered through the catheter into the subarachnoid space. HK-1 (Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-Met-amide) and SP (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-amide) were purchased from Genenet Co. (Fukuoka, Japan) and Sigma Chemical Co. (St. Louis, MO, USA), respectively. The amino acid sequence of HK-1 proposed by Zhang et al. (2000) was adopted in this study, since it remains to be determined whether arginine exists at the N-terminus of HK-1 (Page, 2004). In addition, two peptides, in which Met at the C-termini of HK-1 and SP was replaced by Leu, were designated as [Leu^{11}]-HK-1 (Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-Leu-amide) and [Leu^{11}]-SP (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Leu-amide), respectively, and were purchased from Genenet Co. (Fukuoka, Japan). These four peptides were dissolved in distilled water at 10^{-2} M, and stored at −30 °C until use.

Histamine and 5-HT were purchased from Sigma Chemical Co. (St. Louis, MO, USA), dissolved in saline, and stored at −30 °C until use. These two chemicals were subcutaneously injected into the nape of the neck or injected intradermally into the plantar region of the hind paw.

Assessment of scratching or flinching behavior

To evaluate the induction of scratching behavior, a volume of 10 µl of 10^{-3} M (10 nmol/rat) HK-1 or SP was intrathecally administered through the catheter (Endo et al., 2006), and 50 µl of 2×10^{-3} M (0.5 mg/rat) 5-HT or 2.25×10^{-3} M (0.1 mg/rat) histamine was intradermally injected into the nape of the neck under isoflurane anesthesia (Abbott Laboratories, Chicago, IL, USA) (Naono-Nakayama et al., 2014). Scratchings after the administration of HK-1 or SP and in animals treated with 5-HT or histamine were counted for 5 min and 20 min after the removal of isoflurane, respectively. [Leu^{11}]-HK-1, [Leu^{11}]-SP, HK-1, or SP was intrathecally administered through the catheter, while histamine or 5-HT was subcutaneously injected into the nape of the neck. The effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP was evaluated by examining changes in the number of scratchings induced by the administration of HK-1,

SP, 5-HT or histamine while comparing with that in saline-treated animals. The mean number of scratchings was regarded as the number of scratchings induced by the administration of saline or each chemical.

The effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on the induction of flinching behavior by the intraplantar injection of formalin was also evaluated. Fifty microliters of formalin solution (2% formaldehyde in saline) were injected into the plantar region of the left hind paw under isoflurane anesthesia. After awaking from anesthesia, rats were returned to the testing chamber, and the number of flinchings per min was counted every 2 min until 10 min and every 5 min for 10–60 min after the formalin injection.

Immunohistochemistry of c-Fos

The effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on the induction of c-Fos expression by the injection of histamine, 5-HT, or formalin was evaluated by examining changes in c-Fos immunoreactivity in the dorsal horn of the lumbar spinal cord. After rats treated with these peptides or saline were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), 2×10^{-3} M (0.5 mg/rat) 5-HT, 2.25×10^{-3} M (0.1 mg/rat) histamine, or 2% formalin solution was injected into the unilateral hind paw.

Animals were again anesthetized with an overdose of sodium pentobarbital 2 h after the 5-HT, histamine, or formalin injection, and perfused intracardially with 200 ml saline followed by 500 ml of 4% cold paraformaldehyde in 0.1 M phosphate buffer (PB) for 30 min. The lumbar spinal cord (L4–5) was removed, postfixed for 1 h in the same fixative, and cryoprotected in 10% sucrose in PB for 1 h and then in 30% sucrose in PB overnight. Frozen coronal serial sections of the spinal cord, 50 μm in thickness, were prepared, collected in phosphate-buffered saline (PBS; pH 7.4), and processed as free-floating sections for immunohistochemical staining for c-Fos protein. Immunohistochemical staining of c-Fos was performed as previously described (Naono et al., 2007b). All sections were incubated in hydrogen peroxide and Triton X-100, and the sections were reacted with a polyclonal rabbit anti-c-Fos antibody (1:5,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4 °C after incubation of normal goat serum (SAB kit; Nichirei Biosci. Inc., Tokyo, Japan). The reaction products of the biotinylated goat anti-rabbit antiserum and avidin-conjugated horseradish peroxidase (SAB kit; Nichirei Biosci. Inc., Tokyo, Japan) were visualized using diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co., St. Louis, MO, USA) and hydrogen peroxide, and intensified by the pretreatment with cobalt chloride. Preabsorption of the antibody with a corresponding synthetic peptide (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or omission of the antibody from the protocol abolished staining.

To quantitatively evaluate the effects of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on c-Fos expression induced by 5-HT, histamine, or formalin, cells expressing the c-Fos protein in the dorsal horn of L4–5 were counted. The dorsal horn was divided into lamina I/II lamina III/IV and lamina V/VI. Cells positive to c-Fos were then plotted under bright-field illumination and counted. A single investigator, who was blinded to the type of treatment of each animal, plotted c-Fos-positive cells. At least five animals were used in each experimental group, and 10 sections with the largest number of positive cells per section were collected from each rat. The mean number of positive cells was used to evaluate the effects of the pretreatment with these peptides.

Statistical analysis

Scratching and flinching behaviors were presented in numbers as the mean ± S.E.M. The expression of c-Fos was presented as the number of c-Fos-positive cells as the mean ± S.E.M. Statistical comparisons of the number of scratchings or flinchings and the number of c-Fos-positive cells were performed using an analysis of variance (ANOVA) with repeated measures, followed by Fisher's protected least significant difference (PLSD).

RESULTS

Effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on scratching induced by the intrathecal administration of HK-1 or SP

Scratching behavior was negligible in rats with saline administration, while the intrathecal administration of HK-1 or SP markedly induced scratching behavior. The raw numbers of scratchings for 5 min after the intrathecal administration of 10 μl of 10^{-3} M (10 nmol/rat) HK-1 and SP solution in saline-treated rats were 126.3 ± 8.5 and 110.6 ± 4.6, respectively. These numbers were regarded as the basal level of scratching induced by HK-1 and SP, and represented 100% (Fig. 1, Saline).

To evaluate the effects of the pretreatment with [Leu^{11}]-HK-1 on the induction of scratching by HK-1 and SP, 10 μl of 10^{-3} M (10 nmol/rat) HK-1 or SP was injected into the subarachnoid space through the catheter 5 min after the intrathecal administration of 10 μl of 10^{-5} M, 10^{-4} M, or 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 solution. The increase in the number of scratchings following the administration of 10^{-3} M HK-1 was dose-dependently attenuated by the pretreatment with [Leu^{11}]-HK-1. Indeed, the raw number of scratchings in rats pretreated with 10^{-3} M [Leu^{11}]-HK-1 was 51.0 ± 9.4 and was significantly decreased from the basal level, although the effects of the pretreatment with 10^{-5} M and 10^{-4} M [Leu^{11}]-HK-1 on HK-1-induced scratching were not significant (Fig. 1A, HK-1). The pretreatment with [Leu^{11}]-HK-1 had a dose-dependent effect on SP-induced scratching. However, the effect of the pretreatment with [Leu^{11}]-HK-1 on the induction of scratching by SP was modest and not significant (Fig. 1A, SP).

In contrast, the effect of the pretreatment with [Leu^{11}]-SP on the induction of scratching by HK-1 and SP was markedly different from that of [Leu^{11}]-HK-1. The

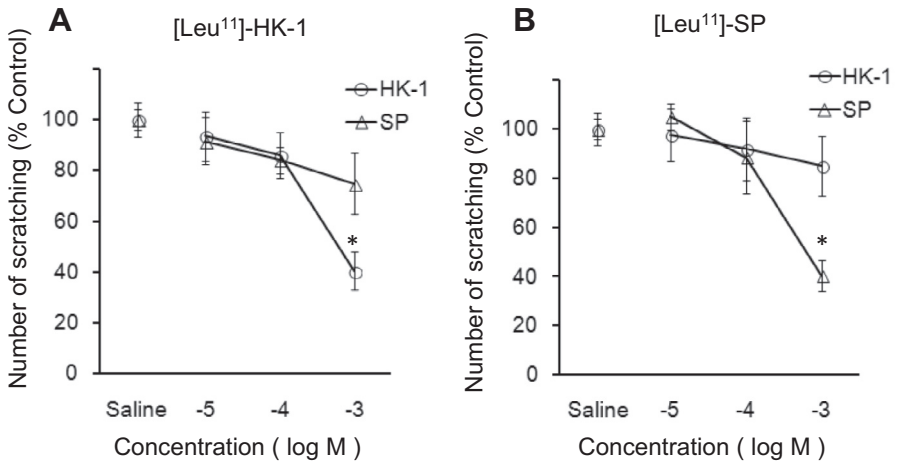

Fig. 1. Effects of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on the induction of scratching by the intrathecal administration of HK-1 or SP. (A) Effects of the pretreatment with different concentrations of [Leu^{11}]-HK-1 on the induction of scratching by HK-1 and SP. The relative number of scratchings induced for 5 min after the intrathecal administration of 10^{-3} M (10 nmol/rat) HK-1 and 10^{-3} M (10 nmol/rat) SP in rats pretreated with saline (n = 6, Saline), 10^{-5} M (n = 6, 0.1 nmol/rat), 10^{-4} M (n = 6, 1 nmol/rat), and 10^{-3} M (n = 6, 10 nmol/rat) [Leu^{11}]-HK-1 5 min before the administration of HK-1 and SP. (B) Effects of the pretreatment with different concentrations of [Leu^{11}]-SP on the induction of scratching by HK-1 and SP. The relative number of scratchings induced for 5 min after the intrathecal administration of 10^{-3} M (10 nmol/rat) HK-1 and 10^{-3} M (10 nmol/rat) SP in rats pretreated with saline (n = 6, Saline), 10^{-5} M (n = 6, 0.1 nmol/rat), 10^{-4} M (n = 6, 1 nmol/rat) and 10^{-3} M (n = 6, 10 nmol/rat) [Leu^{11}]-SP 5 min before the administration of HK-1 and SP. All points represent the number of scratchings ± S.E.M. and significant changes from Control were statistically analyzed by ANOVA with repeated measures, followed by Fisher's protected least significant difference (PLSD) and were represented as $^{*}P < 0.05$.

increase in the number of scratchings following the administration of 10^{-3} M SP was significantly attenuated from the basal level by the pretreatment with 10^{-3} M [Leu^{11}]-SP and the raw number of scratchings was 44.6 ± 6.9, although the decrease in the number of scratchings after the administration of 10^{-5} M and 10^{-4} M [Leu^{11}]-SP was not significant (Fig. 1B, SP). On the other hand, the pretreatment with any concentration of [Leu^{11}]-SP did not significantly affect the number of scratchings induced by 10^{-3} M HK-1 (Fig. 1B, HK-1).

Effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on flinching induced by the formalin injection

The injection of 50 μl of 2% formalin into the plantar region of the hind paw induced flinching behavior, and the induction of flinching behavior in rats pretreated with saline was regarded as the control (Fig. 2A, Control). When the induction of flinching behavior was evaluated by examining changes in the number of flinchings, formalin-induced flinching behavior displayed a typical biphasic response. The number of flinchings just after the formalin injection was marked and then gradually decreased, and was hardly observed 10 min after the formalin injection (Phase I). Flinching reappeared 15 min later, and the number of flinchings gradually increased, with a peak 25 min after the formalin injection, and then gradually decreased (Phase II) (Fig. 2A, Control).

The number of flinchings induced by the formalin injection into the hind paw of rats treated with 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 5 min before the formalin treatment was not significantly different from that in the control. The number of flinchings 30–50 min after the formalin injection was very similar (Fig. 2A, [Leu^{11}]-HK-1). The total number of flinchings in Phase I and Phase II of saline-treated rats was 195.4 ± 56.3 and 847.2 ± 137.2, respectively (Fig. 2B, C, Control), and those in rats pretreated with [Leu^{11}]-HK-1 were 103.0 ± 20.1 and 718.5 ± 62.3, respectively. The reduction in the flinching numbers in [Leu^{11}]-HK-1-pretreated rats was not significantly different from the control in Phase I and Phase II, although the number of flinchings was less than that in saline-treated rats (Fig. 2B, C, [Leu^{11}]-HK-1 + formalin).

On the other hand, the number of formalin-induced flinchings in rats treated with 10^{-3} M (10 nmol/rat) [Leu^{11}]-SP 5 min before the formalin injection was markedly lower than that in the control. The flinching number in Phase I was markedly reduced, while that in Phase II remained low without a peak in the flinching number (Fig. 2A, [Leu^{11}]-SP + formalin). The total number of flinchings in Phase I and Phase II of rats pretreated with [Leu^{11}]-SP was 53.7 ± 10.7 and 383.8 ± 45.2, respectively, and was significantly lower than those in the control (Fig. 2B, C, [Leu^{11}]-SP + formalin).

Effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on c-Fos expression induced by the formalin injection

The immunoreactivity of c-Fos was inconspicuous in the dorsal horn of saline-treated rats, while the subcutaneous administration of 50 μl of 2% formalin to the hind paw markedly enhanced c-Fos immunoreactivity in the medial part of the dorsal horn (Fig. 3A, Control). The enhancement in c-Fos immunoreactivity in the dorsal horn following the formalin injection was markedly attenuated by

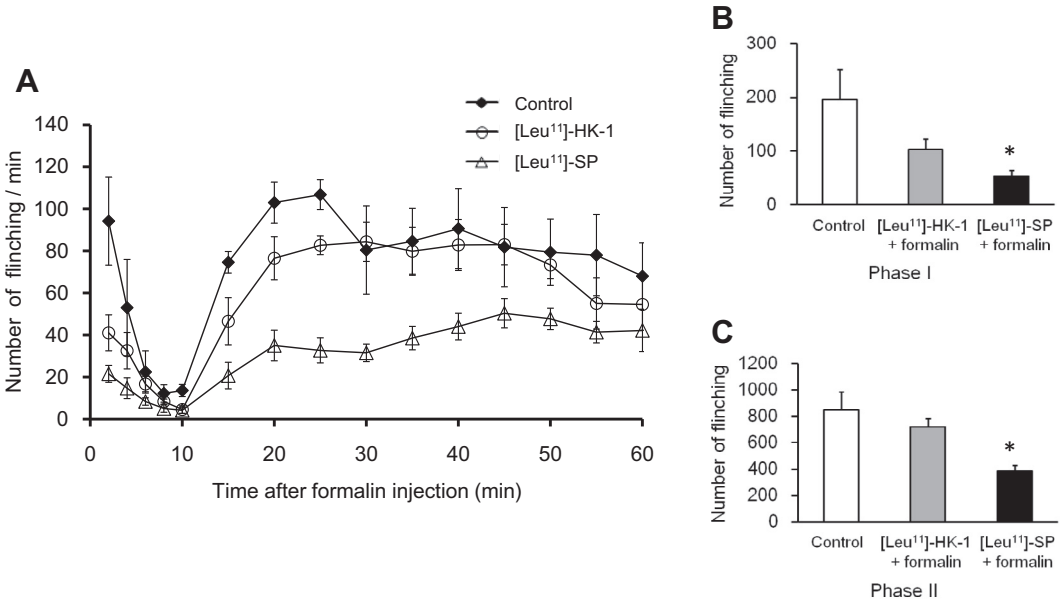

Fig. 2. Effects of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on the induction of flinching behavior by a formalin injection into the paw plantar region. The number of flinchings per min after the injection of 50 μl 2% formalin into the left plantar region in rats pretreated with saline (n = 6, Control), 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 (n = 6, HK-1 (1–5)) and 10^{-3} M (10 nmol/rat) [Leu^{11}]-SP (n = 6, SP (1–5)) 5 min before the formalin injection. The flinching number in rats pretreated with saline was regarded as the basal level. (A) Time course changes in the flinching number per min after the formalin injection in rats pretreated with saline, [Leu^{11}]-HK-1 and [Leu^{11}]-SP. (B) The sum of flinchings in Phase I (0–10 min) and Phase II (15–60 min) after the formalin injection in rats pretreated with saline (n = 6, Control), [Leu^{11}]-HK-1 (n = 6, [Leu^{11}]-HK-1 + formalin) and [Leu^{11}]-SP (n = 6, [Leu^{11}]-SP + formalin). All points represent the flinching number ± S.E.M., and significant changes from the control were statistically analyzed by ANOVA with repeated measures, followed by Fisher's protected least significant difference (PLSD) and were represented as $^{*}P < 0.05$.

the intrathecal administration of [Leu^{11}]-SP 5 min before the formalin injection (Fig. 3C). However, no significant change was observed in the expression of c-Fos by the pretreatment with [Leu^{11}]-HK-1 (Fig. 3B).

The effects of [Leu^{11}]-HK-1 or [Leu^{11}]-SP on formalin-induced c-Fos expression were evaluated by examining changes in the number of c-Fos-positive cells in lamina I/II, lamina III/IV, and lamina V/VI of the dorsal horn. The number of c-Fos-positive cells in each lamina of the spinal cord of saline-treated rats was considered as the basal level, and, indeed, the raw numbers of c-Fos-positive cells in lamina I/II, lamina III/IV, and lamina V/VI were 88.4 ± 2.5, 14.0 ± 2.3 and 19.2 ± 2.8, respectively (Fig. 3D, Control). To clarify the effects of the pretreatment with 10^{-3} M [Leu^{11}]-HK-1 or [Leu^{11}]-SP, 10 μl of each peptide was intrathecally administered 5 min before the formalin injection. The number of c-Fos-positive cells after the formalin injection lamina-dependently decreased by the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP. The number of c-Fos-positive cells in lamina I/II, lamina III/IV, and lamina V/VI in the spinal cord was significantly decreased by the pretreatment with [Leu^{11}]-SP from the basal level, and the raw numbers of c-Fos-positive cells in each lamina were 42.6 ± 3.0, 3.65 ± 0.5 and 4.4 ± 0.5, respectively (Fig. 3D, [Leu^{11}]-SP). On the other hand, the pretreatment with [Leu^{11}]-HK-1 did not significantly change the number of c-Fos-positive cells in lamina I/II in the spinal cord following the formalin injection, whereas the number of c-Fos-positive cells in lamina III/IV and lamina V/VI was significantly decreased (Fig. 3D, [Leu^{11}]-HK-1).

Effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on scratching induced by the subcutaneous injection of 5-HT or histamine

Scratching behavior was markedly induced by the subcutaneous injection of 5-HT into the nape of the rat neck. The raw number of scratchings for 5 min after the subcutaneous administration of 50 μl of 2×10^{-5} M (0.5 mg/rat) 5-HT in saline-treated rats was 357.7 ± 19.3, and this scratching number was regarded as the basal level (Fig. 4 and 5-HT, Control). To clarify the effects of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on 5-HT-induced scratching, 2×10^{-3} M 5-HT was subcutaneously injected into the nape of the neck 5 min after the intrathecal administration of 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 or [Leu^{11}]-SP. The increase in the number of scratchings following the administration of 5-HT was markedly attenuated by the pretreatment with [Leu^{11}]-HK-1, and the raw number of scratchings was 105.3 ± 25.9. This number was significantly lower than the basal level Fig. 4 and 5-HT, [Leu^{11}]-HK-1). The pretreatment with [Leu^{11}]-SP also significantly decreased the number of scratchings induced by 5-HT, and the raw number of scratchings was 54.7 ± 11.6 (Fig. 4 and 5-HT, [Leu^{11}]-SP).

The number of scratchings induced by the subcutaneous injection of 50 μl of 2.25×10^{-3} M

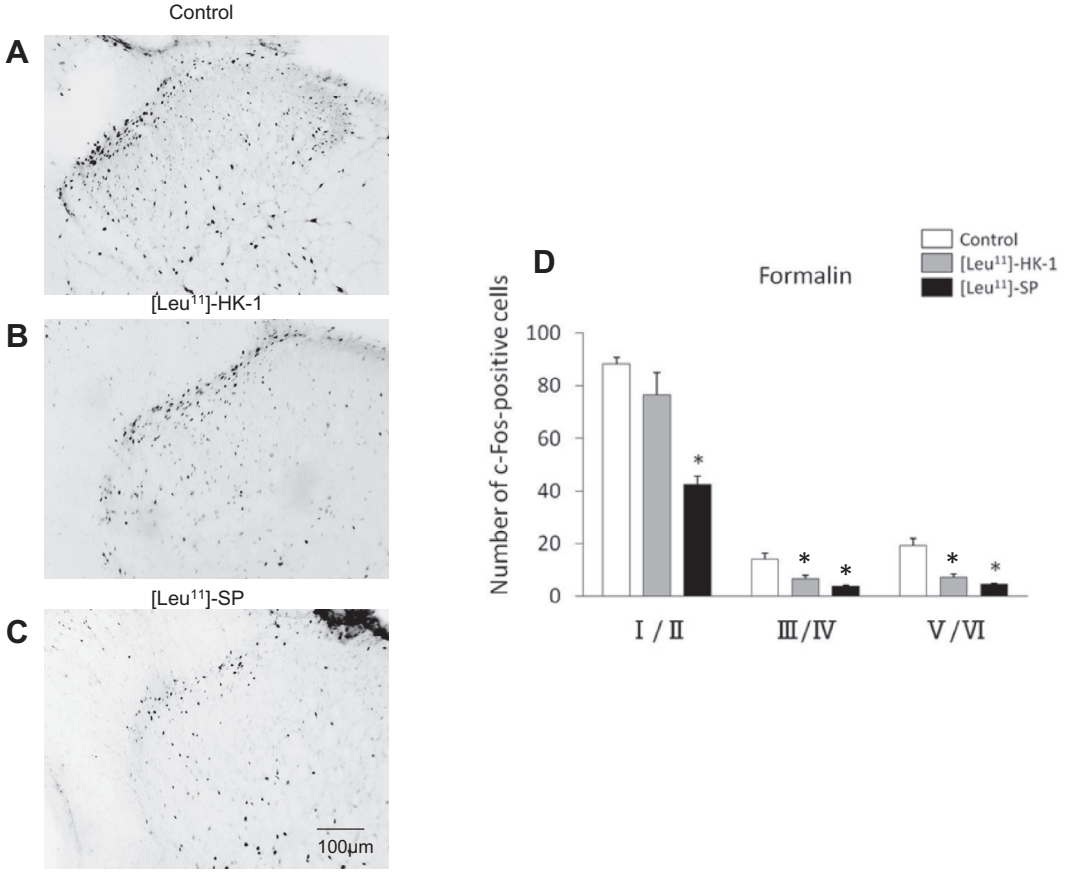

Fig. 3. Effects of the intrathecal administration of [Leu^{11}]-HK-1 or [Leu^{11}]-SP on c-Fos expression by a formalin injection into the paw plantar region. Immunoreactivity of c-Fos in the spinal dorsal horn following the injection of 50 ml 2% formalin into the right hind paw 5 min after the intrathecal administration of saline (A, Control), [Leu^{11}]-HK-1 (B, [Leu^{11}]-SP) or [Leu^{11}]-SP (C, [Leu^{11}]-SP). (D) Number of c-Fos-positive cells in lamina I/II, III/IV, and lamina V/VI of the lumbar dorsal horn after the formalin injection in rats pretreated with saline (n = 5, Control), 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 (n = 5, [Leu^{11}]-HK-1), or 10^{-3} M (10 nmol/rat) [Leu^{11}]-SP (n = 5, [Leu^{11}]-SP). All points represent the number of c-Fos-positive cells ± S.E.M., and significant changes from the control were statistically analyzed by ANOVA with repeated measures, followed by Fisher's protected least significant difference (PLSD) and were represented as $^{*}P < 0.05$.

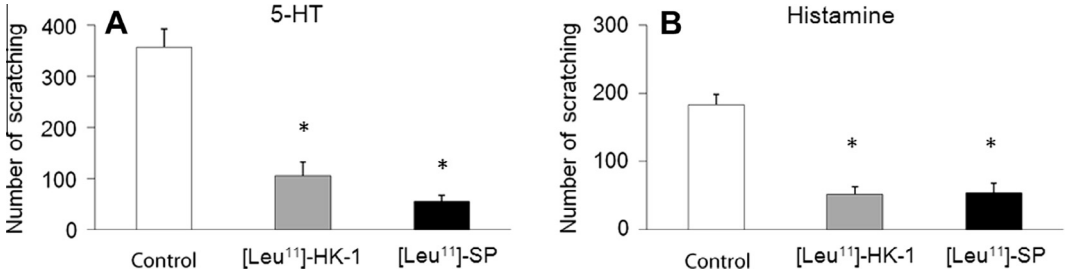

Fig. 4. Effects of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on the induction of scratching by 5-HT or histamine. (A) The number of scratchings induced for 20 min after the subcutaneous administration of 2×10^{-3} M (0.5 mg/rat) 5-HT in rats pretreated with saline (n = 6, Control), 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 (n = 6, [Leu^{11}]-HK-1), and 10^{-3} M (10 nmol/rat) [Leu^{11}]-SP (n = 6, [Leu^{11}]-SP) 5 min before 5-HT administration. (B) The number of scratchings induced for 20 min after subcutaneous administration of 2.25×10^{-3} M (10 mg/rat) histamine in rats pretreated with saline (n = 6, Control), and 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 (n = 6, [Leu^{11}]-HK-1), and 10^{-3} M (10 nmol/rat) [Leu^{11}]-SP (n = 6, [Leu^{11}]-SP) 5 min before the administration of histamine. All points represent the scratching number ± S.E.M., and significant changes from the number of scratchings induced by 5-TH or histamine in saline-pretreated rats were statistically analyzed by ANOVA with repeated measures, followed by Fisher's protected least significant difference (PLSD) and were represented as $^{*}P < 0.05$.

(0.1 mg/rat) histamine solution into the nape of the neck of saline-treated rats was regarded as the basal level (Fig. 4, Histamine, Control), and the raw number of scratchings induced for 5 min was 183.6 ± 14.8. The effects of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on histamine-induced scratching were very similar to those of 5-HT. When 50 μl of 2.25×10^{-3} M histamine was subcutaneously administered 5 min after the intrathecal administration of 10 μl of 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 or [Leu^{11}]-SP, the increase in the number of

scratchings following the administration of histamine was significantly attenuated by the pretreatment with [Leu^{11}]-HK-1 from the basal level, and the raw number of scratchings was 51.6 ± 10.8 (Fig. 4, Histamine, [Leu^{11}]-HK-1). Similarly, the number of histamine-induced scratchings was significantly decreased by the pretreatment with [Leu^{11}]-SP, and the raw number of scratchings was 53.8 ± 14.2 (Fig. 4, Histamine, [Leu^{11}]-SP).

Effect of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on c-Fos expression induced by the subcutaneous injection of 5-HT or histamine

The immunoreactivity of c-Fos was inconspicuous in the dorsal horn of saline-treated rats, while the subcutaneous administration of 50 µl of 2×10^{-3} M (0.5 mg/rat) 5-HT (Fig. 5A) or 2.25×10^{-3} M (0.1 mg/rat) histamine (Fig. 5D) to the hind paw of saline-treated rats markedly enhanced c-Fos immunoreactivity in the medial part of the dorsal horn. The enhanced immunoreactivity of c-Fos in the dorsal horn induced by 5-HT or histamine was markedly attenuated by the intrathecal administration of 10^{-3} M [Leu^{11}]-HK-1 (Fig. 5B, E) or 10^{-3} M [Leu^{11}]-SP (Fig. 5C, F).

The effects of the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP on 5-HT- or histamine-induced c-Fos expression were evaluated by examining changes in the number of c-Fos-positive cells in lamina I/II, lamina III/IV, and lamina V/VI of the dorsal horn. The number of c-Fos-positive cells was low in saline-treated rats, but was increased in lamina I/II following the injection of 2×10^{-5} M (0.5 mg/rat) 5-HT or 2.25×10^{-3} M (0.1 mg/rat) histamine into the hind paw of saline-treated rats. Indeed, the raw numbers of c-Fos-positive cells in lamina I/II, lamina III/IV, and lamina V/VI following the administration of 5-HT were 113.4 ± 2.1, 3.4 ± 0.1, and 2.8 ± 0.2, respectively, while those of histamine were 108.1 ± 3.6, 3.4 ± 0.4, and 4.0 ± 0.4, respectively. The number of c-Fos-positive cells in each lamina was considered as the basal level (Fig. 6, Control).

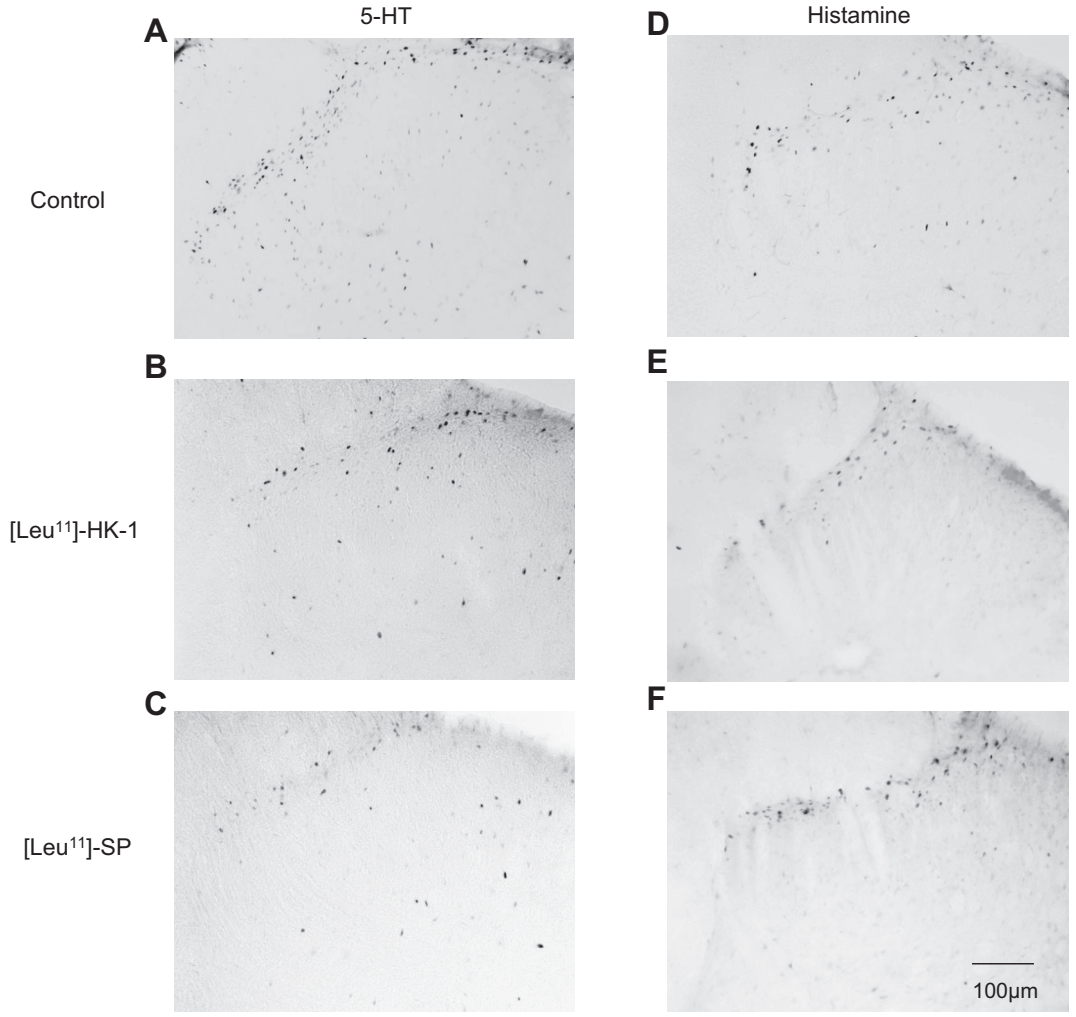

Fig. 5. Immunoreactivity of c-Fos in the spinal dorsal horn following the injection of 5-HT (5-HT) into the left hind paw 5 min after the intrathecal administration of saline (A, Control), 10^{-3} M [Leu^{11}]-HK-1 (B, [Leu^{11}]-HK-1 or 10^{-3} M [Leu^{11}]-SP (C, [Leu^{11}]-SP) and histamine injection (Histamine) into the left hind paw 5 min after the intrathecal administration of saline (D, Control), 10^{-3} M [Leu^{11}]-HK-1 (E, [Leu^{11}]-HK-1, or 10^{-3} M [Leu^{11}]-SP (F, [Leu^{11}]-SP).

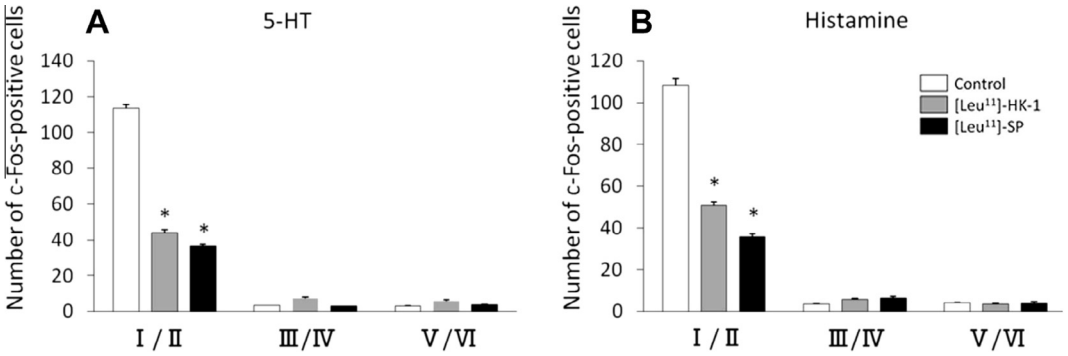

Fig. 6. Effects of the intrathecal administration of [Leu^{11}]-HK-1 or [Leu^{11}]-SP on c-Fos expression by the injection of 5-HT or histamine into the paw plantar region. (A) Number of c-Fos-positive cells in lamina I/II, III/IV, and lamina V/VI of the lumbar dorsal horn after the 5-HT injection in rats pretreated with saline (n = 5, Control), 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 (n = 5, [Leu^{11}]-HK-1), or 10^{-3} M (10 nmol/rat) [Leu^{11}]-SP (n = 5, [Leu^{11}]-SP. (B) Number of c-Fos-positive cells in lamina I/II, III/IV, and lamina V/VI of the lumbar dorsal horn after the histamine injection in rats pretreated with saline (n = 5, Control), 10^{-3} M (10 nmol/rat) [Leu^{11}]-HK-1 (n = 5, [Leu^{11}]-HK-1, or 10^{-3} M (10 nmol/rat) [Leu^{11}]-SP (n = 5, [Leu^{11}]-SP. All points represent the number of c-Fos-positive cells ± S.E.M., and significant changes from the control were statistically analyzed by ANOVA with repeated measures, followed by Fisher's protected least significant difference (PLSD) and were represented as $^{*}P < 0.05$.

To clarify the effects of the pretreatment with 10^{-3} M [Leu^{11}]-HK-1 or 10^{-3} M [Leu^{11}]-SP on 5-HT- or histamine-induced c-Fos expression, 10 µl of each peptide was intrathecally administered 5 min before the administration of 5-HT or histamine. 5-HT- or histamine-induced c-Fos expression was lamina-dependently attenuated by the pretreatment with [Leu^{11}]-HK-1 or [Leu^{11}]-SP and the manner of attenuation was similar to each other. A marked effect of the pretreatment with [Leu^{11}]-HK-1 and [Leu^{11}]-SP on the number of c-Fos-positive cells following the administration of 5-HT or histamine was observed in lamina I/II of the spinal cord, and the number of c-Fos-positive cells was significantly decreased from the basal level. On the other hand, no significant difference was observed between the numbers of c-Fos-positive cells in laminae III/IV and V/VI of saline-treated rats and rats pretreated with [Leu^{11}]-HK-1 or [Leu^{11}]-SP (Fig. 6A, B).

DISCUSSION

The results of the present study demonstrated that HK-1-induced scratching was attenuated by the pretreatment with [Leu^{11}]-HK-1, but not [Leu^{11}]-SP, while SP-induced scratching was attenuated by the pretreatment with [Leu^{11}]-SP, but not [[Leu^{11}]-HK-1. These results suggests that the functions of HK-1 and SP may be blocked by [Leu^{11}]-HK-1 and [Leu^{11}]-SP, respectively; therefore, it appears likely that [Leu^{11}]-HK-1 and [Leu^{11}]-SP are antagonists of the HK-1-preferred receptor and NK1 receptor, respectively, while HK-1 and SP are agonists of the HK-1-preferred receptor and NK1 receptor, respectively. This suggestion is inconsistent with an idea that HK-1 is a full agonist of the NK1 receptor (Morteau et al., 2001; Bellucci et al., 2002; Kurtz et al., 2002). Taken together, a piece of evidence indicates that the HK-1-preferred receptor is very similar, but not identical, to the NK1 receptor (Naono et al., 2007b). Therefore, it is possible that the HK-1-preferred receptor may be derived from the NK1 receptor, since the NK1 receptor consists of two subtypes, long isoform and short isoform, and it has been suggested that SP may bind to the long isoform of the NK1 receptor, while HK-1 may bind to the short isoform of the NK1 receptor (Watanabe et al., 2010). Alternatively, it cannot rule out the possibility that the amino acid sequences consisting of the HK-1 and NK1 receptors may be very similar, although the genes encoding these receptors are different. Now, it remains unclear whether the gene encoding the HK-1-preferred receptor is identical to that of the NK1 receptor.

The attenuating effect of [Leu^{11}]-SP on SP-induced scratching behavior was in agreement with the findings of a previous study (Naono et al., 2009), and [Leu^{11}]-HK-1 also attenuated HK-1-induced scratching. HK-1 and SP share Met at the C-terminus, and the intrathecal administration of these two peptides induced scratching behavior (Endo et al., 2006; Naono et al., 2007a,b, 2008; Naono-Nakayama et al., 2010). On the other hand, [Leu^{11}]-SP and [Leu^{11}]-HK-1 share Leu at the C-terminus, and have an antagonistic effect on the functions of SP and HK-1, respectively. Taken together, it is possible that tachykinin peptides sharing Met at the C-terminus, such as HK-1 and SP, exert an excitatory effect such as scratching behavior, while tachykinin-derived peptides sharing Leu at the C-terminus, such as [Leu^{11}]-HK-1 and [Leu^{11}]-SP, elicit an attenuating effect on the induction of scratching by HK-1 and SP. These results indicate that the amino acid, Met or Leu, at the C-terminus of tachykinin peptides plays a crucial role in the function of these peptides. This idea was based on the findings in studies examining the effects of EKC/D (using the common carboxyl-terminal duodecapeptide in endokinin C and endokinin D); indeed, the C-terminus of this peptide consists of Leu, and the pretreatment with EKC/D attenuated SP-induced scratching behavior (Naono et al., 2007b).

Furthermore, the present study revealed marked differences in the functions of [Leu^{11}]-HK-1 and [Leu^{11}]-SP. Indeed, the pretreatment with [Leu^{11}]-SP and [Leu^{11}]-HK-1 clearly attenuated the induction of scratching behavior by SP and HK-1, respectively, indicating that tachykinin-derived peptides have a more

specific antagonistic effect. A similar finding was recently reported; indeed, SP-induced scratching behavior was markedly attenuated by the N-terminal fragment of SP, SP (1–5), but not HK-1 (1–5) (Naono-Nakayama et al., 2014). In addition, the effects of the pretreatment with EKC/D on SP- and HK-1-induced scratching behavior were also similar to this, since the induction of scratching by SP, but not HK-1, was inhibited by the pretreatment with EKC/D (Naono et al., 2007b). Furthermore, similar to the effects of EKC/D on the induction of scratching behavior by SP, the desensitization induced by SP, but not HK-1, was also attenuated by the pretreatment with EKC/D (Naono et al., 2008). Taken together with these results, it is possible that [Leu^{11}]-SP, SP (1–5), and EKC/D are more specific antagonists of the NK1 receptor. Contrary to the effects of [Leu^{11}]-SP, the pretreatment with [Leu^{11}]-HK-1 produced a marked decrease in the scratching behavior induced by HK-1, but not SP, while the N-terminal fragment of HK-1, HK-1 (1–5), reduced the induction of scratching behavior by HK-1 and SP (Naono-Nakayama et al., 2014). Therefore, these findings suggest that [Leu^{11}]-HK-1, but not HK-1 (1–5), may be a suitable candidate for antagonists of the HK-1-preferred receptor and may be a valuable tool for evaluating the function of HK-1.

Similar to scratching behavior, a marked difference was noted between the effects of the pretreatment with [Leu^{11}]-HK-1 and [Leu^{11}]-SP on the induction of flinching behavior and the enhancement in c-Fos expression following the formalin injection into the plantar region of the hind paw. Formalin-induced flinching behavior and c-Fos expression were similarly attenuated by the intrathecal administration of [Leu^{11}]-SP, but not [Leu^{11}]-HK-1. Taken together with the results of scratching behavior shown in Fig. 1, it seems likely that [Leu^{11}]-SP administered at the spinal cord inhibits the functions of SP released from the central terminals of nociceptive primary afferents by the formalin injection; therefore, SP may have a crucial role in nociceptive processing in the spinal cord following formalin injection. This idea was supported by many previous studies, since formalin-induced flinching was attenuated by the intrathecal administration of NK-1 receptor antagonists (Yamamoto and Yaksh, 1991) and EKC/D (Naono et al., 2007b) as well as small interfering RNA against the NK1 receptor (Naono-Nakayama et al., 2011). On the other hand, the effect of [Leu^{11}]-HK-1 on enhanced flinching behavior and on c-Fos expression following the formalin injection was little, indicating that [Leu^{11}]-HK-1 hardly contributes to nociceptive processing, and the functions of HK-1 differ from SP (Endo et al., 2006; Naono et al., 2007b).

The intrathecal administration of [Leu^{11}]-HK-1 attenuated the induction of scratching behavior following the subcutaneous injection of 5-HT and histamine, pruritic agents, into the nape of the rat neck, whereas no significant change of [Leu^{11}]-HK-1 was observed in flinching behavior induced by the formalin injection, noxious stimulation. This result is consistent with the findings in mice pretreated with bombesin-saporin, which binds with high affinity to the gastrin-releasing peptide (GRP) receptor (Sun et al., 2009) and in mice pretreated with natriuretic polypeptide b (Nppb)-saporin (Mishra and Hoon, 2013). A treatment with bombesin-saporin markedly reduced the induction of scratching by an intradermal injection of pruritic agents such as histamine, compound 48/80, and 5-HT, while little changes were observed in the tests with noxious stimuli (Sun et al., 2009). Similarly, the induction of scratching by pruritic chemicals such as histamine, chloroquine, and 5-HT was significantly reduced in Nppb-saporin-treated animals, whereas no significant difference was noted in noxious thermal and chemical tests between animals treated with Nppb-saporin and saline (Mishra and Hoon, 2013), indicating that GRP and Nppb are involved in pruritic processing, but not nociceptive processing. The similarity between the attenuated effects of [Leu^{11}]-HK-1, bombesin-saporin, and Nppb-saporin on scratching behavior induced by pruritic agents prompted us to assume that HK-1 as well as GRP and Nppb may be involved in pruritic processing; however, the relationship between HK-1, GRP, and Nppb in pruritic processing remains to be elucidated.

The expression of c-Fos induced by the subcutaneous injection of pruritic chemicals such as histamine (Yao et al., 1992; Han et al., 2012), 5-HT or α-Met-5-HT (Nojima et al., 2003; Akiyama et al., 2009; Imamachi et al., 2009), protease-activated receptor-2 agonist (Nakano et al., 2008; Akiyama et al., 2009), endothelin-1 (Imamachi et al., 2009), compound 48/80 (Inan et al., 2009), and chloroquine (Han et al., 2012, 2013) was restrictively distributed at the superficial layer of the dorsal horn. Similarly, in the present study, more than 90% of c-Fos-positive cells following the injection of histamine and 5-HT were localized at the superficial layer of the dorsal horn. The ratio of cells positive to c-Fos in the superficial layer to other layers following the administration of pruritic agents was clearly higher than that after the formalin injection, indicating that the majority of cells responsive to the intradermal injection of pruritic chemicals are distributed in the superficial layer of the spinal dorsal horn (Akiyama et al., 2014). Thus, it seems likely that transmitters or modulators released from primary afferents following pruritic stimuli bind to receptors distributed at the superficial layer of the spinal cord, and the pruritic signals at the spinal cord are conveyed to higher structures in the brain.

In the present study, the induction of scratching behavior and enhancement in c-Fos expression following the intradermal injection of 5-HT or histamine was attenuated by the intrathecal administration of both [Leu^{11}]-HK-1 and [Leu^{11}]-SP. Since the intrathecal administration of [Leu^{11}]-HK-1 and [Leu^{11}]-SP reduced the functions of HK-1 and SP, as shown in Fig. 1, respectively, it is possible that both the HK-1-preferred receptor and NK1 receptor may be involved in pruritic processing in the superficial layer of the spinal dorsal horn. The involvement of NK1 receptor in pruritic processing was also suggested by attenuation of the induction of scratching behavior by 5-HT in rats treated with SP-saporin (Carstens et al., 2010) and by reduction of the itch intensity in patients with chronic pruritus following administration of NK1 receptor antagonist aprepitant

(Ständer et al., 2010). Contrary to the functions of NK1 receptor in pruritic processing, the results of the formalin test demonstrated the involvement of the NK1 receptor in nociceptive processing. Therefore, taken together, it is possible that the HK-1-preferred receptor and the NK1 receptor may be involved in only pruritic processing and in both pruritic and nociceptive processing, respectively. These results indicate that [Leu^{11}]-HK-1 is a specific blocker of the HK-1-preferred receptor involved in pruritic processing.

In summary, the present study demonstrated that the intrathecal administration of [Leu^{11}]-HK-1 attenuated HK-1-induced scratching and the induction of scratching behavior by 5-HT or histamine, indicating that HK-1 is a neurotransmitter or neuromodulator in pruritic processing at the level of the spinal cord. Therefore, it is possible that [Leu^{11}]-HK-1 is a valuable tool for clarifying the mechanisms underlying pruritic processing at the spinal level.

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REFERENCES

Akiyama T, Merrill AW, Zanotto K, Carstens MI, Carstens E (2009) Scratching behavior and Fos expression in superficial dorsal horn elicited by protease-activated receptor agonists and other itch mediators in mice. J Pharmacol Exp Ther 329:945–951.

Akiyama T, Tominaga M, Takamori K, Carstens MI, Carstens E (2014) Roles of glutamate, substance P, and gastrin-releasing peptides as spinal neurotransmitters of histaminergic and nonhistaminergic itch. Pain 155:80–92.

Bellucci F, Carini F, Catalani C, Cucchi P, Lecci A, Meini S, Patacchini R, Quartara L, Ricci R, Tramontana M, Giuliani S, Maggi CA (2002) Pharmacological profile of the novel mammalian tachykinin, hemokinin 1. Br J Pharmacol 135:266–274.

Brown JL, Liu H, Maggio JE, Vigna SR, Mantyh PW, Basbaum AI (1995) Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis. J Comp Neurol 356:327–344.

Carstens EE, Carstens MI, Simons CT, Jinks SL (2010) Dorsal horn neurons expressing NK-1 receptors mediate scratching in rats. Neuroreport 21:303–308.

De Koninck Y, Henry JL (1991) Substance P-mediated slow excitatory postsynaptic potential elicited in dorsal horn neurons *in vivo* by noxious stimulation. Proc Natl Acad Sci U S A 88:11344–11348.

Duffy RA, Hedrick JA, Randolph G, Morgan CA, Cohen-Williams ME, Vassileva G, Lachowicz JE, Laverty M, Maguire M, Shan L-S, Gustafson E, Varty GB (2003) Centrally administered hemokinin-1 (HK-1), a neurokinin NK1 receptor agonist, produces substance P-like behavioral effects in mice and gerbils. Neuropharmacology 45:242–250.

Endo D, Ikeda T, Ishida Y, Yoshioka D, Nishimori T (2006) Effect of intrathecal administration of hemokinin-1 on the withdrawal response to noxious thermal stimulation of the rat hind paw. Neurosci Lett 392:114–117.

Han N, Zu JY, Chai J (2012) Spinal bombesin-recognized neurons mediate more nonhistaminergic than histaminergic sensation of itch in mice. Clin Exp Dermatol 37:290–295.

Han L, Ma C, Liu Q, Weng H-J, Cui Y, Tang Z, Kim Y, Nie H, Qu L, Patel KN, Li Z, McNeil B, He S, Guan Y, Xiao B, LaMotte RH, Dong X (2013) A subpopulation of nociceptors specifically linked to itch. Nat Neurosci 16:174–182.

Henry JL (1976) Effects of substance P on functionally identified units in cat spinal cord. Brain Res 114:439–451.

Hökfelt T, Ljungdahl Å, Terenius L, Elde R, Nilsson G (1977) Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: enkephalin and substance P. Proc Natl Acad Sci USA 74:3081–3085.

Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, Basbaum AI (2009) TRPV1-expressing primary afferents generated behavioral responses to pruritogens via multiple mechanisms. Proc Natl Acad Sci USA 106:11330–11335.

Inan S, Dun NJ, Cowan A (2009) Nalfurafine prevents 5′-guanidinonal trindole- and compound 48/48-induced spinal c-fos expression and attenuates 5′-guanidinonal trindole-elicited scratching behavior in mice. Neuroscience 163:23–33.

Kuraishi Y, Nagasawa T, Hayashi K, Satoh M (1995) Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. Eur J Pharmacol 275:229–233.

Kurtz MM, Wang R, Clements MK, Cascieri MA, Austin CP, Cunningham BR, Chicchi GG, Liu Q (2002) Identification, localization and receptor characterization of novel mammalian substance P-like peptides. Gene 296:205–212.

LaMotte RH, Shimada SG, Sikand P (2011) Mouse models of acute, chemical itch and pain in human. Exp Dermatol 20:778–782.

Matsumura T, Sakai A, Nagano M, Sawada M, Suzuki H, Umino M, Suzuki H (2008) Increase in hemokinin-1 mRNA in the spinal cord during the early phase of a neuropathic pain state. Br J Pharmacol 155:767–774.

Mishra SK, Hoon MA (2013) The cells and circuitry for itch responses in mice. Science 340:968–971.

Morteau O, Lu B, Gerard C, Gerard NP (2001) Hemokinin 1 is a full agonist at the substance P receptor. Nat Immunol 2:1088.

Nakano T, Andoh T, Lee JB, Kuraishi Y (2008) Differential dorsal horn neurons responding to histamine and allergic itch stimuli. Neuroreport 19:723–726.

Naono R, Yoshioka D, Ikeda T, Nakayama T, Nishimori T (2007a) The common carboxyl-terminal region of novel tachykinin peptides contributes to induce desensitization in scratching behavior of rats. Brain Res Bull 71:461–465.

Naono R, Nakayama T, Ikeda T, Matsushima O, Nishimori T (2007b) Leucine at the carboxyl-terminal of endokinin C and D contributes to elicitation of the antagonistic effect on substance P in rat pain processing. Brain Res 1165:71–80.

Naono R, Nakayama T, Ikeda T, Matsushima O, Nishimori T (2008) Pharmacological characterization of desensitization in scratching behavior induced by intrathecal administration of hemokinin-1 in the rat. Neuropeptides 42:47–55.

Naono R, Nakayama T, Ikeda T, Matsushima O, Nishimori T (2009) Effect of the carboxyl-terminal of endokinins on SP-induced pain-related behavior. Biochem Biophy Res Commun 378: 182–185.

Naono-Nakayama R, Sunakawa N, Ikeda T, Nishimori T (2010) Differential effects of substance P or hemokinin-1 on transient receptor potential channels, TRPV1, TRPA1, and TRPM8, in the rat. Neuropeptides 44:57–61.

Naono-Nakayama R, Sunakawa N, Ikeda T, Nishimori T (2011) Knockdown of the tachykinin neurokinin 1 receptor by intrathecal administration of small interfering RNA in rats. Eur J Pharmacol 670:448–457.

Naono-Nakayama R, Ikeda T, Matsushima O, Sameshima H, Takamiya K, Funahashi H, Nishimori T (2014) An amino-terminal fragment of hemokinin-1 has an inhibitory effect on pruritic processing in rats. Neuroscience 259:172–183.

Nojima H, Carstens E (2003) Quantitative assessment of directed hind limb scratching behavior as a rodent itch model. J Neurosci Methods 126:137–143.

Nojima H, Simons CT, Cuellar JM, Carstens MI, Moore JA, Carstens E (2003) Opioid modulation of scratching and spinal c-fos expression evoked by intradermal serotonin. J Neurosci 23:10784–10790.

Page NM (2004) Hemokinins and endokinins. Cell Mol Life Sci 61:1652–1663.
Page NM (2005) New challenges in the study of the mammalian tachykinins. Peptides 26:1356–1368.
Page NM (2006) Characterization of the gene structures, precursor processing and pharmacology of the endokinin peptides. Vascul Pharmacol 45:200–208.
Sakai A, Takasu K, Sawada M, Suzuki H (2012) Hemokinin-1 gene expression is upregulated in microglia activated by lipopolysaccharide through NF-kB and p38 MAPK signaling pathways. PLoS One 7:e32268.
Shimada SG, LaMotte RH (2008) Behavioral differentiation between itch and pain in mouse. Pain 139:681–687.
Ständer S, Siepmann D, Herrgott I, Sunderkötter C, Luger TA (2010) Targeting the neurokinin receptor 1 with aprepitant: a novel antipruritic strategy. PLoS One 5:e10968.
Sun Y-G, Zhao Z-Q, Meng X-L, Yin J, Liu X-Y, Chen Z-F (2009) Cellular basis of itch sensation. Science 325:1531–1534.
Watanabe C, Mizoguchi H, Yonezawa A, Sakurada S (2010) Characterization of intrathecally administered hemokinin-1-induced nociceptive behaviors in mice. Peptides 31:1613–1616.
Yaksh TL, Rudy TA (1976) Chronic catheterization of the spinal subarachnoid space. Physiol Behav 17:1031–1036.
Yamaguchi T, Nagasawa T, Satoh M, Kuraishi Y (1999) Itch-associated response induced by intradermal serotonin through 5-HT2 receptors in mice. Neurosci Lett 35:77–83.
Yamamoto T, Yaksh TL (1991) Stereospecific effects of a nonpeptidic NK1 selective antagonist, CP-96,345: antinociception in the absence of motor dysfunction. Life Sci 49:1955–1963.
Yao GL, Tohyama M, Senba E (1992) Histamine-caused itch induces Fos-like immunoreactivity in dorsal horn neurons: effect of morphine pretreatment. Brain Res 599:333–337.
Yoshioka D, Takebuchi F, Nishimori T, Naono R, Ikeda T, Nakayama T (2006) Intrathecal administration of the common carboxyl-terminal decapeptide in endokinin A and endokinin B evokes scratching behavior and thermal hyperalgesia in the rat. Neurosci Lett 410:193–197.
Zhang Y, Lu L, Furlonger C, Wu GE, Paige CJ (2000) Hemokinin is a hematopoietic-specific tachykinin that regulates B lymphopoiesis. Nat Immunol 1:392–397.
Zimmerman M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109–110.

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