Hemokinin-1-derived peptides have antipruritic effects in rats

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Substance P (SP) is a member of the tachykinin peptide family. Hemokinin-1 (HK-1) was recently identified as a new mammalian tachykinin peptide. SP and HK-1 consist of undecapeptides and share a common carboxyl-terminal (C-terminal), Phe-Xaa-Gly-Leu-Met-amide motif, and more varied amino-terminals (N-terminal). The function of SP in the pain system of the spinal cord has been examined in detail, whereas that of HK-1 remains unclear. Therefore, the effects of [Leu11]-HK-1 on the induction of scratching behavior by the intrathecal administration of HK-1 or SP and scratching behavior by a subcutaneous injection of histamine and serotonin were examined in order to elucidate the function of HK-1. The pretreatment with [Leu11]-HK-1 decreased the induction of scratching behavior by HK-1, but not by SP, while the pretreatment with [Leu11]-SP decreased the induction of scratching behavior by SP, but not by HK-1. Furthermore, the pretreatments with [Leu11]-HK-1 and [Leu11]-SP decreased the frequency of scratching following an intradermal injection of pruritogens, such as serotonin (5-HT) and histamine, into the nape of the neck. The effects of the pretreatment with HK-1 (1-5), an N-terminal fragment peptide, were also examined to determine the function of HK-1. The pretreatment with HK-1 (1-5) attenuated the induction of scratching behavior by HK-1 and SP. In addition, the pretreatment with HK-1 (1-5) attenuated the induction of scratching behavior by a subcutaneous injection of histamine and 5-HT, while the pretreatment with SP (1-5) had a negligible effect on the scratching behavior induced by these compounds. Collectively, these results indicated that HK-1 was involved in pruritic processing because the pretreatment with [Leu11]-HK-1 and HK-1 (1-5) attenuated the induction of scratching behavior by an injection of histamine and 5-HT. Peptide-derived antagonists, such as [Leu11]-HK-1 and HK-1 (1-5), may be unsuitable for the treatment of pruritus because of the difficulties associated with penetrating the blood brain barrier. Therefore, the discovery of chemical compounds that function as antagonists to the HK-1-preferred receptor will become more important in the treatment of pruritic diseases.

Keywords: hemokinin-1; substance P; scratching; pruritus

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Two preprotachykinin genes, TAC1 and TAC3, encode three mammalian tachykinin peptides: substance P (SP; Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2), neurokinin A, and neurokinin B. Hemokinin-1 (HK-1; Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-Met-NH2) was recently identified as a new mammalian tachykinin peptide encoded by the new preprotachykinin gene, TAC4, detected in mouse bone marrow cells [1]. SP and HK-1 consist of undecapeptides and share a common carboxyl terminal (C-terminal), Phe-Xaa-Gly-Leu-Met-amide motif, and more varied amino terminals (N-terminal) [2].
The function of SP in the pain system of the spinal cord has been extensively examined. SP has been detected in and is released from small primary afferents, and is considered to function as a nociceptive transmitter or modulator; however, the function of HK-1 in the spinal cord has not yet been elucidated in detail.

Although the function of HK-1 may be clarified by evaluating the effects of antagonists on the HK-1-preferred receptor or by using knockout mice of the gene encoding the HK-1-preferred receptor, information on available antagonists or the HK-1-preferred receptor is limited. Therefore, we have attempted to discover antagonists of the HK-1-preferred receptor by applying the requirement of SP-derived antagonists to the neurokinin 1 (NK1) receptor because SP is a representative tachykinin peptide and many NK1 receptor antagonists have been reported to date. Spantide I [D-Arg^1, D-Trp^7,9, Leu^11]-SP was initially identified as an antagonist of the SP-derived NK1 receptor [3]. This antagonist is an SP-derived peptide that has some D-type amino acids, and Met at the C terminus is replaced by Leu. However, it remains unclear whether the antagonistic effects of Spantide-I are derived from D-type amino acids or Leu at the C terminus of SP-derived peptides or tachykinin-related peptide derived from a common sequence of endokinin C and endokinin D encoded in the human TAC4 gene [4], consists of Leu at the C terminus of this peptide and a pretreatment with EKC/D was shown to attenuate the function of SP [5]. Furthermore, [Met^11]-EKC/D, in which Leu at the C terminus of EKC/D was replaced by Met, elicited agonistic effects on the NK1 receptor [5], indicating that Leu at the C terminus of these peptides is a determinant for eliciting antagonistic effects on the NK1 receptor. A pretreatment with a peptide synthesized by the replacement of Met at the C terminus of SP with Leu, [Leu^{11}]-SP, was found to decrease the induction of scratching behavior by the intrathecal administration of SP, indicating that this peptide had antagonistic effects on the NK1 receptor [6]. These findings suggest that the antagonistic effects of Spantide I on the NK1 receptor are derived from Leu at the C terminus of this peptide, but not D-type amino acids, and also that antagonists derived from tachykinin peptides may be produced by the replacement of Met at the C terminus of these peptides with Leu.

Therefore, by considering the role of Leu at the C terminus of SP-derived peptides or tachykinin-related peptide, we attempted to make a new antagonist of the HK-1-preferred receptor by replacing Met at the C terminus of HK-1 with Leu, [Leu^{11}]-HK-1, and examined the effects of a pretreatment with [Leu^{11}]-HK-1 on the induction of scratching behavior by the intrathecal administration of HK-1. The pretreatment with [Leu^{11}]-HK-1 decreased the induction of scratching behavior by HK-1, but not by SP, while the pretreatment with [Leu^{11}]-SP reduced SP-induced scratching behavior, but not that by HK-1 [7], indicating that [Leu^{11}]-HK-1 and [Leu^{11}]-SP are suitable antagonists of the HK-1-preferred receptor and NK1 receptor, respectively. The effects of a pretreatment with [Leu^{11}]-HK-1 on nociceptive and pruritic processing were examined in order to elucidate the physiological function of HK-1. When the effects of the pretreatment with [Leu^{11}]-SP and [Leu^{11}]-HK-1 on the formalin test were evaluated, the pretreatment with [Leu^{11}]-SP decreased the frequency of flinching and the number of c-Fos-positive cells in the spinal cord following the subcutaneous injection of formalin into the plantar surface of the hind paw, while the pretreatment with [Leu^{11}]-HK-1 had a negligible effect on the formalin test. Since [Leu^{11}]-SP and [Leu^{11}]-HK-1 had antagonistic effects on the NK1 receptor and HK-1-preferred receptor, respectively, these results suggested that HK-1 was hardly involved in nociceptive processing, whereas SP played a more important role in nociception. In contrast, the pretreatment with [Leu^{11}]-HK-1 decreased the frequency of scratching after the intradermal injection of pruritogens, such as serotonin (5-HT) and histamine, into the nape of the neck. The pretreatment with [Leu^{11}]-SP also reduced the frequency of scratching behavior induced by the same pruritogens. [7] These findings indicated that HK-1 and SP are involved in pruritic processing, and also [Leu^{11}]-HK-1 and [Leu^{11}]-SP may be useful tools for the treatment of patients with pruritus.

The intrathecal administration of N-terminal fragments of SP, SP (1-7), and SP (1-8), has been shown to attenuate the induction of scratching behavior by SP [8,9]. This finding indicates that the N-terminal fragments of SP elicit an inhibitory effect on SP-induced scratching behavior; however, information on the effects of the N-terminal fragment of HK-1 is limited. Thus, we attempted to examine the effects of the intrathecal administration of the N-terminal fragments of HK-1 [10]. Since amino acids in the C-terminal regions of SP and HK-1 have a high homology and the N-terminal regions of these peptides are significantly divergent, HK-1 and SP were divided into two fragments, an N-terminal fragment and C-terminal fragment, based on the similarity of their amino acids. The N-terminal fragments of HK-1 and SP were designed as follows: the N-terminal fragments of HK-1 and SP consisted of five consecutive amino acids from the N terminus, HK-1 (1-5) and SP (1-5), respectively. Although the intrathecal administration of HK-1 (1-5) and SP (1-5) hardly induced scratching behavior, the pretreatment with HK-1 (1-5) attenuated the induction of scratching behavior by HK-1 and SP, indicating that HK-1 (1-5) inhibited the function of HK-1 and SP. On the other
hand, the pretreatment with SP (1-5) attenuated the induction of scratching behavior by SP, but not by HK-1, suggesting that SP (1-5) specifically suppressed the function of SP. In order to determine the involvement of these fragment peptides in nociceptive processing, the effects of pretreatments with these peptides were evaluated by the formalin test. The results of the formalin test revealed that the pretreatments with HK-1 (1-5) and SP (1-5) attenuated the frequency of flinching after an injection of formalin into the plantar surface of rats. The result that SP (1-5) exerted an attenuating effect in the formalin test indicated the involvement of SP in nociceptive processing and is consistent with previous findings obtained using an NK1 receptor antagonist [11], suggesting that SP (1-5) may have antagonistic effects on the NK1 receptor. The pretreatment with HK-1 (1-5) also attenuated the frequency of formalin-induced flinching. Since HK-1 (1-5) suppressed the induction of scratching by HK-1 and SP, the attenuating effects of HK-1 (1-5) in the formalin test suggest that HK-1 (1-5) modulated the function of SP in nociceptive processing or that HK-1 contributed to nociceptive processing. The pretreatment with HK-1 (1-5) attenuated the induction of scratching behavior by a subcutaneous injection of histamine and 5-HT, well-known pruritogens, while the effects of the pretreatment with SP (1-5) on scratching behavior induced by these compounds were negligible. This result indicated that HK-1 is a candidate transmitter in pruritic processing in the spinal cord, but not in nociceptive processing.

Collectively, these results suggest that HK-1 is involved in pruritic processing because the pretreatments with [Leu[11]]-HK-1 and HK-1 (1-5) attenuated the induction of scratching behavior by an injection of histamine and 5-HT, which are well-known pruritogens. On the other hand, it remains unclear whether SP contributes to pruritic processing because the pretreatment with [Leu[11]]-SP hardly affected pruritogen-induced scratching behavior, while SP (1-5) ameliorated the induction of scratching behavior by pruritogens, suggesting that SP is, at least partly, involved in pruritic processing. The administration of SP conjugated to saporin (SP-SAP) was shown to decrease the frequency of scratching induced by 5-HT-[12]. Similarly, in a clinical trial, the severity of pruritus in patients with prurigo or atopic predisposition was significantly ameliorated by a treatment with aprepitant (3-((2R,3S)-3-(P-Fluorophenyl)-2-((alph)-alpha-methyl-3,5-bis(trifluoromethyl)benzyl)oxy)morpholino)methyl)-delta(2)-1,2,4-triazolin-5-one), an NK1 receptor antagonist [13], and the systemic administration of aprepitant decreased the severity of pruritus in patients with metastatic solid tumors treated with biological drugs [14]. Sézary syndrome [15], T-cell lymphoma [16], erlotinib-induced pruritus [17], and Hodgkin’s lymphoma [18]. In addition, a treatment with nalfurafine (TRK-820, (-)-17-(cyclopropylmethyl)-3,14β-dihydroxy-4,5α-epoxy-6β-[N-methyl-trans-3-(3-furyl) acrylamido] morphinan hydrochloride), a κ-opioid receptor agonist, was previously reported to reduce the severity of uremic pruritus in a hemodialysis patient [19,20]. A growing body of clinical information on aprepitant and nalfurafine indicate that the NK1 receptor and κ-opioid receptor are involved in pruritic processing and also that these chemical compounds, but not peptides such as SP and dynorphin, an agonist of the κ-opioid receptor, may be candidates for the treatment of patients with chronic pruritus.

Taken together with the results of [Leu[11]]-HK-1 and a fragment peptide derived from HK-1, a new tachykinin peptide, may be involved in pruritic processing, but not nociceptive processing. To date, two peptides, gastrin-releasing peptide (GRP) [21,22] and neuropeptide natriuretic polypeptide b (Nppb, brain natriuretic peptide (BNP)) [23] have been proposed as proper neuropeptides that function as neurotransmitters of the itch sensation; however, this remains controversial [24,25]. On the other hand, it has yet to be established whether HK-1 is a neurotransmitter of the pruritus sensation. The distribution of this peptide in the central nervous system is restricted to the findings of a blotting study [26,27], therefore, an immunohistochemical study on HK-1 in the spinal and medullary dorsal horns is needed to elucidate the role of HK-1 in pruriceptive processing. Furthermore, the gene encoding the HK-1-preferred receptor has yet to be identified.

Thus, GRP, Nppb, and HK-1 may play a role in pruritic processing. Peptide-derived antagonists, such as [Leu[11]]-HK-1 and the N-terminal fragment of HK-1, may be unsuitable for the treatment of pruritus because of the difficulties associated with penetrating the blood brain barrier. Therefore, the discovery of chemical compounds that function as antagonists or agonists, such as aprepitant and nalfurafine, will become more important in the medical treatment of pruritus, and these chemical compounds may be able to cure patients with severe pruritus. In the near future, chemical compounds that are identified by fully utilizing the pharmacological characteristics of HK-1 and its receptor may become novel medicines for the treatment of pruritic diseases.

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