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Opioid activity of sendide, a tachykinin NK_1 receptor antagonist

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Abstract

Sendide, a tachykinin NK_1 receptor antagonist, was tested for antagonism against scratching, biting and licking responses elicited by intrathecal (i.t.) injections of various tachykinin receptor agonists, N-methyl-D-aspartate (NMDA), somatostatin and bombesin, in mice. Tachykinin NK_1 receptor agonists, substance P, physalaemin and septide, produced a characteristic behavioural response, consisting of scratching, biting and licking. The substance P-induced response was reduced by small doses (0.0625–1.0 pmol) of sendide in a dose-dependent manner. The behavioural response elicited by other tachykinin NK_1 receptor agonists, physalaemin and septide, was also reduced significantly by a small dose (1.0 pmol) of sendide. The inhibitory effect of sendide (1.0 pmol) was not affected by pretreatment with the opioid receptor antagonist, naloxone, at doses up to 4.0 mg/kg. Higher doses of sendide were needed to reduce the behavioural response to neurokinin A, a tachykinin NK_2 receptor agonist, neurokinin B, a tachykinin NK_3 receptor agonist and eledoisin, a tachykinin NK_2/NK_3 receptor agonist. Pretreatment with naloxone (2.0 mg/kg, i.p.) significantly antagonized sendide (1024 pmol)-induced inhibition of the behavioural responses to neurokinin A, neurokinin B and eledoisin. The behaviours elicited by i.t. injection of NMDA, somatostatin or bombesin were also reduced by a higher dose (1024 pmol) of sendide and this sendide effect was reversed by naloxone. These findings suggest that sendide at higher doses may possess opioid activity in addition to an antagonistic action at tachykinin NK_1 receptors in the spinal cord.

Keywords: Tachykinin NK_1 receptor antagonist; Sendide; Opioid activity; Spinal cord; Scratching, biting and licking; (Mouse)

1. Introduction

The undecapeptide, substance P, belongs to a group of related neuropeptides named tachykinins which includes neurokinin A and neurokinin B. There are known to be at least three receptors for the mammalian tachykinins, generally referred to as NK_1, NK_2 and NK_3 (Regoli et al., 1988), which have been cloned and sequenced (Masu et al., 1987; Yokota et al., 1989; Hershey and Krause, 1990; Shigemoto et al., 1990). The endogenous ligands for the three receptors are believed to be substance P, neurokinin A and neurokinin B, respectively, based on their relative agonist potencies (Quirion and Dam, 1988; Helke et al., 1990; Guard and Watson, 1991). Substance P is known to be involved in the spinal processing and transmission of nociceptive primary afferent inputs (Hylden and Wilcox, 1981; Urban and Randic, 1984; Frenk et al., 1988; Fleetwood-Walker et al., 1990; Chen et al., 1991). Intrathecally (i.t.) injected substance P and related compounds can produce reciprocal hindlimb scratching, biting and licking behaviour in mice (Hylden and Wilcox, 1981; Vaught et al., 1984; Takahashi et al., 1987; Sakurada et al., 1987) and tachykinin NK_1 receptor antagonists block the behaviour evoked by i.t. injection of substance P (Sakurada et al., 1992c, 1994). The i.t. administration of agonists for tachykinin NK_1 and/or NK_2 (neurokinin A, D-septide, neurokinin B and eledoisin) receptors also produces, behaviour similar to substance P-induced behaviour in mice (Sakurada et al., 1989, 1992b). Based on the rank order of potency of these mammalian tachykinins, the spinally me-

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diated behavioural response seems to be mediated by a preferential activation of tachykinin NK_1 receptors in the spinal cord (Sakurada et al., 1989). Tachykinin receptors have been the focus of attention of much pharmacological research aimed at the discovery of specific peptide and non-peptide tachykinin receptor antagonists. The first non-peptide tachykinin NK_1 receptor antagonist, CP-96,345 [(2*S*,3*S*)-*cis*-2-(diphenyl-methyl)-*N*-[(2-methoxy-phenyl)-methyl]-1-azabicyclo[2,2,2]octan-3-amine] has been shown to act as a competitive tachykinin NK_1 receptor antagonist at central and peripheral substance P binding sites, and in in vitro and in vivo functional assays (McLean et al., 1991; Snider et al., 1991). Recently, a peptide NK_1 receptor antagonist, sendide (Try-D-Phe-Phe-D-His-Leu-Met-NH_2), has been developed, which has high affinity for tachykinin NK_1 receptors in the mouse spinal cord (Sakurada et al., 1992c). It has been shown that this compound competitively antagonizes the spinally mediated behavioural response induced by i.t. injection of substance P. Binding studies have revealed that sendide displaces the binding of [^{3}H]substance P to mouse spinal cord membranes with extremely high potency. Thus, the recent development of selective tachykinin receptor antagonists has enabled investigation of the physiological role of neurokinins (Longmore et al., 1995; Sakurada et al., 1997).

There is evidence that an interaction between neurokinins and opioid systems may exist in the dorsal spinal cord. Opioid receptor agonists were found to inhibit the substance P-induced response in a dose-dependent way which is naloxone-reversible (Hylden and Wilcox, 1982; Takahashi et al., 1987; Sakurada et al., 1988; Johnston and Chahl, 1991). Some substance P analogues have been shown to produce naloxone-reversible antinociception (Post and Folkers, 1985) and inhibit tachykinin NK_2- and/or NK_3- receptor agonist-induced scratching, biting and licking, which are also reversed by pretreatment with naloxone (Sakurada et al., 1992b). Thus, the aim of the present study was to investigate, using naloxone, an opioid receptor antagonist, whether the spinal action of sendide is mediated through an opioid receptor.

2. Material and methods

The subjects were experimentally naive, male ddY mice (Shizuoka Laboratory Center, Japan) weighing an average of 23 g at the time of experiment. They were provided with free access to both food and water. The colony room was maintained at 22 ± 0.5°C with an alternating 12-h light–dark cycle. All animals were used only once. This study was conducted during the light phase of the cycle. Studies on the behavioural experiments were performed with the approval of the Ethics Committee of Animal Experiment in Tohoku College of Pharmacy.

Injections (i.t.) were made in unanesthetized mice at the L5 and L6 intervertebral space by the technique of Hylden and Wilcox (1980). A volume of 5 μl was injected i.t. with a 28-gauge needle connected to a 50-μl Hamilton microsyringe, while the animal was lightly restrained to maintain the position of the needle. Puncture of the dura was behaviourally indicated by a slight flick of the tail.

Before the i.t. injection, individual animals were adapted for 60 min to a plastic cage ($22 \times 15 \times 12.5$ cm) which also served as the observation chamber. Immediately following the i.t. injection of each receptor agonist, the mice were placed in the transparent cage and observed for 5 min. The total duration (s) of reciprocal movements of hindlimb scratching directed toward the flank, biting and/or licking of hind-legs and lower abdomen was recorded with a stopwatch. Like scratching, biting and/or licking were vigorous behaviours alternately directed to the left and the right.

The compounds administered were substance P, physalaemin, neurokinin A, neurokinin B, somatostatin, bombesin (Peptide Institute, Osaka, Japan), eledoisin (Peninsula Laboratories, Belmont, CA, USA), *N*-methyl-D-aspartate (NMDA) (Nakalai Tesque, Kyoto, Japan), and naloxone hydrochloride (Sigma, St. Louis, MO, USA). [Tyr^6, D-Phe^7, D-His^9]substance P-(6-11) (sendide) and [pGlu^6, L-Pro^9]substance P (6-11) (septide) were synthetized by conventional solid phase methods in our laboratory. For i.t. injections, these compounds were dissolved in sterile artificial cerebrospinal fluid (CSF) containing 126.6 mM NaCl, 2.5 mM KCl, 2.0 mM $MgCl_2$ and 1.3 mM $CaCl_2$. Sendide was co-injected with tachykinin NK receptor agonists, NMDA, somatostatin or bombesin in a total volume of 5 μl. Naloxone hydrochloride (Sigma) was dissolved in saline and administered intraperitoneally (i.p.) 10 min prior to injections of various agonists.

The results are presented as the means and S.E.M. Significant differences between groups were determined by Dunnett's test for multiple comparisons after analysis of variance (ANOVA). A P value of less than 0.05 was taken to indicate significance.

3. Results

3.1. Effects of sendide on the behavioural response to substance P, neurokinin A and neurokinin B

Fig. 1 shows the effectiveness of sendide for antagonizing the behavioural response elicited by i.t. injections of substance P (100 pmol), neurokinin A (400 pmol) and neurokinin B (1000 pmol). Sendide, given simultaneously with substance P, reduced the substance P-induced behavioural response with an ID_{50} of 0.3 pmol (0.1–0.8 pmol; 95% confidence limits when co-injected i.t.) (Fig. 1,

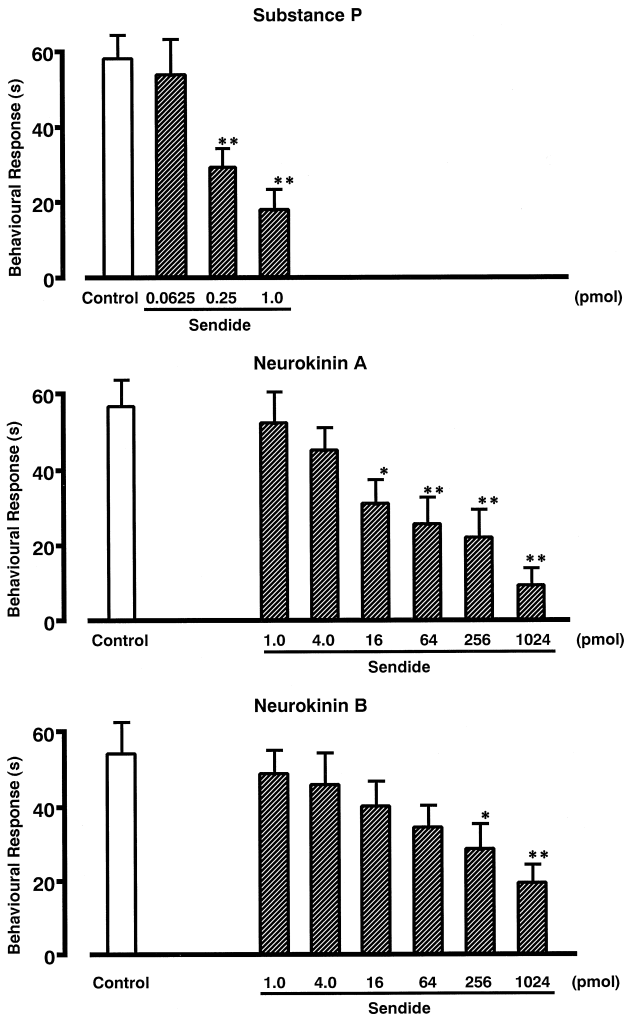

Fig. 1. Effect of sendide on the behavioural response evoked by substance P, neurokinin A and neurokinin B in mice. The duration of scratching, biting and licking induced by substance P (100 pmol), neurokinin A (400 pmol) or neurokinin B (1000 pmol) was determined over a 5-min period, starting immediately after intrathecal injection. Sendide was co-administered intrathecally with each agonist. The data are given as the means ± S.E.M. for groups of 10 mice. $^{**}P < 0.01$, $^{*}P < 0.05$ when compared to each agonist alone (control).

upper panel). A significant effect of sendide was observed against substance P from 0.25 pmol ($P < 0.01$) onwards. Sendide co-injected with neurokinin A or neurokinin B produced a dose-dependent reduction of each agonist-induced response at doses much higher than those needed to reduce the substance P response (Fig. 1, middle and lower panels). The ID_{50} values of sendide were 50.0 (20.0–160.0) pmol and 140.0 (30.0–600.0) pmol against neurokinin A and neurokinin B, respectively.

3.2. Effects of naloxone pretreatment on the inhibitory action of sendide

The inhibitory action of sendide on tachykinin NK_1, NK_2 and NK_3 receptor agonist-induced behavioural responses was tested in mice pretreated with naloxone, an opioid receptor antagonist. As shown in Fig. 2A, naloxone in the dose-range 0.5–4.0 mg/kg, i.p. gave no significant antagonism against sendide (1.0 pmol) which reduced markedly the substance P (100 pmol)-induced behavioural response. Similar results were obtained in the case of other tachykinin NK_1 receptor agonists, physalaemin (2.0 pmol) and septide (5.0 pmol) (Fig. 2B). In contrast, pretreatment with naloxone (2.0 mg/kg, i.p.) resulted in a significant antagonism against sendide (1024 pmol) in the behavioural response elicited by neurokinin A (400 pmol), neurokinin B (1000 pmol) and eledoisin (5.0 pmol) (Fig. 3).

When NMDA, somatostatin and bombesin were injected i.t., all mice exhibited a substance P-like behavioural response consisting of vigorous scratching, biting and licking. The duration of the agonistic action of NMDA (0.3 nmol) was similar to that with substance P.

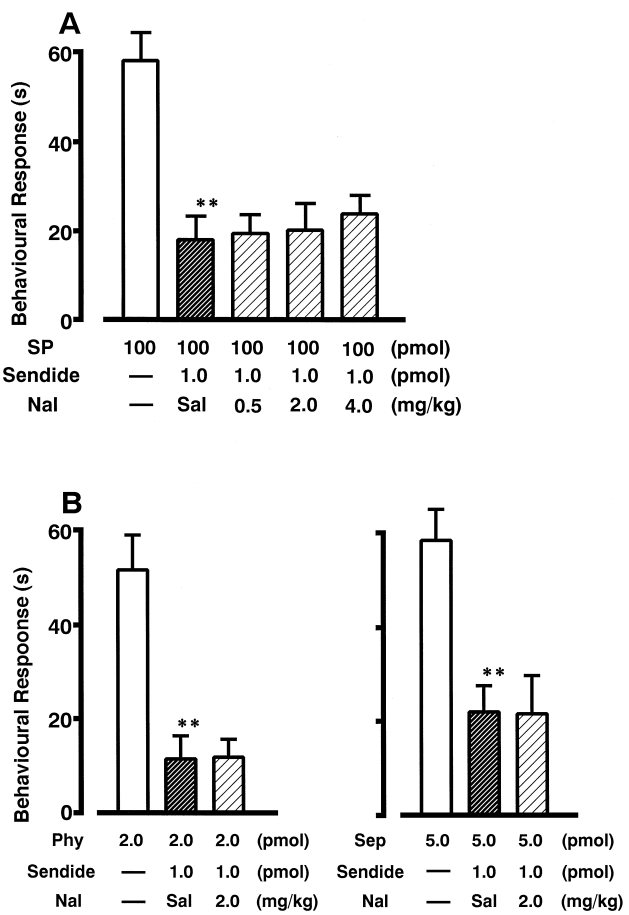

Fig. 2. Effect of naloxone pretreatment on the inhibitory action of sendide as assayed from the NK_1 receptor agonist-induced behavioural response. Sendide was co-administered intrathecally with each agonist. Naloxone (Nal) or saline (Sal) was administered i.p. 10 min before i.t. injection of substance P (SP; panel A), physalaemin (Phy; panel B) and septide (Sep; panel C). The data are given as the means ± S.E.M. for groups of 10 mice. $^{**}P < 0.01$ when compared to each agonist alone.

The behavioural response elicited by somatostatin (500 pmol) and bombesin (1.0 pmol) lasted longer than the response to substance P; the response induced by somatostatin and bombesin lasted for 20 min and 35 min, respectively. The response induced by both peptides peaked at 5–10 min post-injection.

Sendide (1024 pmol), co-administered with NMDA (0.3 nmol), caused a significant reduction of the NMDA-induced response (Fig. 4A). The somatostatin- and bombesin-induced responses were also reduced significantly by a high dose (1024 pmol) of sendide (Fig. 4B and C). Pretreatment with naloxone (2.0 mg/kg, i.p.) resulted in significant antagonism against sendide in the NMDA-, somatostatin- and bombesin-induced behavioural response.

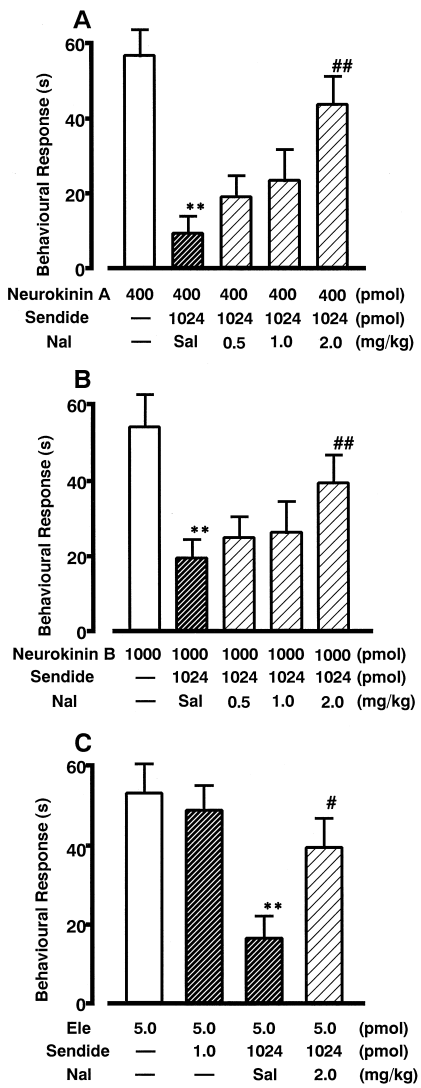

Fig. 3. Effect of naloxone pretreatment on the inhibitory action of sendide as assayed by the NK_2 and/or NK_3 receptor agonist-induced behavioural response. Sendide was co-administered intrathecally with each agonist. Naloxone (Nal) or saline (Sal) was administered i.p. 10 min before i.t. injection of neurokinin A (panel A), neurokinin B (panel B) and eledoisin (Ele; panel C). The data are given as the means ± S.E.M. for groups of 10 mice. $^{**}P < 0.01$ when compared to each agonist alone. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ when compared to sendide plus each agonist.

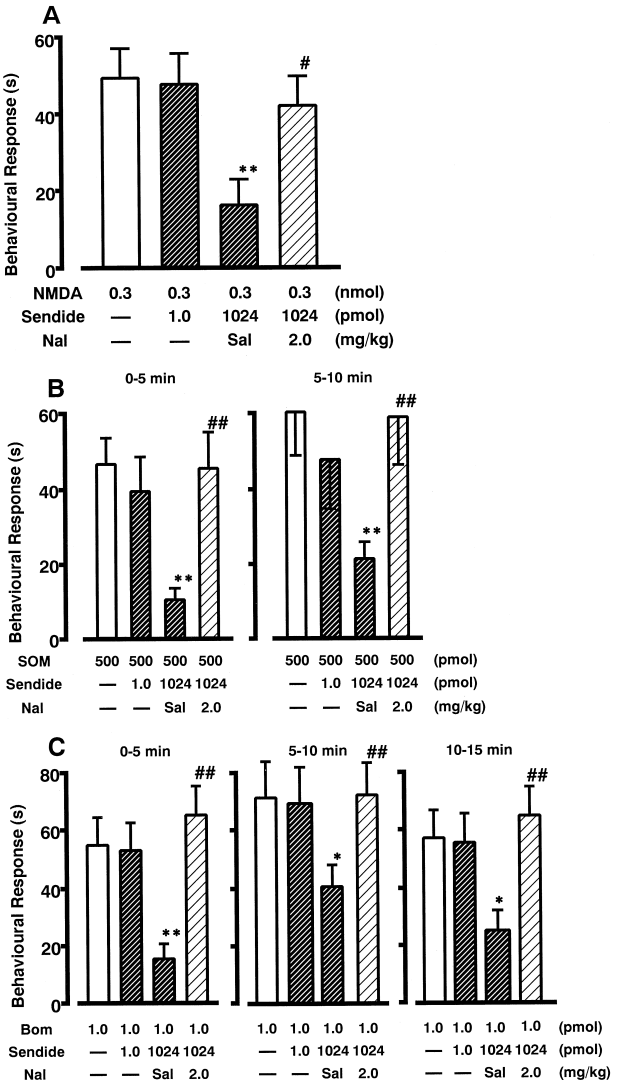

Fig. 4. Effect of naloxone pretreatment on the inhibitory action of sendide as assayed by the *N*-methyl-D-aspartate (NMDA)-, somatostatin- and bombesin-induced behavioural response. Sendide was co-administered intrathecally with each agonist. Naloxone (Nal) or saline (Sal) was administered i.p. 10 min before i.t. injection of NMDA (panel A), somatostatin (SOM; panel B) and bomebesin (Bom; panel C). These data are given as the means ± S.E.M. for groups of 10 mice. $^{**}P < 0.01$, $^{*}P < 0.05$ when compared to each agonist alone. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ when compared to sendide plus agonist.

4. Discussion

The present study clearly illustrated a dual action of sendide in the spinally mediated behavioural model in mice. An antagonistic effect of sendide on the scratching, biting and licking response induced by tachykinin NK_1 receptor agonists was produced with the small pmol dose range (less than 1.0 pmol) of the peptide antagonist. Much

higher doses of sendide were required to antagonize the behavioural response to tachykinin NK_2 and/or NK_3 receptor agonists. These results are consistent with those of previous studies (Sakurada et al., 1992c, Sakurada et al., 1994) showing that i.t. co-administration of sendide in a lower pmol range does not inhibit the behavioural response to agonists for the tachykinin NK_2 and/or NK_3 receptor, whereas at higher doses it produces significant inhibition of the characteristic response. In the present study, sendide at higher doses inhibited the behavioural response induced not only by tachykinin NK_2 and/or NK_3 receptor agonists but also by somatostatin, NMDA and bombesin, suggesting that sendide, injected i.t. at higher doses, could inhibit non-selectively the spinally mediated behavioural response. Though i.t. injected NMDA produces scratching, biting and licking behaviour resembling that seen with substance P (Aanonsen and Wilcox, 1986), the NMDA-induced behavioural response is mainly mediated through NMDA receptors in the spinal cord, but not through tachykinin NK_1 receptors (Sakurada et al., 1990). The present results support this possibility by providing evidence that 1.0 pmol of sendide with inhibitory activity for spinal tachykinin NK_1 receptors did not antagonize the NMDA-induced behavioural response. It is of importance to point out that the duration of the tachykinin NK_1 antagonistic effects of sendide, a peptidic antagonist, is similar to that of the effect of CP-96,345, a non-peptidic antagonist; the effect of sendide at doses of 4.0 pmol and 8.0 pmol lasted for 120 min and over 240 min, respectively (Sakurada et al., 1994). Sendide with a long duration of action may be a metabolically stable analogue of substance P-(6-11) when exposed to various peptidases in the spinal cord (Sakurada et al., 1994). It is possible, then, that sendide at a higher dose (1024 pmol) may be also able to reduce the long-lasting behavioural response induced by somatostatin and bombesin.

The interaction between neurokinin and opioid peptides in the spinal cord has been well documented (Frederickson et al., 1978; Hayes and Tyres, 1979; Doi and Jurna, 1981; Hylden and Wilcox, 1982; Willcockson et al., 1984; Takahashi et al., 1987; Sakurada et al., 1988; Larson, 1988; Skilling et al., 1990). Recent pharmacological data show that substance P markedly potentiates the antinociceptive effects of morphine following i.t. co-administration in rats (Kream et al., 1993). This potentiated antinociceptive effect is blocked by prior treatment with naloxone. Taken together, these earlier results suggest that some effects induced by i.t. substance P involve activation of opioid receptors in the spinal cord. This concept is supported by the present finding that the spinal action of sendide at high doses on the responses evoked by tachykinin NK_2 and/or NK_3 receptor agonists (neurokinin A, neurokinin B and eledoisin), somatostatin, NMDA and bombesin was reversed by pretreatment with naloxone (2.0 mg/kg). These results suggest that the inhibitory effect of sendide in high doses but not low doses may be mediated in part by opioid mechanisms in the spinal cord. Similarly, it was reported that the inhibitory effect of i.t. injected spantide, a first generation tachykinin antagonist, on the behavioural response induced by tachykinin NK_2 and/or NK_3 receptor agonists is antagonized by pretreatment with naloxone, though naloxone alone does not affect the tachykinin NK_1 receptor agonist-induced response (Sakurada et al., 1992b). In addition, we have shown that pretreatment with naloxone results in a dose-dependent antagonistic effect on the antinociception induced by a large dose (6.0 nmol) of spantide in the early and late phases of the paw-licking response with 2.0% formalin (Sakurada et al., 1992a). Results of prior binding studies have also shown that spantide has a low but measurable affinity for binding sites of [^{3}H]naloxone on the spinal cord and brain membranes (Sakurada et al., 1992b; Tan-No et al., 1995). The i.t. injection of [D-Trp^{7,9}, Leu^{11}]substance P produces antinociceptive effects in the mouse tail-flick and hot-plate tests, and this is reversed by a relatively high dose (5 mg/kg) of the opioid receptor antagonist, naloxone (Post and Folkers, 1985). Thus, it is fully reasonable to speculate that a peptide compound may possess dual actions, i.e., antagonist at tachykinin receptors and agonist at opioid receptors. Although the discovery of an antagonist active on both tachykinin NK_1 and NK_2 receptors has already been reported (Morimoto et al., 1992; Murai et al., 1992; Robineau et al., 1995; Kudlacz et al., 1996), this appears to be the first attempt to create a peptide compound displaying opioid activity in addition to tachykinin NK_1 receptor antagonistic activity. Sendide, acting on both opioid and tachykinin NK_1 receptors, could be clinically more useful for the treatment of chronic pain than highly specific tachykinin NK_1 or NK_2 receptor antagonists.

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