Substance P Induction of Itch-Associated Response Mediated by Cutaneous NK1 Tachykinin Receptors in Mice

TSUGUNOBU ANDOH, TETSURO NAGASAWA, MASAMICHI SATOH and YASUSHI KURAISHI

Department of Applied Pharmacology, Faculty of Pharmaceutical Sciences (T.A., Y.K.), Department of Clinical Application (T.N.), Research Institute for Wakan-yaku, Toyama Medical & Pharmaceutical University, Toyama and Department of Molecular Pharmacology (M.S.), Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

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ABSTRACT

Our experiments were conducted to determine whether substance P (SP) would elicit an itch sensation mediated by mast cells in mice. An intradermal injection of SP (10–135 µg site−1) into the rostral back of the ICR mouse dose-dependently produced scratching of the injected site. The SP (135 µg site−1 = 100 nmol site−1) induced scratching was inhibited by capsaicin (repeated administration) and naloxone: features being similar to itch in humans. SP elicited scratching in mast cell-deficient (WBB6F1/W/W) mice as well as control (+/+). Pretreatment with compound 48/80 produced similar degrees of inhibition of SP-induced scratching in mast cell-deficient mice as well as control (+/) and ICR mice. Intradermal injections of the NK1 receptor agonist GR73632 produced dose-dependent scratching, while the NK2 agonist GR64349 and the NK3 agonist senktide were without effects. SP-induced scratching was inhibited by the NK1 receptor antagonists spantide and L-668,169, but not by the NK2 antagonist L-659,877. The results suggest that scratching of the mouse induced by an i.d. injection of SP is itch-associated response. The SP action may be mediated at least partly by cutaneous NK1 receptors, and mast cells may not be key factors in SP-induced itching.

Itch is a sensation that provokes a desire to scratch. It is the most common symptom of cutaneous diseases (e.g., atopic dermatitis, contact dermatitis, urticaria) and accompanies several systemic disorders (e.g., chronic renal failure, cholestasis), but its underlying mechanisms are far from being understood. This sensation is produced experimentally by several endogenous substances such as histamine, SP, vasoactive intestinal peptide and neurotensin (Hägermark, 1992). SP is one of the most potent pruritogenic endogenous peptides (Hägermark et al., 1978) and speculated to be in involved in some pruritic diseases (Farber et al., 1986). Basic peptides generally degranulate mast cells and SP produces the degranulation as well (Devillier et al., 1989; Ebertz et al., 1987), and itch induced by SP is thought to be mediated by histamine released from mast cells (Hägermark et al., 1978). We have recently found that when applied to the skin of the mouse, SP elicits scratching of the treated skin and that histamine was without apparent effects (Kuraishi et al., 1995). These findings suggest the possibility that histamine is not an itch mediator in the mouse and that SP elicited scratching through histamine-independent mechanisms. Therefore, in our experiments, we examined whether scratching induced by SP would be due to an itch sensation and whether mast cells would play an important role in SP-induced itching.

Methods

Animals. In most experiments, male ICR mice (Japan SLC, Ltd., Shizuoka, Japan) of 5 to 6 wk of age, weighing 24 to 26 g, were used and in some experiments, male mast cell-deficient mice (WBB6F1/W/W; 10 wk old, average body weight = 25.4 g) and the control ones (WBB6F1/+; 10 wk old, average body weight = 28.4 g) were used. They were housed under controlled temperature (23–25°C) and light (lights on from 08:00 to 20:00). Food and water were freely available.

Drugs. SP (Peptide Institute, Minoh, Japan), compound 48/80 (Sigma Chemical Co., St. Louis), naloxone hydrochloride (Sigma), and spantide (Peptide Institute) were dissolved in physiological saline. GR73632, GR64349, senktide, L-668,169, and L-659,877 were from Research Biochemicals International (Natick, MA) and dissolved in physiological saline. Capsaicin (Sigma) was dissolved in physiological saline containing 10% ethyl alcohol.

Procedure. Before behavioral recording, the mice (four animals per observation) were put into an acrylic cage (26 × 18 × 30 cm) composed of four cells (13 × 9 × 30 cm) for at least 1 hr for acclimation. Immediately after intradermal injection, they were put back to

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ABBREVIATIONS: GR64349, Lys-Asp-Ser-Phe-Val-Gly-R-y-lactam-Leu-Met-NH2; GR73632, NH2-(CH2)6-CO-Phe-Phe-Pro-NM-Leu-Met-NH2; L-659,877, cyclo(Gln-Trp-Phe-Leu-Met); L-668,169, cyclo(Glu-O-Trp(NMe) Phe(R)Gly [ANC-2]Leu-Met); NK, neurokinin; RM-ANOVA, repeated measures analysis of variance; SP, substance P.
the same cell and their behavior was videotaped using an 8-mm video camera for 1 hr with any experimenter kept out from the observation room. Playing back of the video served for counting scratching behavior. The mouse generally showed several scratching by the hind paws for about 1 sec and a series of these movements was counted as one bout of scratching (Kuraishi et al., 1995). SP was injected i.d. in a volume of 50 μl into the rostral part of the back (around interscapular level), the hair of which had been removed on the day before the injection, except one experiment in which SP was injected into the caudal back. Increasing doses (50, 50, 100, 150 and 200 mg kg⁻¹) of capsaicin was injected s.c. into the caudal part of the back daily for 5 days under ether anesthesia, in light of ethical considerations (Zimmerman, 1985), and SP was injected the day after the last capsaicin treatment. Naloxone hydrochloride (1 mg kg⁻¹) was administered s.c. 15 min before SP injection. Compound 48/80 (100 μg site⁻¹) was injected s.c. into the rostral part of the back in a volume of 200 μl, and 2 or 3 hr later SP was injected to the same region. In one experiment, to determine the duration of edema after compound 48/80 treatment, this agent (10 μg in a volume of 20 μl) was injected i.d. into the ear, the thickness of which was measured using a dial thickness gauge (Ozaki MFG, Tokyo, Japan). Spantide, L-668,169 and L-659,877 were injected i.d. together with SP. The degree of inhibition of scratching was calculated with reference to the number of scratches per 60 min and for the average number of control group to be 100%.

**Data analysis.** Data were analyzed by RM-ANOVA and post hoc Dunnett’s test; a P < .05 value was considered significant. Data were presented as the mean and s.e.

**Results**

**Some characteristics of SP-induced scratching.** When injected i.d. into the rostral back, SP elicited scratching of the skin around the injected site by the hind paws. Figure 1 shows an example of scratching responses to SP (135 μg site⁻¹). The mouse showed the first scratching 1 min 35 sec after the injection and then intermittent scratching. SP (10–135 μg site⁻¹) dose-dependently elicited scratching of the treated skin (fig. 2C–E). The effect of a dose of 1 μg site⁻¹ was not apparent (similar to that of saline; fig. 2A) and the effect of a dose of 300 μg site⁻¹ was similar to that of 135 μg site⁻¹ (fig. 2B and F). The average latency of the scratching after SP at a dose 135 μg site⁻¹ was 2.1 ± 0.3 min (n = 8) and those at doses of 100, 135 and 300 μg site⁻¹ were similar to each other. The effects of SP (100–300 μg site⁻¹) peaked in the initial 10-min period and almost subsided by 30 min after injection. As a dose of 135 μg site⁻¹ (= 100 nmol site⁻¹) produced the maximal effect, this dose was used in subsequent studies.

An i.d. injection of SP (100 nmol site⁻¹) into the rostral back did not apparently elicit scratching of the face (fig. 2G). In addition, when injected into the caudal back, a region which the mouse can not scratch, SP (100 nmol site⁻¹) did not elicit scratching of the rostral back (fig. 2H).

Capsaicin pretreatment resulted in an apparent reduction of scratching induced by SP at a dose of 100 nmol site⁻¹ (fig. 3); application of RM-ANOVA demonstrated significant main effect of capsaicin (P < .05) and group × time interaction (P < .01). Pretreatment with naloxone (1 mg kg⁻¹) markedly suppressed the scratching induced by SP at a dose of 100

![Fig. 1. An example of hind-paw scratching after an i.d. injection of SP. SP at a dose of 135 μg site⁻¹ (= 100 nmol site⁻¹) was injected i.d. into the rostral back of the ICR mouse at time 0. The animal showed several hind-paw scratches for about 1 sec, which is shown by each bar.](image1)

![Fig. 2. Dose-response and site of the scratch-inducing action of SP. A–F. The mice were given an i.d. injection of saline (A) or SP at doses of 1 (B), 10 (C), 100 (D), 135 (E) and 300 μg site⁻¹ (F) into the rostral back of the ICR mouse and the scratching of the skin around the injected site was counted. G. The scratching of the face of the same mice as that of E, respectively. H. The mice were given an intradermal injection of SP (135 μg site⁻¹) into the caudal back and the scratching of the rostral back was counted. n = 8 (A–E, G and H) and 12 (F).](image2)

![Fig. 3. Inhibition by capsaicin of SP-induced scratching. The ICR mice were given s.c. injections of increasing doses (50, 50, 100, 150 and 200 mg kg⁻¹) of capsaicin (B; n = 12) or vehicle (A; n = 13) daily for 5 days. The day after the last capsaicin treatment, SP (100 nmol kg⁻¹) was injected i.d. RM-ANOVA. Main effect of capsaicin, F(1,23) = 5.56, P < .05; group × time interaction, F(5,115) = 4.21, P < .01.](image3)
nmol site\(^{-1}\) (fig. 4); RM-ANOVA revealed significant main effect of naloxyone (P < .001) and group \(\times\) time interaction (P < .0001).

**Effects of compound 48/80.** A s.c. injection of compound 48/80 (100 \(\mu g\) site\(^{-1}\)) into the rostral back of the ICR mouse elicited scratching of the injected site, which almost subsided by 90 min after the injection. When injected into the ear, compound 48/80 caused acute local edema, which peaked at 15 min and almost subsided by 2 hr; the thickness of the ear was 0.52 \pm 0.01, 1.21 \pm 0.08 and 0.66 \pm 0.01 mm \((n = 6)\) before and 15 min and 2 hr after compound 48/80 treatment. Thus, SP (100 nmol site\(^{-1}\)) was i.d. injected into the same region as compound 48/80 injection 2 hr after its pretreatment. Subcutaneous pretreatment with compound 48/80 substantially inhibited SP-induced scratching (fig. 5); RM-ANOVA revealed significant main effect of compound 48/80 \((P < .05)\) and group \(\times\) time interaction \((P < .001)\). The degree of inhibition by compound 48/80 was 59.3 \pm 12.9\% \((n = 8)\).

SP (100 nmol site\(^{-1}\)) substantially elicited scratching in mast cell-deficient mice as well as the control (+/+) ones (fig. 6A and C). The average latency of the first scratching was 1.5 \pm 0.3 \((n = 8)\) and 2.3 \pm 0.4 min \((n = 8)\) in the mast cell-deficient (WBB6F1 W/W\(^{v}\)) and control (+/+) mice, respectively. Pretreatment with compound 48/80 (100 \(\mu g\) site\(^{-1}\), 3 hr before) significantly suppressed scratching induced by SP at a dose of 100 nmol site\(^{-1}\) in the mast cell-deficient mice (main effect, \(P < .05\); group \(\times\) time interaction, \(P < .01\); RM-ANOVA) and the control ones (main effect, \(P < .01\); group \(\times\) time interaction, \(P < .001\); RM-ANOVA) (fig. 6B and C). The degree of suppression by compound 48/80 was 58.7 \pm 5.6 \((n = 8)\) and 60.7 \pm 5.0\% \((n = 8)\) in mast cell-deficient and control mice, respectively.

**Effects of tachykinin receptor agonists and antagonists.** When injected i.d. into the rostral back of the ICR mice, the NK\(_1\) receptor agonist GR73632 (10, 30, 100 nmol site\(^{-1}\)) produced a dose-related scratching of the injected skin (fig. 7). The average latency of scratching following a dose of 100 nmol site\(^{-1}\) was 2.5 \pm 1.0 min \((n = 8)\), and the time-course was similar to that of SP at the same dose (see fig. 2E). In contrast, the NK\(_{2}\) agonist GR64349 and the NK\(_{3}\) agonist senktide were inactive at doses of 10, 30 and 100 nmol site\(^{-1}\) (fig. 7).

Scratching induced by SP (100 nmol site\(^{-1}\)) was significantly (main effect, \(P < .05\)) inhibited by simultaneous injec-

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**Fig. 4.** Inhibition by naloxyone of SP-induced scratching. The ICR mice were given a s.c. injection of saline (A) or naloxyone (B) at a dose of 1 mg kg\(^{-1}\) and 15 min later an intradermal injection of SP (100 nmol site\(^{-1}\)). \(n = 8\) each group. RM-ANOVA, Main effect of naloxyone, \(F(1,14) = 21.59, P < .001\); group \(\times\) time interaction, \(F(5,70) = 7.58, P < .0001\).

**Fig. 5.** Inhibition by compound 48/80 of SP-induced scratching. The scratching behavior of ICR mice was counted after an i.d. injection of SP (100 nmol site\(^{-1}\)) to the rostral back where saline (A) or compound 48/80 (B) at a dose of 100 \(\mu g\) site\(^{-1}\) was s.c. injected 2 hr before SP injection. \(n = 8\) each group. RM-ANOVA, Main effect of compound 48/80 \((F(1,14) = 4.63, P < .05\); group \(\times\) time interaction, \(F(5,70) = 5.68, P < .001\).

**Fig. 6.** Comparison of SP-induced scratching and its inhibition by compound 48/80 between the mast cell-deficient and the control mice. SP (100 nmol site\(^{-1}\)) was injected i.d. into the mast cell-deficient mice (WBB6F1 W/W\(^{v}\); A and B) and control ones (WBB6F1 +/-; C and D). B and D, The mice were pretreated with subcutaneous injection of compound 48/80 (100 \(\mu g\) site\(^{-1}\)) 3 hr before SP injection. \(n = 8\) each group. RM-ANOVA: (A and B) main effect of compound 48/80, \(F(1,14) = 7.47, P < .05\); group \(\times\) time interaction, \(F(5,70) = 3.33, P < .01\); C and D, Main effect of compound 48/80, \(F(1,14) = 10.37, P < .01\); group \(\times\) time interaction, \(F(5,70) = 6.77, P < .0001\).

**Fig. 7.** Scratch-inducing activities of selective agonists for tachykinin receptor subtypes. The ICR mice were given an intradermal injection of GR73632 (NK\(_1\) agonist), GR64349 (NK\(_{2}\) agonist) or senktide (NK\(_{3}\) agonist) at doses of 10 (A, D, G), 30 (B, E, F) and 100 nmol site\(^{-1}\) (C, F, I) into the rostral back. \(n = 8\) each group.
Fig. 8. Inhibition by spantide of SP-induced scratching. The ICR mice were given an intradermal injection of SP (100 nmol site\(^{-1}\)) alone (A) or together with spantide at doses of 0.05 (B) and 0.5 nmol site\(^{-1}\) (C); n = 8 each group. RM-ANOVA, Main effect of spantide, F(2,21) = 4.17, P < .05.

Fig. 9. Effects of antagonists against either NK1 or NK2 tachykinin receptors on SP-induced scratching. The ICR mice were given an i.d. injection of SP (100 nmol site\(^{-1}\)) alone (CNT) or together with the NK1 antagonist L-668,169 or the NK2 antagonist L-659,877. n = 8 each group. *P < .05 as compared with CNT.

Discussion

Cutaneous administration of SP elicits scratching by the hind paws in mice (Kuraishi et al., 1995; present experiment). Our major purpose was to determine whether such scratching is due to an itch sensation of the treated skin. Scratching and scratching-like behavior were reported to be induced by intracerebroventricular and intrathecal injections of SP (Ravard et al., 1994). However, an intradermal injection of SP (100 nmol site\(^{-1}\)) into the rostral back elicited scratching of the injected site but not the facial scratching. In addition, an injection of SP at the same dose into the caudal back did not elicit scratching of the rostral back. These results suggest that this neuropeptide induced scratching through local skin stimulation.

As itch is a subjective sensation, it is hard to determine whether scratching is due to an itch sensation. Therefore, we conducted two series of experiments to examine whether scratching induced by i.d. SP would share features of human itching. Repeated pretreatment with capsaicin partially but significantly inhibited SP-induced scratching, a finding suggesting that the SP action is at least partly mediated by capsaicin-sensitive primary afferents. In this context, several kinds of human itching were claimed to be alleviated by repeated treatment with capsaicin. For example, experimental itch induced by histamine (Toth-Kasa et al., 1986) and by capsaicin itself (Green and Shaffer, 1993) is inhibited by capsaicin treatment. Repeated treatment with capsaicin cream also suppresses itch of patients with pruritic diseases such as chronic renal failure with hemodialysis treatment (Breneman et al., 1992), pruritic psoriasis (Ellis et al., 1993), naloxone-pretreated asthmatics (Wallengren, 1991) and hydroxyethyl starch-induced pruritus (Szeimies et al., 1994).

SP-induced scratching was markedly inhibited by the opioid antagonist naloxone, suggesting the involvement of the opioidergic systems. Considering that naloxone enhances, rather than inhibits, pain behavioral responses (Jacob and Ramabadran, 1978; Sugimoto et al., 1986), the inhibition by naloxone of behavioral response to SP may rule out the possibility that this response was due to a pain sensation. This view is in accord with our previous observation that painful stimulation did not elicit scratching (Kuraishi et al., 1995). Naloxone ameliorates itch sensation of patients with pruritic disease, especially cholestasis (Bernstein and Swift, 1979; Bergasa et al., 1992) and inhibits scratching activity of the patients (Bergasa et al., 1992). In addition, naloxone elevates the itch threshold for histamine stimulation in human subjects (Bernstein et al., 1982), although the duration of itch induced by histamine was claimed not to be altered by naloxone (Fjellner and Hägermark, 1983). Taking account of these findings in human, our result is consistent with the idea that SP-induced scratching is due to pruritogenic stimulation (probably an itch sensation) of the treated skin.

Itching is the most common side effect of epidural or intrathecal injection of opioid analogues, especially morphine (for review see Ballantyne et al., 1988), which is inhibited by naloxone (Saiah et al., 1994). Direct intracranial injections of opioids, especially mu receptor agonists, induce naloxone-reversible scratching in animals (Thomas et al., 1992; Thomas and Hammond, 1995; Tohda et al., 1997). In mice, an intracisternal injection of morphine (0.1–3 nmol per mouse) produces a dose-dependent facial scratching (Tohda et al., 1997), although an i.d. injection of this opioid (3 and 30 nmol/mouse) does not produce scratching of the injected site (Tohda et al., 1996). These findings taken together suggest that opioid peptide(s) are involved in transmission or facilitatory regulation of itch signaling in the central nervous system and that naloxone blocks central opioidergic function to inhibit SP-induced scratching. All these findings taken together suggest that SP-induced scratching is due to an itch sensation.

Compound 48/80 itself is pruritogenic (Wahlgren et al., 1990) and subcutaneous pretreatment with compound 48/80...
inhibits SP-induced itching in human subjects (Hägermark et al., 1978). Similarly in mice, compound 48/80 itself induced scratching (Kuraishi et al., 1995; present experiment) and the pretreatment inhibited SP-induced scratching. These similarities provide support for the idea that SP-induced scratching is due to itch stimulation. Although compound 48/80 caused acute local edema, it almost subsided by 2 hr. Therefore, the inhibition of the SP action by compound 48/80 pretreatment might not be due to the dilution of the injected SP by extravascular fluid. As compound 48/80 and SP produce the degranulation of mast cells (Ebertz et al., 1987; Lowman et al., 1988), the simplest explanation of the inhibition by compound 48/80 of SP-induced scratching is that compound 48/80 depleted mast cell histamine that is responsible for itch. However, SP elicited scratching even in mast cell-deficient (WBB6F1/W+/-) mice and the frequency of scratching was similar to that of control +/- mice. Considering that few or no mature mast cells are detected in the skin of naive WBB6F1/W+/- mice (Theoharides et al., 1993), this raises the possibility that mast cells are not essential to the SP action. Pretreatment with compound 48/80 inhibited SP-induced scratching in mast cell-deficient mice as well as control +/- and ICR mice. In our preliminary experiments, an intradermal injection of compound 48/80 (30 μg site ~1) apparently elicited scratching in the mast cell-deficient mice (309 ± 30 hr~1) as well as control +/- mice (182 ± 25 hr~1) and ICR mice (224 ± 50 hr~1). These findings taken together suggest that compound 48/80 acts mainly on cells other than mast cells to elicit scratching and to inhibit SP action. In this context, compound 48/80 was claimed to act directly on capsaicin-sensitive primary afferents (Eglezos et al., 1992). Although the precise site(s) of these actions of compound 48/80 remain unclear, it should be noted that inhibition by compound 48/80 does not suggest the exclusive involvement of mast cells.

The mice scratched after an i.d. injection of the tachykinin NK1 receptor agonist GR73632, although the NK2 agonist GR64349 and the NK3 agonist senktide were without effects. In addition, the scratch-inducing action of SP was suppressed by coadministration of the NK1 antagonists spantide and L-668,169, but not by the NK2 antagonist L-659,877. These results suggest that tachykinin NK1 receptors in the skin are involved in the SP action. The finding that the SP action was inhibited by the NK2 antagonist L-668,169 also in mast cell-deficient mice suggests that NK1 receptors, a target of intraepidermal SP, are present other than mast cells. SP releases histamine from human skin mast cells (Ebertz et al., 1987; Lowman et al., 1988) and rat peritoneal mast cells (Fettrell et al., 1982; Devillier et al., 1989). The histamine-releasing effect may not be due to the activation of tachykinin receptors on the mast cells, but instead due to the direct action on G protein (Devillier et al., 1989; Mousli et al., 1990). The stimulation of tachykinin NK2 receptors was claimed to release histamine from a murine mast cell line (Krumins and Broomfield, 1993). Therefore, the involvement of NK1 receptors in SP-induced scratching is consistent with the view that mast cells do not play an important role in the behavioral action of SP.

There are several possible sites of action of SP (the location of NK1 receptors) in the skin. One possible site is intracutaneous terminals of primary sensory neurons. NK1 receptor mRNA is expressed in the mouse dorsal root ganglia (Andoh et al., 1996). SP increases intracellular Ca2+ concentrations in cultured mouse dorsal root ganglion neurons, which express NK1 receptor mRNA (Tsurumi et al., 1997), and this SP action is suppressed by NK1 receptor antagonists (T. Andoh and Y. Kuraishi, unpublished observation). These findings suggest that SP acts directly on the peripheral terminals of primary sensory neurons through NK1 receptors, although it remains unknown whether such primary sensory neurons convey itch signals. The other cells such as macrophages (Lucey et al., 1994), keratinocytes (Koizumi et al., 1994) and endothelial cells (Bowden et al., 1994) are also potential sites of action of intradermal SP. Although the precise mechanisms of SP-induced scratching are unclear, endogenous factors released from such cells may regulate the SP action.

Although low doses of spantide antagonized the SP-induced scratching, the higher doses increased the SP action. This agent has no agonist activity for NK1 receptors, but it shows some affinity for opioid receptors (Sakurada et al., 1992) and produces histamine-mediated vascular effects (Hoover, 1991). Therefore, although the precise mechanisms are unclear, pharmacological actions other than NK1 receptor blockade may be involved in the increasing effect of spantide on the SP action. In conclusion, an i.d. injection of SP into the rostral back of the mouse elicited scratching, which may be itch-associated response. The results suggest that the SP action is at least partly mediated NK1 receptors in the skin. Mast cells do not play an important role in the SP-induced response, although their regulatory roles cannot be denied. This may be an animal model of itch, which is useful in studying the mechanisms of itch and in developing new kinds of antipruritic drugs.

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Send reprint requests to: Dr. Yasushi Kuraishi, Department of Applied Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical & Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan.
