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# Scratching behavior induced by pruritogenic but not algesiogenic agents in mice

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**Abstract**

We compared the behavioral effects of treatment with pruritogenic and algesiogenic agents in mice. The animals were given subcutaneous injections of pruritogenic agents, compound 48/80 (3–100 μg), substance P (10–300 μg) and histamine (3–300 μg), and algesiogenic agents, capsaicin (30 and 100 μg) and dilute formalin (5 mg of formaldehyde), into the rostral back, and scratching of the injected site by the hind paws was counted. Compound 48/80 and substance P dose dependently elicited the scratching behavior, but histamine, capsaicin and dilute formalin were without significant effects at the doses examined. These results suggest that compound 48/80- and substance P-induced scratching of the injected site is due to itch, but not to pain. The data did not provide support for the idea that histamine produces itch in the mouse.

*Keywords:* Scratching; Itch; Pain; Compound 48/80; Substance P; Histamine

## 1. Introduction

Although pruritus is the main and unpleasant symptom of cutaneous diseases and accompanies several visceral disorders, such as chronic renal failure, hepatic cholestasis, and diabetes mellitus, its underlying mechanisms remain unknown. To shed light on the physiological and pathological mechanisms of pruritus, we need animal models of itch. However, there are no reliable animal models available (Woodward et al., 1985). One reason for this might be due to the difficulties in behavioral animal experiments on pruritus. For example, although itch is a sensation that is associated with a strong desire to scratch, the mouse licks, but not scratches, its hind paw when it is subcutaneously (s.c.) injected with the pruritogenic agent compound 48/80 (unpublished observation), a behavioral response similar to that following treatment with the algesiogenic agent formalin (Hunskaar et al., 1985). Pruritus may be a main symptom in canine allergy (Woodward et al., 1985), but intradermal injections of histamine and compound 48/80 cause a painful reaction rather than itching behaviors (Schwartzman, 1965). Humans can distinguish ticklish stimuli from prickly ones, while many mammals respond with a similar stereotypical behavior (see McMahon and Koltzenburg, 1992). In addition, the mouse shows scratching of the ear and body during grooming, which is hard to consider as itch-associated behavior.

Since itch is a subjective sensation and animals do not describe their sensory experiences, for the measurement of itch in animal behavioral experiments, we should use itch-related behaviors that are elicited only by pruritogenic stimuli but not by other sensory stimuli such as painful ones and that are rarely observed in untreated animals. As mentioned above, itch is a sensation that provokes a desire to scratch the stimulated area, probably to scratch away irritants from the skin. Clinically, scratch is used as an objective measure of itch (Savin et al., 1973; Felix and Shuster, 1975; Aoki et al., 1980; Summerfield and Welch, 1980). Therefore, as a first step in developing an animal model of itch, we compared the behavioral effects, especially scratch-eliciting ones, of treatment with pruritogenic and algesiogenic agents in mice. Here, we report that s.c.

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injections of pruritogenic agents, but not algesiogenic ones, into the rostral part of the back produced scratching of the injected site by the hind paws, which probably is an itch-associated behavior.

## 2. Materials and methods

### 2.1. Materials

Compound 48/80 (Sigma, St. Louis, USA), substance P (Peptide Institute, Minoh, Japan), histamine (Sigma, St. Louis, USA), and formalin (Wako Pure Chemical Ind., Osaka, Japan) were dissolved in or diluted with physiological saline. Capsaicin (Sigma, St. Louis, USA) was dissolved in physiological saline containing 50% dimethyl sulfoxide. All these agents were s.c. injected into the back at the interscapular level in a volume of 0.10 ml.

### 2.2. Behavioral experiments

Male ddY mice of 4 weeks of age, weighing 18–23 g, were used in the experiments. 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. Before the experiment, the animals were put into an acrylic cage (22 × 22 × 24 cm) for about 10 min for acclimation. Immediately after s.c. injection, they (generally five animals/cage) were put back into the same cage and, for the observation of scratching, behaviors were recorded using an 8-mm video camera (CCD-700V, Sony, Tokyo, Japan) under unmanned conditions. Scratching of the injected site by the hind paws was counted and that of other sites such as ears was disregarded. Each mouse was used for only one experiment. The mice generally showed several scratches for about 1 s and a series of these behaviors was counted as one incident of scratching at 10-min intervals.

### 2.3. Data processing

Statistical comparisons were made using one-way analysis of variance and Dunnett's post-hoc test or for data without normal distribution Kruskal-Wallis statistic on ranks and Dunn's post-hoc test; the calculation was done using software SigmaStat (Jandel, San Rafael, USA) and $P < 0.05$ was considered significant. The means of data are presented together with S.E.

## 3. Results

### 3.1. Compound 48/80-induced scratching

Compound 48/80 has been shown to produce an itch sensation in humans (Armstrong et al., 1953; Hägermark et al., 1978; Fjellner and Hägermark, 1981). When sixteen mice were given compound 48/80 (50 μg, s.c.) into the rostral part of the back, all showed scratching of the injected site by the hind paws. The first scratching was observed within 5 min after injection in all mice examined and then scratching appeared intermittently. The frequency of behaviors other than scratching, for example grooming and forelimb motions, was not apparently different between compound 48/80- and saline-treated mice.

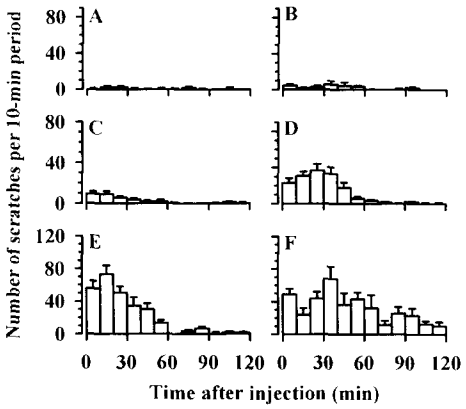


Fig. 1. Time course of scratching after an s.c. injection of compound 48/80. Mice were given an injection of saline (A, $n = 10$) or compound 48/80 at doses of 3 (B, $n = 10$), 10 (C, $n = 20$), 30 (D, $n = 20$), 50 (E, $n = 16$) and 100 μg (F, $n = 10$). Values represent the means and S.E.

Fig. 1 shows the time course of scratching behaviors for 2 h after the injection of compound 48/80. When compared with saline, compound 48/80 at doses of 10–100 μg produced apparent scratching, without effects at a dose of 3 μg. The effects of compound 48/80 (10–50 μg) peaked within 30 min and had almost subsided by 60 min, while that of 100 μg of compound 48/80 lasted for more than 60 min. When the number of scratches in 60 min after compound 48/80 was plotted against the dose, the effect was dose-dependent from 10 to 50 μg; the effect of 100 μg was not apparently different from that of 50 μg (Fig. 2).

### 3.2. Behavioral effects of substance P and histamine

Substance P and histamine as well as compound 48/80 produce an itch sensation in humans (Armstrong et al., 1953; Hägermark et al., 1978; Fjellner and Hägermark, 1981). Injections of substance P at s.c. doses of 100 and 300 μg elicited slight but significant scratching, without effects at lower doses of 10 and 30 μg (Fig. 2). The first scratching after substance P (300 μg) was observed within 5 min in all mice examined ($n = 5$) and the effects of substance P (100 and 300 μg) peaked in the first 10-min period and had almost subsided by 30 min following injection. Grooming and

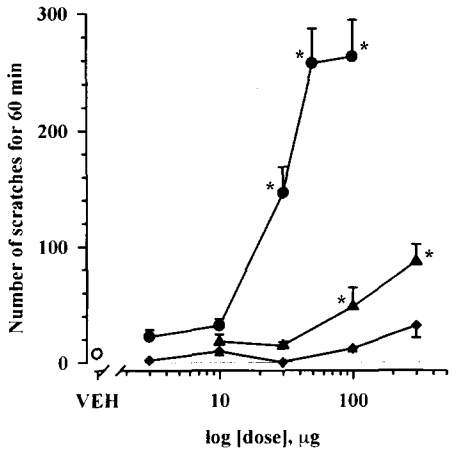


Fig. 2. Dose-response curves for the scratch-inducing effect of compound 48/80, substance P and histamine. Mice were given an s.c. injection of compound 48/80 (●, $n = 10$–20), substance P (▲, $n = 5$–6), histamine (◆, $n = 5$–10) or saline (VEH, ○, $n = 10$). The number of scratches in 60 min was plotted against the dose. Values represent the means and S.E. * $P < 0.05$ when compared with VEH (Dunn's post-hoc test).

forelimb motions were not apparently different between substance P- and saline-treated mice.

Injections of histamine at s.c. doses of 3–300 μg did not have a significant scratching-inducing effect (Fig. 2). Although following the maximum dose tested (300 μg) the number of scratches in 60 min was 31.8 ± 10.5 ($n = 5$), there were few scratches in 20 min following injection. Histamine at the doses tested did not produce any apparent alterations in gross behaviors, such as sedation, and motor functions, such as ataxia and weakness of the hindlimbs.

### 3.3. Effects of capsaicin and formalin

To determine whether the painful stimulation of the rostral part of the back would elicit scratching behavior, we examined the behavioral effects of s.c. injections of algesiogenic agents, capsaicin and formalin (Fig. 3). In contrast to compound 48/80 and substance P, capsaicin (30 and 100 μg) and dilute formalin (5 mg of formaldehyde) did not induce significant scratching behavior following injection. Although a higher dose (300 μg) of capsaicin resulted in the death of all animals tested ($n = 5$) during the observation period, capsaicin and dilute formalin at the doses tested did not apparently alter gross behaviors and motor functions.

## 4. Discussion

The main findings of the present study are that, when injected into the rostral part of the back of the mouse, pruritogenic, but not algesiogenic, agents elicited scratching of the injected site by the hind paws. In human subjects, application of 500 μg/ml of compound 48/80 on the blister base (Armstrong et al., 1953), an intradermal injection of 0.3–10 μg/ml (Fjellner and Hägermark, 1981), and an s.c. injection of 10 μg/ml (Hägermark et al., 1978) have been shown to cause an itch sensation the duration of which is relatively short, for example several minutes (Armstrong et al., 1953). In the present experiments, s.c. injections of compound 48/80 at doses of 10–50 μg (100–500 μg/ml) produced scratching behavior in mice in a dose-dependent manner. These concentrations are greater than itch-producing ones in humans and the duration of the scratching was longer than that of the itch sensation in human subjects. Intradermal injections of substance P at doses of 0.1–10 μM (corresponding to about 0.13–13 μg/ml) produce an itch sensation in humans (Hägermark et al., 1978; Fjellner and Hägermark, 1981). Although higher concentrations (1–3 mg/ml) were needed, substance P produced scratching in mice. On the other hand, injections of capsaicin (30 and 100 μg) and dilute formalin (5 mg of formaldehyde) into the rostral back did not elicit scratching behavior. When s.c. injected into the hind paw, these algesiogenic agents elicit licking of the treated paw (Hunskaar et al., 1985; Sakurada et al., 1992), a behavior considered to be a pain-related response. Taken together, these findings suggest that scratching of the treated rostral back by the hind paws is due to itch, but not to pain.

Capsaicin acts on primary afferents to release substance P (for review, see Holzer, 1991), but, in the present experiments, substance P, but not capsaicin, elicited scratching in mice. The precise reason why

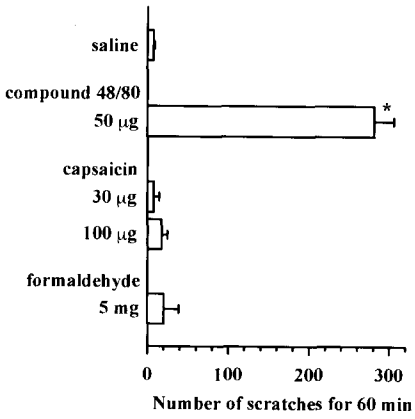


Fig. 3. Scratching following s.c. injections of compound 48/80, capsaicin and formaldehyde. Mice were given an injection of compound 48/80 (50 μg, $n = 19$), capsaicin (30 and 50 μg, $n = 5$ each group), dilute formalin (5 mg of formaldehyde, $n = 5$) or saline ($n = 10$) and the number of scratchings was counted for 60 min. Values represent the means and S.E. * $P < 0.05$ when compared with saline (Dunnett's post-hoc test).

capsaicin did not induce scratching is not clear. However, when applied on the human skin, capsaicin produces a burning pain, but not an itch sensation (Tóth-Kása et al., 1986; Breneman et al., 1992). Taking account of the 'selectivity theory' of neural mechanisms of itch (McMahon and Koltzenburg, 1992), the widespread activation of nociceptive primary afferents with capsaicin might mask the activity of 'itch-signaling' primary afferents induced by released substance P.

Intracranial injections of substance P at relatively low doses induce hindlimb scratching in mice (Dobry et al., 1981) and rats (Van Wimersma Greidanus and Maigret, 1988). However, it is unlikely that peripherally administered substance P acted upon the central nervous system to elicit the scratching. The reasons for this are as follows: first, in the present experiments, substance P at s.c. doses of 100 and 300 μg (corresponding to about 5 and 15 mg/kg, respectively) produced scratching of the injected site in mice, while this peptide at intravenous doses up to 50 mg/kg does not elicit scratching in the same species (Dobry et al., 1981). Second, an intracranial injection of substance P elicits grooming behavior (Dobry et al., 1981; Van Wimersma Greidanus and Maigret, 1988), while an s.c. injection of substance P did not. Third, in our preliminary experiments, substance P (135 μg) injected into the caudal part of the back did not induce scratching of the rostral part of the back. These findings taken together suggest that scratching of the injected site following s.c. injection of substance P is due to a scratch-inducing sensation at the injected skin.

Compound 48/80 and substance P release mediators from the skin mast cells (Barrett et al., 1985; Ebertz et al., 1987; Lowman et al., 1988). Therefore, one of the conceivable mechanisms for the scratch-inducing effects of compound 48/80 and substance P is that these agents act upon the mast cells to release mediators, which produce scratching behavior. Histamine is present in the mast cells and has been thought to be an important mediator of itch. When applied on the blister base at concentrations of 100–1000 μg/ml (Armstrong et al., 1953) or intradermally injected at concentrations of 0.3-10 μg/ml (Hägermark et al., 1978; Fjellner and Hägermark, 1981), histamine produces an itch sensation in humans. In the present experiments, however, s.c. injections of histamine at doses of 3–300 μg (30–3000 μg/ml) did not apparently elicit the scratching behavior. Although the precise reason why histamine could not elicit scratching is unclear, one explanation is that, at least in the mouse, histamine is not a strong itch-producing mediator and that scratching induced by compound 48/80 and substance P is mediated by mediators other than histamine. In support of this view, the histamine $H_1$ receptor antagonist chlorcyclidine inhibits the flare response, but not the itch sensation, induced by an intradermal injection of substance P at a concentration of 10 μM, corresponding to about 13 μg/ml (Hägermark et al., 1978). In addition, it is claimed that histamine is not a main mediator of itch in many pruritogenic diseases (Krause and Shuster, 1983; Wahlgren et al., 1990; Hägermark, 1992). Another explanation is that there are species differences in itch mediators and that histamine produces itch in humans but not in mice. In this context, serotonin, another chemical mediator of murine mast cells, apparently elicits scratching in rats (Berendsen and Broekkamp, 1991) and mice (unpublished observation), although it produces mild itch in humans (Fjellner and Hägermark, 1979). In any case, histamine does not induce a desire to scratch in the mouse.

In summary, the present results suggest that scratching of the rostral back injected with compound 48/80 and substance P by the hind paws may be due to itch, an impulse to scratch, but not due to pain. Such pseudo-itch behavior may be a useful index for physiological and pharmacological studies on itch and antipruritic agents. The data did not provide support for the idea that histamine applied peripherally produces itch in the mouse.

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