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Involvement of unique mechanisms in the induction of scratching behavior in BALB/c mice by compound 48/80

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Abstract

Compound 48/80 induced scratching behavior in BALB/c mice, and the role of mast cell mediators in this behavior was examined. Mouse scratching behavior was detected and evaluated using a new apparatus, MicroAct. Compound 48/80 increased the incidence of scratching behavior and scratching time in a dose-dependent manner, accompanied by a potent activation of mast cells and a potent increase in vascular permeability. Dibucaine and μ-opioid receptor antagonists inhibited the scratching behavior. Although histamine H_1 receptor antagonists potently inhibited the vascular permeability increase, they did not affect the scratching behavior. Methysergide inhibited the scratching behavior slightly without affecting the vascular permeability increase, whereas cyproheptadine inhibited both. A cyclooxygenase inhibitor, a 5-lipoxygenase-activating protein inhibitor and a PAF receptor antagonist did not affect the scratching behavior. High doses of serotonin induced scratching behavior less frequently than did compound 48/80. Furthermore, mast cell-deficient WBB6F1-W/W^v mice exhibited frequent scratching behavior after injection of compound 48/80. These results clearly indicate that compound 48/80 can induce scratching behavior in mice independent of mast cell mediators.

Keywords: Scratching behavior; (Mouse); Compound 48/80; Histamine; 5-HT (5-hydroxypryptamine, serotonin); Mast cell; MicroAct

1. Introduction

Compound 48/80 is a potent activator of connective tissue-type mast cells and/or skin mast cells (Enerbäck, 1966; Koibuchi et al., 1985; Benyon et al., 1989), and the cutaneous reaction caused by compound 48/80 has been discussed in relation to mast cell mediators such as histamine (Metys et al., 1988; Juhlin and Pihl-Lundin, 1992). In humans, intradermal injection of compound 48/80 induces itch as well as flare and protein extravasation (Stahle and Hagermark, 1984; Wahlgren et al., 1991; Rukwied et al., 2000). The itch evoked in healthy people is attenuated by histamine H_1 receptor antagonists, demonstrating the involvement of skin mast cell activation (Rukwied et al., 2000). Histamine derived from skin mast cells seems to be the most important mediator of itch in many skin diseases. In contrast, the itch evoked by compound 48/80 in atopic dermatitis patients is not affected by histamine H_1 receptor antagonists, indicating that histamine derived from mast cells does not play an important role (Wahlgren, 1991; Rukwied et al., 2000). The histamine-induced cutaneous reaction in both healthy people and atopic dermatitis patients is attenuated by histamine H_1 receptor antagonists, but the cutaneous response in atopic dermatitis patients is reduced (Rukwied et al., 2000). Therefore, compound 48/80 seems to induce itch in atopic dermatitis patients independent of histamine. Compound 48/80 induces a high incidence of scratching behavior in mice, and the scratching behavior is sometimes used as an index for the study of itch (Kuraishi et al., 1995; Kim et al., 1999; Inagaki et al., 2000b). As reported previously, however, histamine induces frequent scratching behavior only in a few strains of mice such as ICR but not in other strains of mice (Kuraishi et al., 1995; Inagaki et al., 2000a, 2001), although compound 48/80 is a potent inducer of scratching behavior even in mice that do not exhibit frequent scratching behavior in response to histamine (Kuraishi et al., 1995). These reports suggest that compound 48/80 could induce scratching behavior in mice independent of histamine, and that some similarities may be present between histamine-insensitive mice and atopic dermatitis patients. In

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the present study, therefore, to elucidate the role of mast cell mediators we examined the scratching behavior caused by compound 48/80 in mice. We used BALB/c mice because histamine does not induce frequent scratching behavior in this strain.

Scratching behavior is used as an index for the study of itch not only in experimental animals but also in humans (Endo et al., 1997; Bijak et al., 2001). However, quantitative and reliable evaluation of scratching behavior is sometimes difficult in both experimental animals and humans. Recently, we developed a new apparatus, MicroAct, which detects and evaluates mouse-scratching behavior automatically and objectively. We used this apparatus in the present study.

2. Materials and methods

2.1. Animals

Male BALB/c, WBB6F1-+/+ and WBB6F1-W/W^v mice, 8–10 weeks of age, obtained from Japan SLC (Hamamatsu, Japan) were used. Experiments were undertaken following the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animal Science (1987).

2.2. Agents and reagents

Compound 48/80 (Sigma, St. Louis, MO, USA) and serotonin (serotonin–creatinin sulfate, Merck, NJ, USA) were dissolved in saline and used for inducing scratching behavior. A local anesthetic, dibucaine (hydrochloride, Sigma), μ-opioid receptor antagonists, naloxone (hydrochloride, Sigma) and naltrexone (hydrochloride, Sigma), histamine H_1 receptor antagonists, terfenadine (Sigma) and ketotifen (fumarate, Sigma), serotonin receptor antagonists, cyproheptadine (hydrochloride, Sigma) and methysergide (maleate, Sigma), a cyclooxygenase inhibitor, indomethacin (Sigma), and a 5-lipoxygenase-activating protein inhibitor, 3-[1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid (MK-886, Wako, Osaka, Japan) (Gillard et al., 1989; Dixon et al., 1990) were obtained commercially. A PAF receptor antagonist, 4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (Y-24180) (Terasawa et al., 1990; Takehara et al., 1990), was a generous gift from Yoshitomi Pharmaceutical Industries, Fukuoka, Japan. These agents were dissolved in saline or suspended in saline containing sodium carboxymethylcellulose and administered to mice intraperitoneally except for dibucaine, which was applied topically, and naloxone, which was given intravenously.

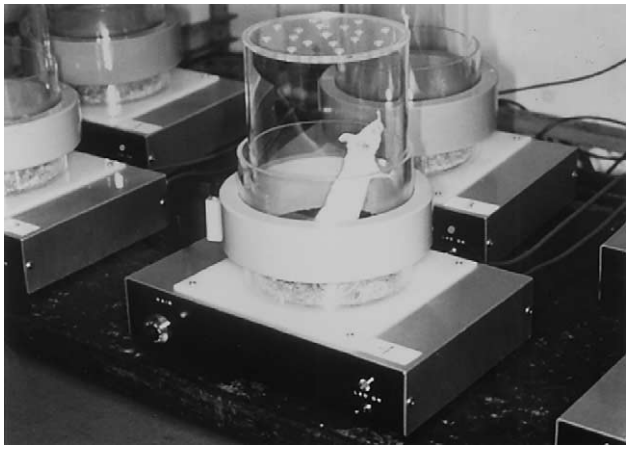

Fig. 1. Appearance of MicroAct. An observation chamber surrounded by a round coil is placed on an amplifier.

Table 1
Analysis parameters of MicroAct for detecting waves corresponding to consecutive scratching behavior in mice

Threshold	0.05 V
Event gap	0.05 s
Minimum duration	0.25 s
Maximum frequency	20 Hz
Minimum frequency	5 Hz

Repeated waves of 5–20 Hz with a duration of over 0.25 s were detected. If two waves were separated by over 0.05 s, they were not considered as consecutive.

2.3. Induction and evaluation of scratching behavior

The rostral part of the skin on the back of mice was clipped, and 20 μl of compound 48/80 solution or serotonin solution was injected intradermally. Control mice received a saline injection instead. Immediately after the injection, the mice were placed in the observation chamber and their behavior was observed for 60 min.

In the present study, mouse-scratching behavior was automatically detected and objectively evaluated with a new apparatus, MicroAct (Neuroscience, Tokyo, Japan). A small magnet (1 mm in diameter, 3 mm long) was inserted subcutaneously into both hind paws under ether anesthesia at least 1 h before the start of observation. It was confirmed that the operation did not affect the behavior of the mouse. The mouse with magnets was placed in the observation chamber (11 cm in diameter, 18 cm high), which was surrounded by a round coil (Fig. 1). The electric current induced in the coil by the movement of magnets attached to the hind paws was amplified and recorded. Then, characteristic waves reflecting scratching behavior were detected by a computer. Parameters for the detection of consecutive scratching behavior shown in Table 1 were established on the basis of our repeated preliminary experiments. Although a mouse scratches very quickly and beats by the hind paw are usually repeated several times, the apparatus could detect each beat. Under the present experimental condition, the apparatus detected consecutive scratching behavior consisting of three or more beats. Results of scratching

behavior are given as both incidences of consecutive scratching behavior and total scratching time in 60 min. In some experiments, mouse behavior was recorded by a video camera in addition to MicroAct and scratching behavior was counted by an observer as reported previously (Kuraishi et al., 1995; Inagaki et al., 1999).

2.4. Measurement of vascular permeability increase

The increase in vascular permeability caused by compound 48/80 was assessed as reported previously (Inagaki et al., 1986a,b). In brief, 1.25 mg of Evans blue dye was injected intravenously at the same time as compound 48/80 was injected. Mice were killed 30 min later and the reaction site was excised. The skin specimen was dissolved in 0.7 ml of 1 N KOH solution, and 9.3 ml of a mixture of 0.6 N H_3PO_4 solution and acetone (5:13) was added. After vigorous shaking, the precipitates were filtered off and the amount of dye extracted was measured colorimetrically at 620 nm.

2.5. Histopathological observation

Compound 48/80 at a dose of 100 μg was injected intradermally into the back skin of BALB/c mice and a skin specimen was excised 30 min later. Skin sections were prepared by normal methods and stained with hematoxylin and eosin or toluidine blue.

2.6. Statistics

The data are expressed as the mean values with standard error. The statistical significance of differences was evaluated by Tukey–Kramer's multiple comparison test using InStat Program (GraphPad Software, San Diego, CA, USA). When the P value was smaller than 0.05, the difference was considered to be significant.

3. Results

3.1. Scratching behavior induced by compound 48/80 in BALB/c mice and evaluation by MicroAct

Compound 48/80 at doses of 10–300 μg was injected intradermally into the back skin of BALB/c mice, and induced scratching behavior was evaluated for 60 min by both an observer and MicroAct. A comparison between evaluations made by an observer and MicroAct is shown in Fig. 2. Consecutive scratching behavior occurred most frequently in the first 10 min after injection of compound 48/80 and the total occurrence increased according to the dose of compound 48/80. The evaluation by MicroAct in the time-course study coincided very well with that by an observer, except for during the first 10 min. The evaluation in the first 10 min was slightly higher in the case of MicroAct than in the case of an observer. The total

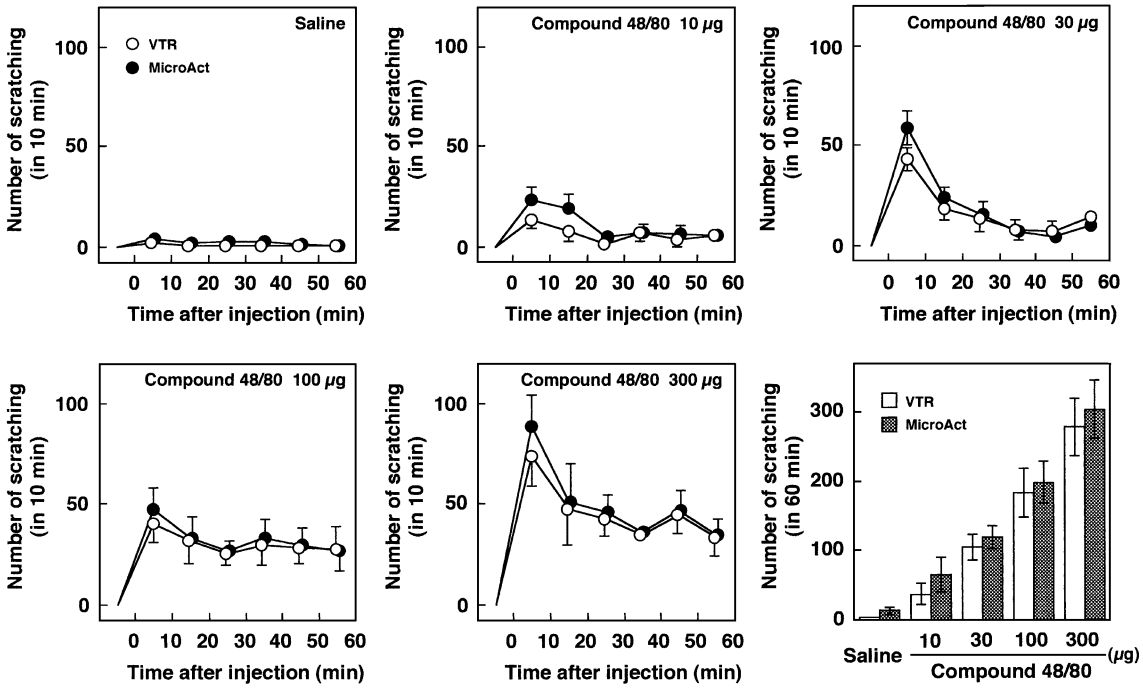

Fig. 2. Comparison between the observations of scratching behavior made by an observer and MicroAct. Compound 48/80 at doses of 10–300 μg or saline in a volume of 20 μl was injected into the shaved back skin of BALB/c mice and scratching behavior was observed for 60 min. Each value represents the mean ± S.E.M. for five mice. VTR: mouse behavior was recorded by a video camera and evaluated by an observer.

Table 2
Incidence of scratching behavior and scratching time caused by compound 48/80 in mice

	Number of scratchings	Scratching time (s)
Saline	13 ± 6	6.2 ± 3.2
Compound 48/80		
10 μg	66 ± 25	25.7 ± 9.7
30 μg	119 ± 16	50.0 ± 4.5
100 μg	198 ± 30	89.9 ± 17.2
300 μg	303 ± 43	144.4 ± 27.2

Compound 48/80 at doses of 10–300 μg or saline in a volume of 20 μl was injected into shaved back skin of BALB/c mice and the scratching behavior was evaluated for 60 min by MicroAct. Each value represents the mean ± S.E.M. for five mice.

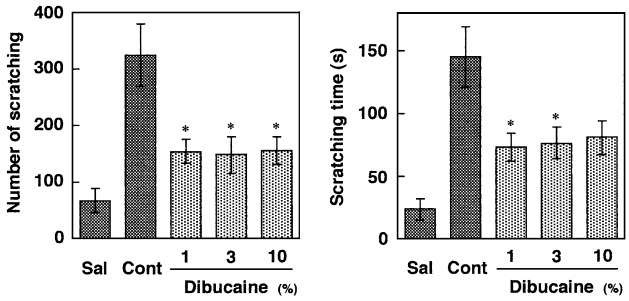

Fig. 4. Effects of dibucaine on compound 48/80-induced scratching behavior in BALB/c mice. Scratching behavior was induced by injecting 100 μg compound 48/80 intradermally into the shaved back skin and was observed for 60 min. Dibucaine at concentrations of 1–10% was applied topically 30 min before compound 48/80 injection. Each value represents the mean ± S.E.M. for five to seven mice. * $P < 0.05$.

incidence of consecutive scratching behavior in 60 min was also slightly higher in the case of MicroAct than in the case of an observer.

The results of scratching behavior evaluated by MicroAct are expressed as the scratching time as well as the incidence. As shown in Table 2, both the incidence of consecutive scratching behavior and total scratching time in 60 min increased dose dependently and the dose–response relationships were almost comparable. When 100 μg compound 48/80 was injected, scratching behavior consisting of four beats appeared most frequently and the mean beats in an event was 6.1. Scratching behavior lasting between 0.3 and 0.4 s appeared most frequently and the mean duration was 0.45 s. The mean scratching speed was 13.6 beats/s and 97.6% of total scratching behavior was between 8 and 19 beats/s.

Results of histopathological observation are shown in Fig. 3. Injection of 100 μg compound 48/80 caused potent edema and vascular dilatation (Fig. 3B). Mast cells observed in the dermis were completely degranulated after the injection of compound 48/80 (Fig. 3D).

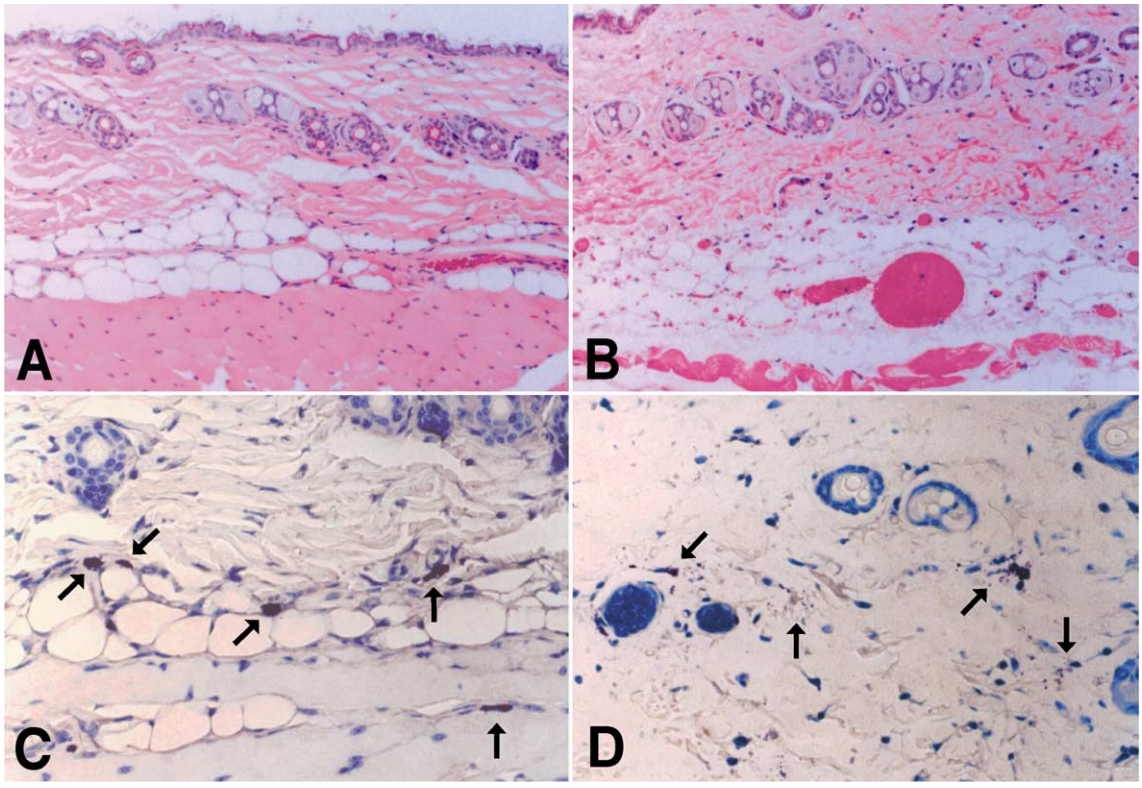

Fig. 3. Histopathological pictures of BALB/c mouse skin. (A) Hematoxylin–eosin staining, control skin, ×170. (B) Hematoxylin–eosin staining, compound 48/80 injected, ×170. (C) Toluidine blue staining, control skin, ×340. (D) Toluidine blue staining, compound 48/80 injected, ×340. Arrows: intact mast cells (C) and traces of mast cell degranulation (D).

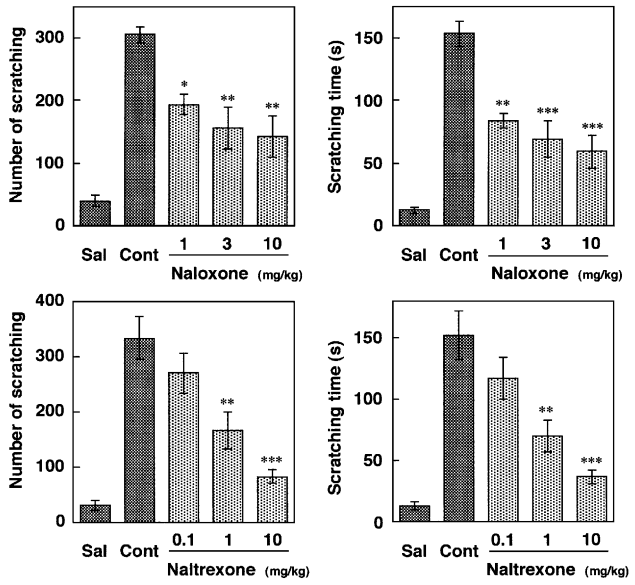

Fig. 5. Effects of naloxone and naltrexone on compound 48/80-induced scratching behavior in BALB/c mice. Scratching behavior was induced by injecting 100 μg compound 48/80 intradermally into the shaved back skin and was observed for 60 min. Naloxone and naltrexone at doses of 0.1–10 mg/kg were given intravenously and intraperitoneally, respectively, 30 min before compound 48/80 injection. Each value represents the mean ± S.E.M. for six or seven mice. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

3.2. Effects of drugs on the scratching behavior and vascular permeability increase in BALB/c mice

Scratching behavior and an increase in vascular permeability were induced by injecting 100 μg compound 48/80, and the effects of various drugs on the responses were investigated.

The results for dibucaine are shown in Fig. 4. Dibucaine at concentrations of 1–10% was topically applied to the skin sites 30 min before injection of compound 48/80. Dibucaine inhibited the scratching behavior (both the incidence and scratching time) significantly.

The results for the μ-opioid receptor antagonists are shown in Fig. 5. Naloxone and naltrexone were administered intravenously and intraperitoneally, respectively, 30 min before injection of compound 48/80. Both antagonists apparently inhibited the scratching behavior dose dependently. The inhibition of scratching and the scratching time was comparable.

The results for the histamine H_1 receptor antagonists, ketotifen and terfenadine, are shown in Fig. 6. Both drugs at doses of 0.1–10 mg/kg were administered intraperitoneally 60 min before injection of compound 48/80. The histamine H_1 receptor antagonists potently inhibited the dye leakage caused by compound 48/80. In contrast, neither of the drugs affected scratching behavior.

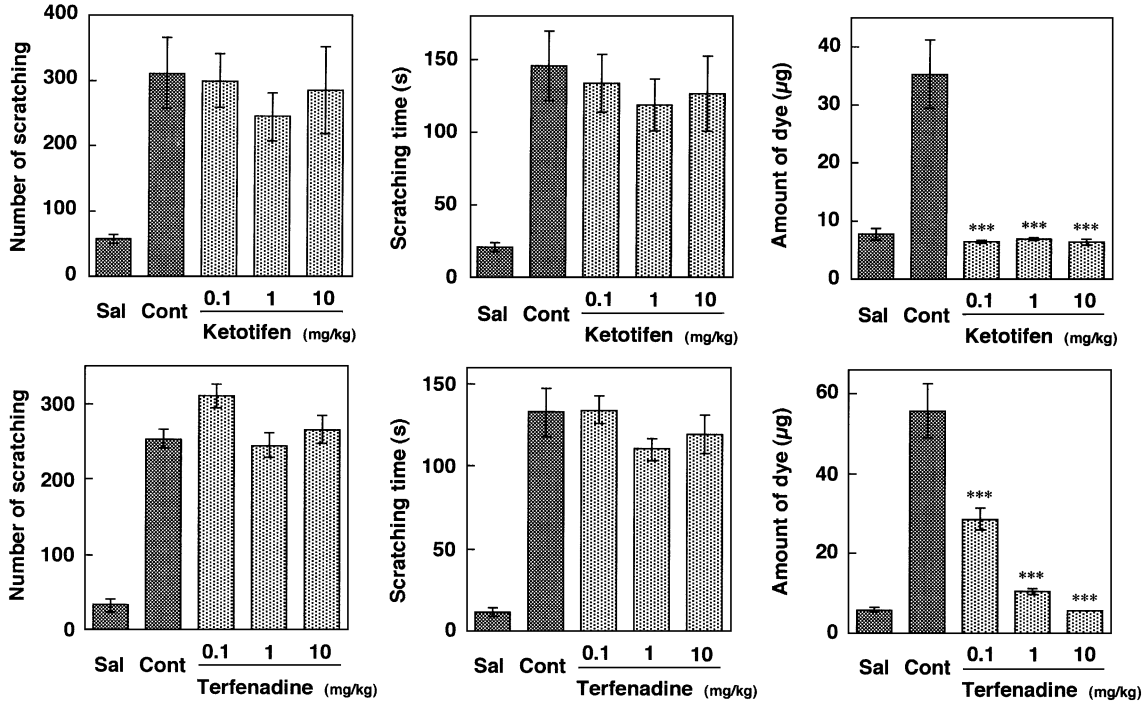

Fig. 6. Effects of ketotifen and terfenadine on compound 48/80-induced scratching behavior and vascular permeability in BALB/c mice. Compound 48/80 at a dose of 100 μg was injected intradermally into the shaved back skin of mice. Scratching behavior was observed for 60 min and vascular permeability was evaluated 30 min after compound 48/80 injection. Ketotifen and terfenadine at doses of 0.1–10 mg/kg were given intraperitoneally 60 min before compound 48/80 injection. Each value represents the mean ± S.E.M. for five to seven mice. *** $P<0.001$.

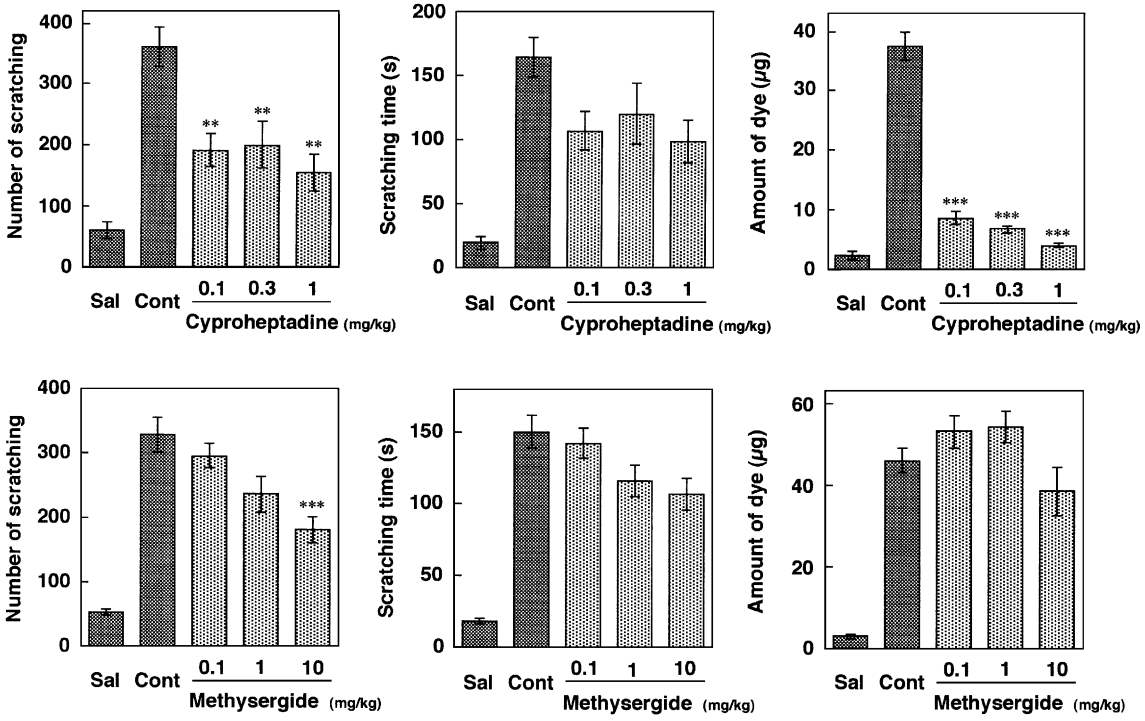

Fig. 7. Effects of cyproheptadine and methysergide on compound 48/80-induced scratching behavior and vascular permeability in BALB/c mice. Compound 48/80 at a dose of 100 μg was injected intradermally into the shaved back skin of mice. Scratching behavior was observed for 60 min and vascular permeability was evaluated 30 min after compound 48/80 injection. Cyproheptadine at doses of 0.1–1 mg/kg and methysergide at doses of 0.1–10 mg/kg were given intraperitoneally 60 min before compound 48/80 injection. Each value represents the mean ± S.E.M. for six mice. $^{**}P<0.01$, $^{***}P<0.001$.

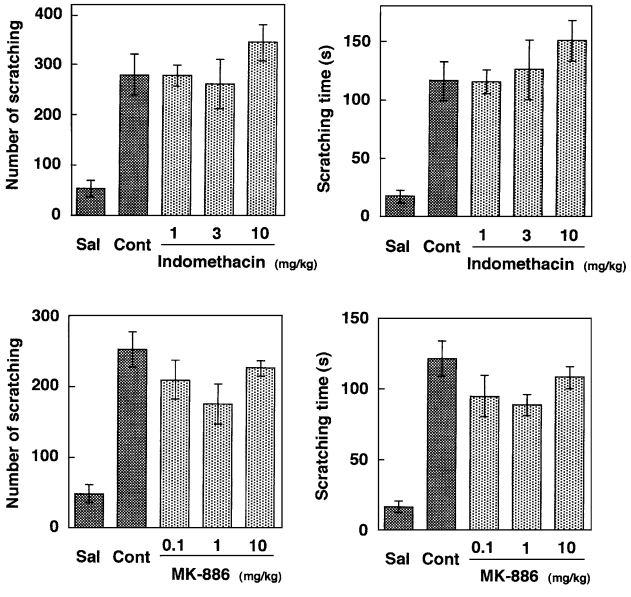

Fig. 8. Effects of indomethacin and MK-886 on compound 48/80-induced scratching behavior in BALB/c mice. Scratching behavior was induced by injecting 100 μg compound 48/80 intradermally into the shaved back skin and was observed for 60 min. Indomethacin at doses of 1–10 mg/kg was given intraperitoneally 30 min before compound 48/80 injection. MK-886 at doses of 0.1–10 mg/kg was given intraperitoneally 60 min before. Each value represents the mean ± S.E.M. for five or seven mice.

The results for cyproheptadine and methysergide are shown in Fig. 7. Cyproheptadine at doses of 0.1–1 mg/kg and methysergide at doses of 0.1–10 mg/kg were administered intraperitoneally 60 min before injection of compound 48/80. Cyproheptadine potently inhibited the dye leakage. The agent inhibited the incidence of scratching behavior significantly, whereas it showed a tendency to

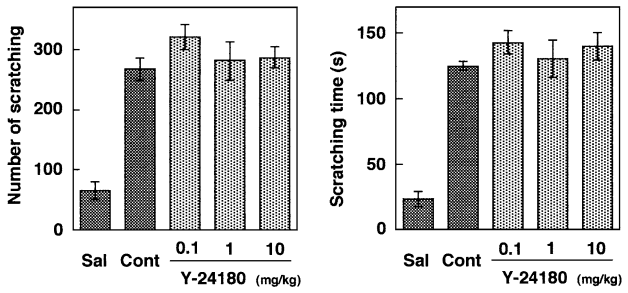

Fig. 9. Effects of Y-24180 on compound 48/80-induced scratching behavior in BALB/c mice. Scratching behavior was induced by injecting 100 μg compound 48/80 intradermally into the shaved back skin and was observed for 60 min. Y-24180 at doses of 0.1–10 mg/kg was given intraperitoneally 60 min before compound 48/80 injection. Each value represents the mean ± S.E.M. for six mice.

inhibit scratching time. In contrast, methysergide failed to affect the dye leakage caused by compound 48/80. Although the agent partially inhibited the incidence of scratching behavior, it only showed a tendency to inhibit scratching time.

The results for indomethacin and MK-886 are shown in Fig. 8. Indomethacin at doses of 1–10 mg/kg and MK-886 at doses of 0.1–10 mg/kg were given intraperitoneally 30 and 60 min, respectively, before injection of compound 48/80. Neither drug affected the scratching behavior caused by compound 48/80. The results for Y-24180 are shown in Fig. 9. Y-24180 at doses of 0.1–10 mg/kg was given intraperitoneally 60 min before injection of compound 48/80. Y-24180 also failed to affect scratching behavior.

3.3. Induction of scratching behavior by serotonin in BALB/c mice

Serotonin at doses of 100 and 300 nmol was injected intradermally into the back skin of BALB/c mice and scratching behavior was evaluated for 60 min. As shown in Fig. 10, although both doses of serotonin caused frequent scratching behavior, the incidence of scratching behavior and scratching time was comparable to each other and a less than those caused by 100 μg compound 48/80.

3.4. Induction of scratching behavior by compound 48/80 in mast cell-deficient WBB6F1-W/W^v mice

Scratching behavior was observed in mast cell-deficient WBB6F1-W/W^v mice and control WBB6F1-+/+ mice after injection of 10 or 30 μg compound 48/80. As shown in Fig. 11, WBB6F1-W/W^v mice exhibited frequent scratching behavior upon injection of compound 48/80, and both the incidence and time exceeded those of control WBB6F1-+/+ mice. The incidence of scratching behavior and scratching time caused by 30 μg compound 48/80 in WBB6F1-W/W^v mice was almost comparable to those caused by 100 μg compound 48/80 in BALB/c mice.

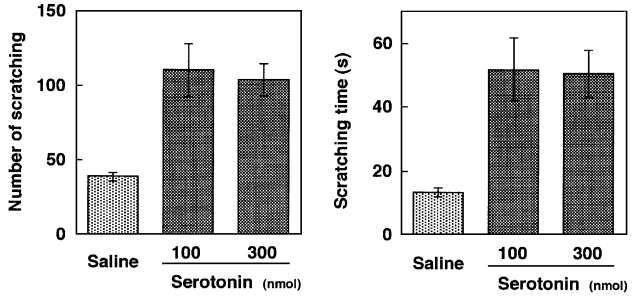

Fig. 10. Scratching behavior caused by serotonin in BALB/c mice. Scratching behavior was induced by injecting 100 or 300 nmol serotonin intradermally into the shaved back skin and was observed for 60 min. Each value represents the mean ± S.E.M. for five mice.

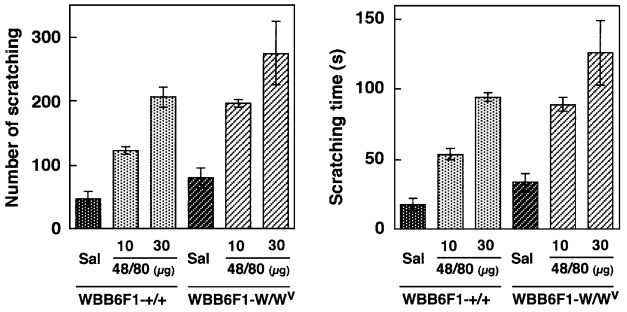

Fig. 11. Scratching behavior caused by compound 48/80 in WBB6F1-+/+ and W/W^v mice. Scratching behavior was induced by injecting 10 or 30 μg compound 48/80 intradermally into the shaved back skin and was observed for 60 min. Each value represents the mean ± S.E.M. for five mice.

4. Discussion

In the present study, we used a new apparatus, MicroAct, to evaluate mouse scratching behavior. All hind-paw scratching detected by an observer was also detected by MicroAct. However, the evaluation made by MicroAct was slightly higher than that by an observer. The difference was apparent in the first 10 min after injection of compound 48/80. In the present study, the observer did not count incomplete scratching behavior that sometimes appeared during recovery from ether anesthesia, and this may explain the difference, at least in part. However, as MicroAct detects some behaviors independent of scratching, involvement of noise cannot be excluded. However, the proportion of noise seems to be relatively small and it was estimated in our preliminary experiment as being about 5% of the total incidence when scratching behavior was induced by 100 μg compound 48/80. Furthermore, in our repeated experiments, we confirmed that the evaluation by MicroAct is highly reproducible. Therefore, MicroAct seems to be a reliable apparatus for the objective evaluation of mouse-scratching behavior.

MicroAct provides information not only on the incidence of consecutive scratching behavior but also on the scratching time and the number of beats involved. The present results are expressed as both the incidence and the duration of scratching. In most cases, both evaluations coincided very well, suggesting that the two indices are equally useful. However, some differences were observed in the results for methysergide and cyproheptadine. To characterize the indices, we need additional experiments.

In the present study, we found that compound 48/80 induced a high incidence of scratching behavior in BALB/c mice independent of histamine, although mast cells were actually degranulated. Serotonin seems to participate in the induction of scratching behavior whereas lipid mediators

such as prostaglandins, leukotrienes and PAF do not play important roles. Furthermore, compound 48/80 induced frequent scratching behavior in mast cell-deficient WBB6F1-W/W^v mice, demonstrating that compound 48/80 could induce scratching behavior independent of mast cells. The present results indicate, therefore, that compound 48/80 could induce scratching behavior through unique mechanisms other than mast cell activation.

Compound 48/80 at doses of 10–300 μg induced frequent scratching behavior in BALB/c mice in a dose-dependent manner. Because ICR mice, which respond to histamine, exhibit frequent scratching behavior upon injection of 3 μg compound 48/80 (Inagaki et al., 1999), BALB/c mice seem to be a lower responder strain than the ICR strain. The compound 48/80-induced scratching behavior in BALB/c mice was apparently inhibited by a local anesthetic and by μ-opioid receptor antagonists, suggesting that the scratching behavior has features similar to that of itch in humans (Shuttleworth et al., 1988; Ballantyne et al., 1988; Bergasa et al., 1995). Scratching behavior in mice, which appears to be associated with an allergic cutaneous reaction, and can be evoked by injection of serotonin, was inhibited by local anesthetics or μ-opioid receptor antagonists (Musoh et al., 1997; Yamaguchi et al., 1999, 2001; Inagaki et al., 2000b). Scratching behavior caused by compound 48/80 has a similar character and we detected it using MicroAct.

Compound 48/80 caused a potent activation of mast cells and a potent increase in vascular permeability. The vascular permeability increase could be inhibited completely by histamine H_1 receptor antagonists, indicating that histamine released from activated mast cells plays an important role in the increase in vascular permeability elicited by compound 48/80. In spite of the potent mast cell activation, however, the histamine H_1 receptor antagonists could not attenuate the scratching behavior caused by compound 48/80. The results coincide well with our previous results showing that histamine does not induce frequent scratching behavior in BALB/c mice (Inagaki et al., 2000a, 2001), and indicate that histamine was not a causative factor in scratching behavior.

Rodent mast cells contain substantial amounts of serotonin, which is released from mast cells as well as histamine (Friedrich et al., 1984; Weitzman et al., 1985). Serotonin increases vascular permeability potently and induces scratching behavior in many strains of mice (Inagaki et al., 2001). In the present study, methysergide, a serotonin antagonist, only showed a tendency to inhibit the vascular permeability increase caused by compound 48/80 injection. As serotonin causes a potent increase in vascular permeability (Inagaki et al., 1986a), the amount of serotonin released from mast cells upon compound 48/80 injection seems to be small. Furthermore, a relatively large dose (100 and 300 nmol) of serotonin induced scratching behavior less frequently than did 100 μg compound 48/80. Although methysergide actually inhibited the incidence of scratching behavior caused by compound 48/80 significantly, the inhibition was only partial and the scratching time was not inhibited significantly by the agent. These results, therefore, collectively suggest that serotonin plays only a minor role in the induction of scratching behavior by compound 48/80 in BALB/c mice. In contrast to methysergide, cyproheptadine, an inhibitor of both serotonin and histamine, potently inhibited the increase in vascular permeability. The antihistamine action of cyproheptadine seems to be responsible for the inhibition. Cyproheptadine, however, inhibited the scratching behavior more potently than did methysergide. As histamine H_1 receptor antagonists failed to inhibit the scratching behavior in the present study, the histamine H_1 receptor antagonistic property does not seem to play an important role in the inhibition of scratching. Although the differences in doses of methysergide and cyproheptadine should be considered carefully, cyproheptadine may possess an anti-scratch property independent of antihistamine and antiserotonin actions.

The participation of lipid mediators in the induction of scratching behavior by compound 48/80 was examined using a cyclooxygenase inhibitor, indomethacin, 5-lipoxygenase-activating protein inhibitor, MK-886 (Gillard et al., 1989; Dixon et al., 1990), and PAF receptor antagonist, Y-24180 (Terasawa et al., 1990). All these agents failed to affect the scratching behavior caused by compound 48/80 in BALB/c mice, although prostaglandins, leukotriene B_4 and PAF are reported to be possible mediators of scratching behavior in experimental animals (Woodward et al., 1995, 1996; Andoh et al., 2001). Under the present experimental condition, therefore, it is considered that prostaglandins, leukotrienes and PAF do not play important roles in scratching behavior.

The results of the pharmacological studies revealed that mast cell mediators are not involved in the induction by compound 48/80 of scratching behavior in BALB/c mice, except for serotonin, which may have a minor role. So next, the participation of the mast cell itself was examined using mast cell-deficient WBB6F1-W/W^v mice. The present results clearly indicate that compound 48/80 could induce frequent scratching behavior in the mast cell-deficient mice. It is interesting to note that compound 48/80 induced scratching behavior more frequently in WBB6F1-W/W^v mice than in control WBB6F1-+/+ mice. Furthermore, WBB6F1-W/W^v mice are better responders to compound 48/80 than are BALB/c mice, although the mechanism remains to be examined. It is apparent, therefore, that compound 48/80 could induce scratching behavior independent of mast cells.

In conclusion, compound 48/80 could induce frequent scratching behavior independent of mast cell activation. In atopic dermatitis patients, compound 48/80 induces itch that cannot be attenuated by histamine H_1 receptor antagonists (Rukwied et al., 2000). Some similarities may be present between compound 48/80-induced scratching behavior in BALB/c mice and itch in atopic dermatitis patients. To elucidate the mechanism involved, additional experiments should be performed.

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