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European Journal of Pharmacology 495 (2004) 159–165

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Evaluation of antipruritic effects of several agents on scratching behavior by NC/Nga mice

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Received 9 February 2004; received in revised form 17 May 2004; accepted 25 May 2004

Abstract

We investigated the effects of several agents on the established itching model in NC/Nga mice, model of atopic dermatitis-like disease, to elucidate related characteristics. The number of spontaneous scratching behaviors (the duration time is over 1.5 s) by NC/Nga mice with severe skin lesions was measured before and after administration of agents for 24 h. The scratching behavior by NC/Nga mice was significantly suppressed by administration of dexamethasone or tacrolimus, but not by chlorpheniramine maleate or cyproheptadine hydrochloride. These results suggest that this method shows a good correlation with the effectiveness of drugs prescribed for itching in humans with atopic dermatitis, and histamine and serotonin do not play an important role in causing the scratching behavior seen by NC/Nga mice. The scratching behavior was also significantly suppressed by naloxone hydrochloride, dibucaine or capsaicin. These results suggest that the scratching behavior seen in this model is caused by itching signal transmission through neural system. Furthermore, we found that theophylline, pinacidil or limaprost had scratching suppression effects in this model.

Keywords: Atopic dermatitis; NC/Nga, mouse; Scratching behavior; Itching; MicroAct

1. Introduction

Itching is a characteristic symptom in various forms of dermatosis, especially atopic dermatitis; consequently, it constitutes a major diagnostic criteria (Hanifin and Rajka, 1980; Williams et al., 1994). It is well known that the existence of the itch–scratch cycle, i.e., the strong actions of scratching facilitates the susceptibility to more itching and the aggravations of skin lesions in patients with atopic dermatitis (Kimura and Miyazawa, 1989; Wahlgren, 1999). Therefore, the most effective strategy for preventing this aggravation of skin lesions and upgrading the quality of life for patients with atopic dermatitis is reduction of itching and scratching (Caroline, 1999).

It is necessary to establish an appropriate animal model to develop effective medications and to elucidate mechanisms related to itching in atopic dermatitis. NC/Nga mice were originally established as an inbred strain from Japanese fancy mice (Kondo et al., 1964). Under conventional conditions, NC/Nga mice facilitated the development of spontaneous skin lesions with the diagnostic characteristics of high concentrations of total immunoglobulin E in plasma and invasion of inflammatory cells into the skin lesions (Matsuda et al., 1997; Suto et al., 1999). NC/Nga mice with severe skin lesions frequently scratch their face, ears and the rostral part of the back using their hind paws (Tohda et al., 1997). All these features are similar to events seen in patients with atopic dermatitis, hence NC/Nga mice are considered to be a suitable model of human atopic dermatitis. In a previous study, we investigated the spontaneous scratching pattern by NC/Nga mice in detail and designed a new method for evaluating agents which suppress scratching behavior associated with itching sensation (Takano et al., 2003), the outline of which is as follows: we focused on differences in patterns of scratching behavior between some strains of mice with normal skin (ICR, BALB/c and NC/Nga) and NC/Nga mice with severe skin lesions. The characteristic of scratching patterns by NC/Nga mice with normal skin as well as ICR and BALB/c mice was non-sustained (scratching duration is 0.3–0.5 s) and with a small amplitude. On the other hand, the characteristic of scratching pattern by NC/Nga mice with

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doi:10.1016/j.ejphar.2004.05.038

severe skin lesions was persistent (scratching duration is over 1.5 s) with a large amplitude, which is apparently morbid. We defined the pattern of scratching with persistent component as an atopic dermatitis-like behavior and recorded the number of persistent components. Using this method, we examined the effects of a glucocorticoid, an immunosuppressant and antihistamines, which are commonly prescribed for the patients with atopic dermatitis, on the spontaneous and persistent scratching behavior by NC/Nga mice. Oral administration of a glucocorticoid or an immunosuppressant significantly suppressed the scratching behavior by NC/Nga, but did not suppress the scratching behavior induced by histamine injection in ICR mice or that induced by ovalbumin-active cutaneous anaphylaxis in BALB/c mice. In contrast, antihistamines significantly suppressed the scratching behavior induced by histamine injection in ICR mice or that induced by ovalbumin active cutaneous anaphylaxis in BALB/c mice, but did not suppress scratching behavior by NC/Nga mice. These results showed a good correlation with the therapeutic activity of drugs in atopic dermatitis and urticaria in humans, and our established method may serve as a useful model for evaluating antipruritic drugs and for studying mechanisms involved in itching of atopic dermatitis.

In the present study, we investigated the effects of several types of agents, prescribed for allergic diseases or which act on nerve systems, to elucidate which agents were effective in this itching model, and to clarify mechanisms of the scratching behavior seen by NC/Nga mice.

2. Materials and method

2.1. Animals

Male NC/Nga mice purchased from SLC Japan (Shizuoka, Japan) were all housed under conditions of controlled temperature (23 ± 3 °C), humidity (55 ± 15%) and lighting (lights on from 7:00 to 19:00 h). To increase the incidence of dermatitis-related scratching behavior, they

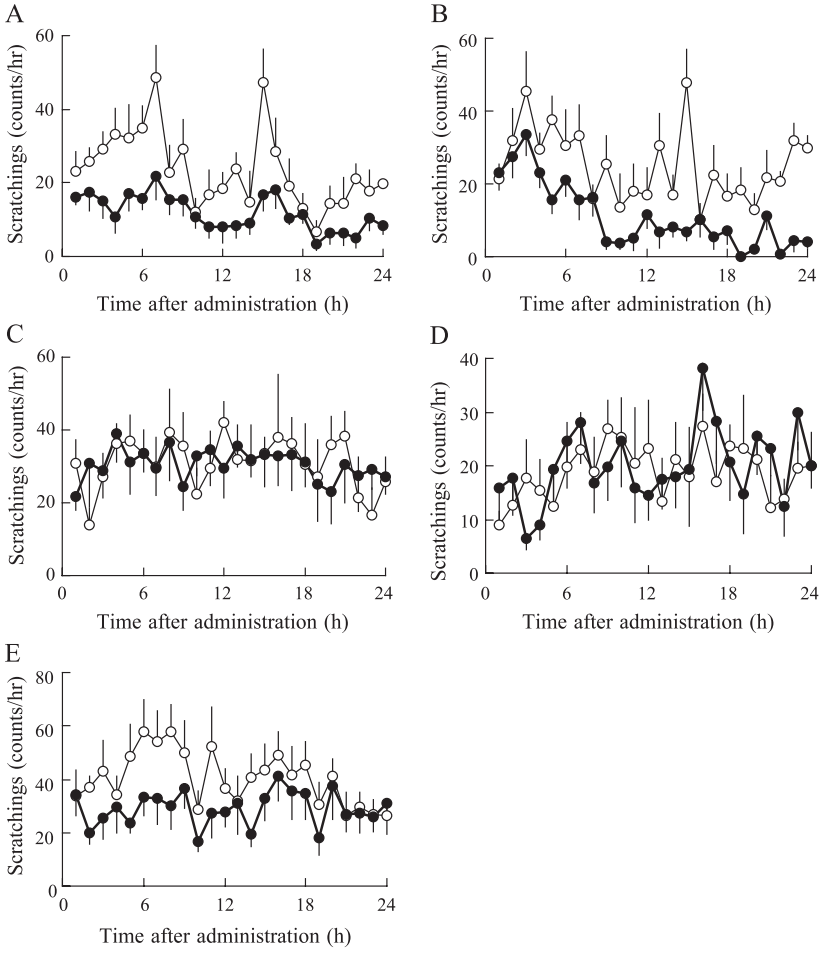

Fig. 1. Effects of dexamethasone, tacrolimus, chlorpheniramine, cyproheptadine and theophylline on spontaneous scratching behavior by NC/Nga mice (hourly counts). (A) Dexamethasone 0.1%; (B) tacrolimus 0.1%; (C) chlorpheniramine maleate 10 mg/kg; (D) cyproheptadine hydrochloride 3 mg/kg; (E) theophylline 30 mg/kg. Previous scratchings were measured for 24 h after vehicle administration (PRE, open circles); then the next day, agents were administered and scratching behavior was measured continuously for 24 h (POST, closed circles). Values are the mean and S.E.M. for six to eight mice.

were kept together with mice of the same strain having severe skin lesions and then used at 15–20 weeks of age. Food and tap water were provided ad libitum. All studies reported here have been reviewed by the Taisho Pharmaceutical Animal Care Committee and have met the Japanese Experimental Animal Research Association Standards as defined in the Guidelines for Animal Experiments (1987).

2.2. Materials

Dexamethasone, capsaicin (Wako, Osaka, Japan), tacrolimus (Prograf®, purchased from Fujisawa, Japan), dibucaine (Toyama Kagaku Kogyo, Tokyo, Japan) and limaprost (synthesized at Taisho Pharmaceutical, Saitama, Japan) were dissolved in ethanol (Kokusan Kagaku, Tokyo, Japan), and 200 μl of this solution was applied to the rostral part of the back of the mice. Naloxone hydrochloride (Sigma, St. Louis, USA) was dissolved in physiological saline and injected subcutaneously into the rostral part of the back of the mice. Chlorpheniramine maleate (Wako), cyproheptadine hydrochloride, pinacidil and theophylline (Sigma) were suspended in 1% (v/v) Tween-80 (Wako) and administered orally.

2.3. Measurement of spontaneous scratching behavior in NC/Nga mice

The spontaneous scratching behavior by NC/Nga mice was measured as we reported earlier (Takano et al., 2003), i.e., previous scratching was measured for 24 h after vehicle administration (before drug treatment, PRE). Then the next day, the agents were administered, and scratching behavior was measured for 24 h (after drug treatment, POST). For measurements, a small magnet (1 mm in diameter, 3 mm long) was implanted subcutaneously into both the hind paws of each mouse under ether anesthesia at least 6 h before the measurement of scratching. The mouse was placed in an observation chamber (11 cm in diameter, 18 cm high), which was surrounded by a round coil. The electric current induced in the coil by the movement of magnets attached to the hind paws was amplified and recorded. The number of scratching behaviors was measured using a new apparatus, MicroAct (Neuroscience, Tokyo, Japan), which can detect and evaluate scratching behavior of mice automatically and objectively (Inagaki et al., 2002, 2003). Analysis parameters of MicroAct for detecting waves were: threshold, 0.1 V; event gap, 0.2 s; minimum duration, 1.5 s; maximum frequency, 20 Hz; and minimum frequency, 2 Hz. It was confirmed that the vehicle administration did not affect the scratching behavior by NC/Nga mice (oral administration of 1% Tween-80 was PRE, 671.3 ± 101.7; POST, 665.2 ± 92.1 times/24 h. Topical application of ethanol was PRE, 679.9 ± 67.4; POST, 777.6 ± 60.9 times/24 h. Subcutaneous administration of saline was PRE, 828.8 ± 80.3; POST, 839.3 ± 83.4 times/24 h).

2.4. Data analysis

Experimental values are given as means and S.E.M. Statistical comparisons were made using Student's paired *t*-test for spontaneous scratchings by NC/Nga mice; a $P < 0.05$ value was considered as having statistical significance.

3. Results

3.1. Effects of dexamethasone, tacrolimus, chlorpheniramine, cyproheptadine and theophylline on spontaneous scratching behavior by NC/Nga mice

The scratching behavior was suppressed by dexamethasone (Fig. 1A) or tacrolimus (Fig. 1B) for a long duration, and the total counts of scratchings for 24 h were significantly suppressed compared to pre-treatment (Fig. 2). Also, it was suppressed by theophylline for 10 h (from 1 to 11 h after the administration, Fig. 1E), and the total counts of scratchings were significantly suppressed (Fig. 2). On the other hand, chlorpheniramine maleate (Figs. 1C and 2) or cyproheptadine hydrochloride (Figs. 1D and 2) did not suppress it at any hour or even the total counts.

3.2. Effects of naloxone, dibucaine, capsaicin, pinacidil and limaprost on spontaneous scratching behavior by NC/Nga mice

Naloxone hydrochloride suppressed scratching behavior for 10 h after administration (Fig. 3A). Dibucaine suppressed it at two periods of time (1–4 h and 14–24 h after

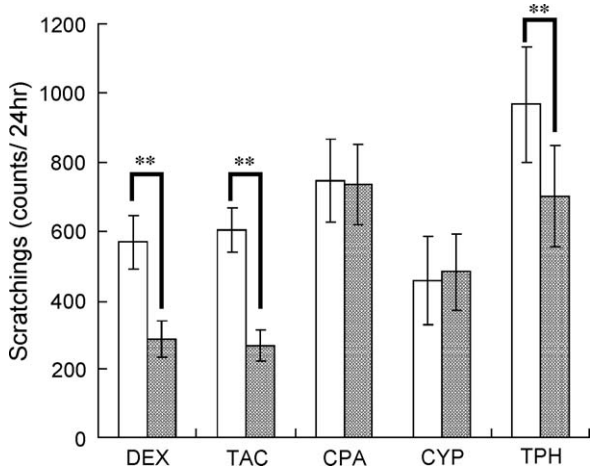

Fig. 2. Effects of dexamethasone, tacrolimus, chlorpheniramine, cyproheptadine and theophylline on spontaneous scratching behavior by NC/Nga mice (total counts). DEX, dexamethasone 0.1%; TAC, tacrolimus 0.1%; CPA, chlorpheniramine maleate 10 mg/kg; CYP, cyproheptadine hydrochloride 3 mg/kg; TPH, theophylline 30 mg/kg. Previous scratchings were measured for 24 h after vehicle administration (PRE, open columns); then the next day, agents were administered and scratching behavior was measured continuously for 24 h (POST, closed columns). Values are the mean and S.E.M. for six to eight mice. $^{**}P < 0.01$ when compared with PRE.

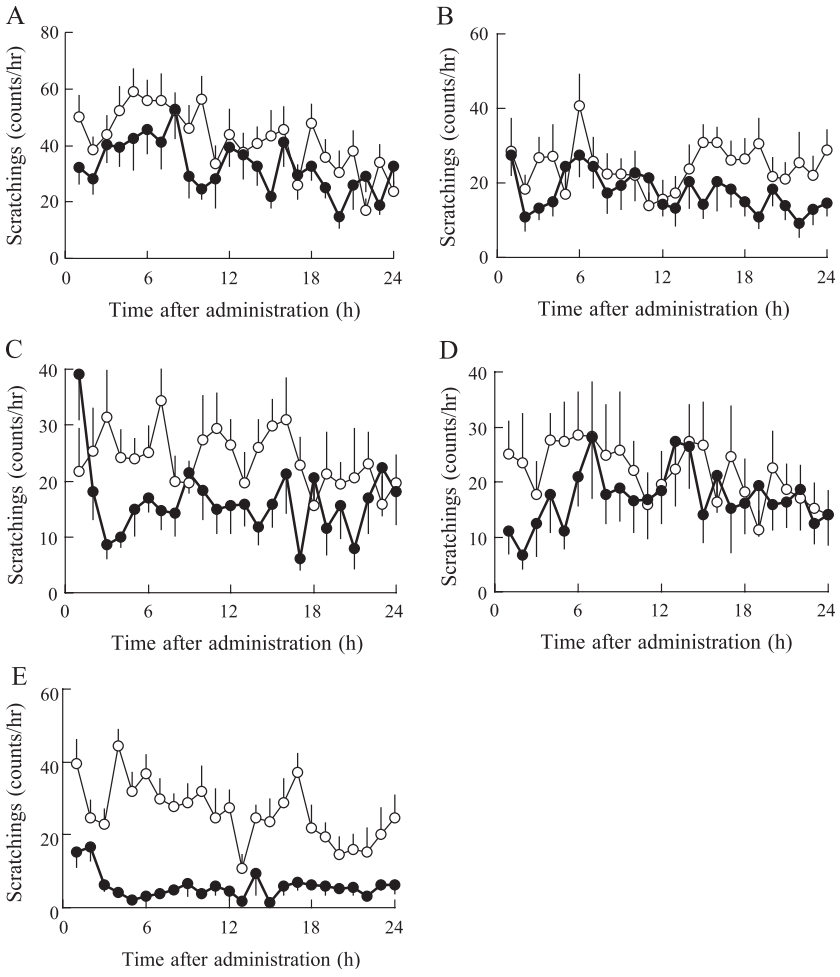

Fig. 3. Effects of naloxone, dibucaine, capsaicin, pinacidil and limaprost on spontaneous scratching behavior by NC/Nga mice (hourly counts). (A) naloxone hydrochloride 1 mg/kg; (B) dibucaine 1%; (C) capsaicin 0.1%; (D) pinacidil 10 mg/kg; (E) limaprost 0.01%. Previous scratchings were measured for 24 h after vehicle administration (PRE, open circles); then the next day, agents were administered and scratching behavior was measured continuously for 24 h (POST, closed circles). Values are the mean and S.E.M. for six to eight mice.

the administration, Fig. 3B). Capsaicin suppressed it from 2 h after the administration (Fig. 3C), and pinacidil suppressed it for 5 h after administration (Fig. 3D). Limaprost markedly suppressed it for a long duration (Fig. 3E). The total counts of scratchings for 24 h were significantly suppressed by the above five drugs, compared to pretreatment (Fig. 4).

4. Discussion

Glucocorticoids and immunosuppressants have therapeutic effects for subjects with atopic dermatitis (Nakagawa et al., 1994; Hanifin and Tofte, 1999; Assmann et al., 2001; Hiroi, 2001). There had been few reports on antipruritic effects of glucocorticoids and immunosuppressants on in vivo models of atopic itching. In our previous study, oral administration of dexamethasone or tacrolimus significantly suppressed the scratching behavior by NC/Nga mice (Takano et al., 2003). Topical application of these agents in a concentration of clinical use also significantly suppressed the scratching behavior in the present study. The suppressive effects were higher than in case of oral administration and continued for a long duration. These results indicate that dexamethasone or tacrolimus mostly act at the peripheral, and cytokines such as those from T cells may play an important role in causing the scratching behavior seen by NC/Nga mice. Histamine and serotonin are regarded as mediators causing itch in humans (Hägermark, 1992; Wahlgren, 1992; Greaves and Wall, 1996) and can also elicit scratching in mice following skin administration (Kuraishi et al., 1995; Inagaki et al., 1999; Yamaguchi et al., 1999). However, histamine H_1 receptor antagonists generally do not inhibit the itching and scratching of patients with atopic dermatitis, hence histamine is not considered to be a major pruritogen in atopic dermatitis (Berth-Jones and Graham-Brown, 1989; Wahlgren et al., 1990; Hägermark and Wahlgren, 1996; Klein and Clark, 1999; Munday et al., 2002). Moreover, we can find few reports that serotonin receptor antagonists are effective for the itching of atopic dermatitis. In this study, chlorpheniramine

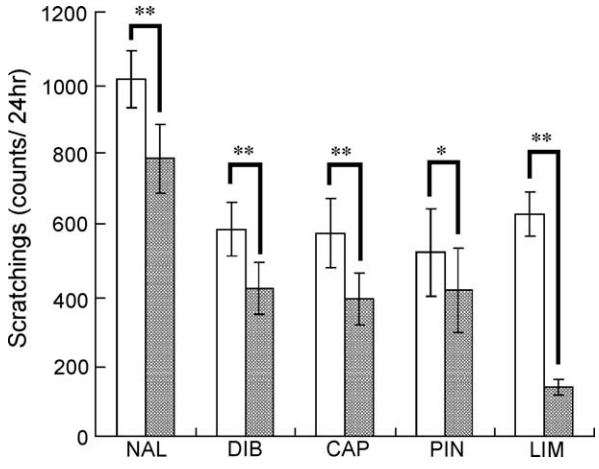

Fig. 4. Effects of naloxone, dibucaine, capsaicin, pinacidil and limaprost on spontaneous scratching behavior by NC/Nga mice (total counts). NAL, naloxone hydrochloride 1 mg/kg; DIB, dibucaine 1%; CAP, capsaicin 0.1%; PIN, pinacidil 10 mg/kg; LIM, limaprost 0.01%. Previous scratchings were measured for 24 h after vehicle administration (PRE, open columns); then the next day, agents were administered and scratching behavior was measured continuously for 24 h (POST, closed columns). Values are the mean and S.E.M. for six to eight mice. $*P<0.05$, $**P<0.01$ when compared with PRE.

maleate, a histamine H_1 receptor antagonist, or cyproheptadine hydrochloride, a serotonin receptor antagonist, did not suppress the scratching behavior by NC/Nga mice. These results suggest that this scratching model in mouse shows a good correlation with the therapeutic activity of drugs in cases of atopic dermatitis in humans, and the mechanism of spontaneous scratching of NC/Nga mice resembles that of itching of atopic dermatitis in humans.

The scratching behavior of NC/Nga mice was significantly suppressed by theophylline, a drug used clinically to treat asthma and chronic bronchitis. It is considered that the major mechanism of action of theophylline in smooth muscle relaxation is to increase cyclic adenosine monophosphate (cAMP) following phosphodiesterase inhibition (Rabe et al., 1995). In another experiment, forskolin, which increases intracellular cAMP significantly suppressed the scratching behavior by NC/Nga mice (PRE, 275.3 ± 50.0; POST, 162.0 ± 26.0 times/24 h). These results suggest that theophylline suppresses scratching behavior by elevating cAMP in cells of NC/Nga mice skin. However, of course, as this theory is presently speculative, further study is needed to determine the precise mechanism of the inhibitory action.

In humans, itching is one of the adverse effects by administering morphine, a μ-opioid receptor agonist (Cousins and Mather, 1984; Ballantyne et al., 1988). On the other hand, μ-opioid receptor antagonists suppress the itch sensation in humans (Bernstein et al., 1982; Monroe, 1989) and scratching behavior in mice (Inagaki et al., 2000; Yamaguchi et al., 2001). In the present study, the scratching behavior by NC/Nga mice was significantly suppressed by the subcutaneous administration of naloxone. Therefore, it is indicated that this behavior observed in the scratching model was a response associated with itching sensation, and the itching signal is transmitted through central nervous system.

Topical application of dibucaine, a topical anesthetic agent, or capsaicin, which desensitizes nerve endings, significantly suppressed the scratching behavior by NC/Nga mice. Topical anesthetic agents have antipruritic effects in humans (Dalili and Adriani, 1971) and mice (Inagaki et al., 2002), and capsaicin also has antipruritic effects in humans (Folster-Holst and Brasch, 1996) and mice (Andoh et al., 1998). These results suggest that the itching signal is transmitted by peripheral sensory neurons, and thereby the scratching behavior seen in this model is caused just as in humans.

The scratching behavior was significantly suppressed by pinacidil, a K^+ channel opener. K^+ channel openers affect not only cardiac, smooth and skeletal muscles, but also nerve cells. These openers can also decrease neuronal excitability and interfere with neurotransmission (Alzheimer and ten Bruggencate, 1988; Longman and Hamilton, 1992; Lawson, 1996). The itching signal in skin is transmitted by sensory neurons, especially unmyelinated C-fibers, to the dorsal horn (Schmelz et al., 1997; Yosipovitch et al., 2003). Therefore, it was considered that the antipruritic effect of pinacidil was associated with disruption of neurotransmission effects.

In our preliminary experiment, we examined the effect of indomethacin, a representative of non-steroid anti-inflammatory drugs, to confirm the inhibiting effect of prostanoids on scratching behavior in NC/Nga. Topical application of indomethacin (0.1%) significantly enhanced the scratching behavior of NC/Nga mice (PRE, 349.6 ± 27.0; POST, 563.3 ± 92.2 times/24 h). From this result, we considered that prostaglandins might have antipruritic activity, so we examined the effect of limaprost, a prostaglandin E_1 analogue in this study. As we had expected, the scratching behavior was significantly suppressed by topical application of limaprost. In another experiment, prostaglandin E_2 also suppressed the scratching behavior of NC/Nga mice (PRE, 392.8 ± 54.1; POST, 172.5 ± 38.3 times/24 h). These results suggest that prostaglandins have the potential to suppress scratching and itching related to atopic dermatitis. There are reports that prostaglandins do not cause itching, but do enhance histamine-induced itching when given together with histamine (Greaves and McDonald-Gibson, 1973; Hägermark et al., 1977).

In summary, we confirmed the antipruritic effects of several agents on the established itching model NC/Nga mice. The behavior by NC/Nga mice was suppressed by administration of dexamethasone or tacrolimus, but not by chlorpheniramine maleate or cyproheptadine hydrochloride. These results suggest that this method shows a good correlation with the effectiveness of drugs in humans with atopic dermatitis, and cytokines, not histamine and serotonin, play an important role in causing the scratching behavior seen by NC/Nga mice. The behavior was also suppressed by naloxone hydrochloride, dibucaine or capsaicin. These results

suggest that the scratching behavior seen in this model is caused by itching signal transmission through neural system. Furthermore, we found that theophylline, pinacidil or limaprost had antipruritic effects on the itching associated atopic dermatitis. These agents will prove to be useful therapeutics for the itching patient with atopic dermatitis. We intend to further explore the antipruritic effect of various medications using this method.

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