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Prostanoid DP_1 receptor agonist inhibits the pruritic activity in NC/Nga mice with atopic dermatitis

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Abstract

NC/Nga mice have similar pathological and behavioral features of human atopic dermatitis and are used as a model of the disease. Under conventional circumstances, spontaneous and persistent scratching is frequent and can lead to the onset of skin inflammation. We examined the effects of several prostanoids and their related compounds on the scratching behavior of NC/Nga mice. Among them, topically applied prostaglandin D_2, prostaglandin E_1, prostaglandin E_2 and prostaglandin I_2 significantly suppressed the scratching, the order of inhibitory activities being prostaglandin $D_2 \gg$ prostaglandin I_2 > prostaglandin E_1 = prostaglandin E_2. Prostaglandin D_2 metabolite, prostaglandin J_2 also significantly suppressed the scratching but not so 13,14-dihydro-15-keto-prostaglandin D_2, and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. The order of the inhibitory activities of these prostaglandin D_2 metabolites depended on affinity of the prostanoid DP_1 receptor but not on the DP_2 receptor (chemoattractant receptor-homologous molecule expressed on T helper2 cells, $CRTH_2$) and PPAR-γ receptors. Likewise, topically applied arachidonic acid significantly suppressed the scratching while indomethacin enhanced it. Pretreatment of arachidonic acid increased the skin prostaglandins (prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$) contents, but indomethacin decreased the prostaglandin D_2 and prostaglandin E_2 contents. On the other hand, prostaglandin D_2 and indomethacin had no apparent effects on histamine-induced scratching of ICR mice. These results suggested that prostaglandin D_2 plays a physiological role in inhibiting pruritis of NC/Nga mice via their specific prostanoid DP_1 receptors, and that prostaglandin D_2 and/or a prostanoid DP_1 receptor agonist may have therapeutic effects for cases of consecutive skin inflammation.

Keywords: Prostaglandin D_2; Scratch; Atopic dermatitis; NC/Nga, mouse; Pruritus

1. Introduction

Itching is a characteristic symptom in various forms of dermatitis, especially atopic dermatitis, consequently it constitutes a major diagnostic criterion (Hanifin and Rajka, 1980) for humans. It is well known that existence of the Itch–Scratch cycle, which means that scratching facilitates the itching and aggravates the skin lesions in patients with atopic dermatitis (Kimura and Miyazawa, 1989; Wahlgren, 1999). It has been considered that one of the most effective strategies for preventing aggravation of skin lesions and upgrading the quality of life for patients with atopic dermatitis is to reduce the itching and scratching (Caroline, 1999). Histamine is one of the mediators related to itching in humans (Wahlgren, 1992). However, histamine H_1 receptor antagonists generally do not have a sufficient inhibitory effect on 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). On the other hand, corticosteroids and immuno-suppressants do have therapeutic effects for subjects with atopic dermatitis (Nakagawa et al., 1994;

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Hanifin and Tofte, 1999; Hirai, 2001). However, high frequencies of adverse events such as skin atrophy and irritation can occur with use of these drugs (Smith, 1995; Assmann et al., 2001).

It is important to establish a pertinent animal model when developing novel medications, and also to elucidate mechanisms of itching which occur in atopic dermatitis. NC/Nga mice were originally established as an inbred strain in Japan (Kondo et al., 1964). Under conventional conditions, NC/Nga mice had spontaneous skin lesions with diagnostic characteristics of high concentrations of total IgE in the plasma and invasion of inflammatory cells into the skin lesions (Matsuda et al., 1997; Suto et al., 1999). Furthermore, skin-lesioned NC/Nga mice frequently scratch their face, ears, and rostral part of their back using their hind paws (Tohda et al., 1997). All these features are similar to events seen in the patients with atopic dermatitis. Hence, NC/Nga mice are thought to be a suitable animal model of human atopic dermatitis. We investigated the spontaneous scratching pattern of NC/Nga mice and designed a new method to study the scratching behavior (Takano et al., 2003). This method also can be used to screen agents which suppress itching. Pretreatment with dexamethasone or tacrolimus (FK-506, Kino et al., 1987) significantly suppressed spontaneous scratching behavior of NC/Nga mice but did not affect the scratching behavior induced by histamine. In contrast, pretreatment with chlorpheniramine or ketotifen significantly suppressed the scratching behavior induced by histamine in NC/Nga mice but did not affect the spontaneous scratching behavior of the mice. These results show a good correlation with the therapeutic activity of drugs in atopic dermatitis and urtication, respectively, in humans. Therefore, this behavioral evaluation method may serve as a useful model to reveal the antipruritic effects of drugs and for studying mechanisms of atopic dermatitis.

Prostaglandins, the major arachidonic acid metabolite released from various tissues, are generally recognized to be a potent mediator which enhances pain and inflammation. The actions of prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2 and thromboxaneA_2 are mediated by stimulation of prostanoid DP, EP_{1-4}, FP, IP and TP receptors, respectively (Coleman et al., 1994). Prostaglandin D_2 is the major cyclooxygenase product in a variety of biological processes, platelet aggregation, relaxation of vascular and nerve cell function (Giles and Leff, 1988). Moreover, prostaglandin D_2 is also produced by allergen-activated mast cells and has been implicated in various allergic diseases as a proinflammatory lipid mediator (Lewis et al., 1982), but the actual roles in various inflammatory diseases are unclear. However, there is limited information on physiological functions of pruritus by these prostaglandins. We examined the effects of several prostaglandins and their related compounds on the spontaneous scratching behavior in NC/Nga mice in attempts to elucidate regulatory mechanisms.

2. Materials and method

2.1. Animals

Male NC/Nga and ICR 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 07:00 to 19:00), and then used for study. NC/Nga mice with severe skin lesions were used at 15–20 weeks of age. Food and tap water were provided ad libitum for all mice. 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

Arachidonic acid and indomethacin were obtained from Sigma-Aldrich (St. Louis, MO), Prostaglandin D_2, Prostaglandin E_1, Prostaglandin E_2, Prostaglandin $F_{2\alpha}$, Prostaglandin I_2, 9,11-dideoxy-9_α,11_α-methanoepoxy prostaglandin $F_{2\alpha}$ (U-46619), Prostaglandin J_2, 13,14-dihydro-15-keto-prostaglandin D_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 were obtained from Cayman Chemical (Ann Arbor, MI). These drugs were dissolved in ethanol (Kokusan Kagaku, Tokyo, Japan) at each concentration (w/v%) and applied to the skin of the back and neck of the mice. Histamine dihydrochloride from Wako Junyaku (Osaka, Japan) was dissolved in physiological saline and injected intradermally into the rostral part of the back of the mice.

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

Spontaneous scratching behavior by skin-lesioned NC/Nga mice was measured for 24 h (15:00–15:00) on the following day. For evaluation of drug effects, the scratching behavior was measured for 24 h (before drug treatment; Pre), then the next day, the agents were administered, and the scratching behavior was continuously measured for 24 h (after drug treatment; Post). For the measurements, a small magnet (1.0-mm diameter, 3.0 mm long) was implanted subcutaneously into both hind paws of each mouse under ether anesthesia applied 2 h before the measurement of scratching. The mouse was placed in an observation chamber (11-cm diameter, 18 cm high) 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. Scratching behavior was automatically detected and objectively evaluated using MicroAct (Neuroscience, Tokyo, Japan). Analysis parameters for detecting waves were Threshold; 0.1 V, Event gap; 0.2 s, Minimum duration 1.5 s, Maximum frequency; 20 Hz, Minimum frequency; 2 Hz.

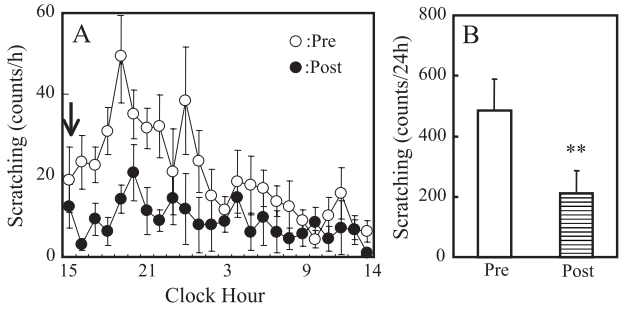

Fig. 1. Effect of prostaglandin D_2 on spontaneous scratching behavior of skin-lesioned NC/Nga mice. (A) Typical intra-day inhibition pattern of topically applied prostaglandin D_2 on spontaneous scratching behavior of NC/Nga mice. The number of scratching by NC/Nga mice was measured for the first 24 h (○: Pre), followed by the next 24 h after 0.01% prostaglandin D_2 application (●: Post). ↓: Application of drug. Data represent intra-day scratching counts at each clock hour. (B) Total scratchings for 24 h of pre- or post-treatment of 0.01% prostaglandin D_2. $^{**}P<0.01$ when compared with Pre value (paired Student's *t*-test).

2.4. Measurement of histamine-induced scratching behavior in ICR mice

Male ICR mice 6 weeks of age were used. To elicit scratching behavior, 5 μg of histamine was injected intradermally into the rostral part of the back of mice, as reported (Takano et al., 2003). Immediately after the injection, the mice were placed in an observation chamber and scratching behavior was monitored for 30 min. And indomethacin or prostaglandin D_2 was applied to the skin 1 h before the histamine injection. Scratching behavior was automatically detected and objectively evaluated using MicroAct. Analysis parameters for detecting waves were Threshold; 0.1 V, Event gap; 0.2 s, Minimum duration 0.3 s, Maximum frequency; 20 Hz, Minimum frequency; 2 Hz.

2.5. Measurement of skin prostaglandins contents in NC/Nga mice

Mice were topically treated with 200 μl of 0.1% indomethacin or 0.1% arachidonic acid in ethanol on their shaven backs. One hour later, mice were injected with indomethacin (10 mg/kg) to prevent further production of prostanoids and their back skins were excised. The skin was minced and homogenized in ice-cold phosphate-buffered saline containing 10 μmol indomethacin with a Polytron tissue homogenizer for 30 s. Four milliliters of acetone was added to the sample and vortexed, and the precipitate was removed by centrifugation at 3000 rpm for 10 min at 4 °C. The supernatant was carefully poured into a test tube and evaporated to dryness under a stream of nitrogen and resuspended in enzyme-immunoassay buffer. The amounts of prostaglandin E_2, prostaglandin D_2, 6-keto-prostaglandin $F_{1\alpha}$ were measured for each prostanoid using specific enzyme-immunoassay (EIA) kits (Cayman Chemical). The amounts of prostaglandin $F_{2\alpha}$ were measured using prostaglandin $F_{2\alpha}$ EIA kits (R&D Systems, Minneapolis, MN, USA).

2.6. Data analysis

Experimental values are given as means±S.E.M. Student's paired *t*-test for spontaneous scratching of NC/Nga mice. Student's *t*-test for skin prostaglandins contents of NC/Nga mice and histamine-induced scratching of ICR mice. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ values were considered as having statistical significance.

3. Results

3.1. Effects of several prostaglandins on spontaneous scratching behavior by NC/Nga mice

The general pattern of spontaneous scratching behavior of skin-lesioned NC/Nga mice showed a peak around 19:00 to 24:00 then decreased gradually (Fig. 1A). Application of 0.2 ml of ethanol to the skin had no significant effect on this scratching behavior (Table 1). Significant suppression by prostaglandin D_2 was observed at night from the intra-day suppression pattern of scratching behavior (Fig. 1A). Topical treatment of prostaglandin D_2 inhibited the scratching behavior and the counts of scratching were significantly inhibited by the treatment of 0.01% prostaglandin D_2 (212.0±74.3 counts/24 h) compared to pre-treatment (485.0±102.9 counts/24 h) (Fig. 1B). The

Table 1
Anti-pruritic effects of prostaglandin D_2-related compounds in NC/Nga mice

Compound	Concentration %	Receptor type	Inhibition %
Vehicle	–	–	−16.9±7.5
Prostaglandin D_2	0.01	Prostanoid DP_1, DP_2 agonist	54.3 ± 14.9^{b}
Prostaglandin J_2	0.1	Prostanoid DP_1, DP_2 agonist	21.4 ± 6.2^{a}
13,14-Dihydro-15-keto-prostaglandin D_2	0.1	Prostanoid DP_2 agonist	6.2±10.8
15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2	0.1	Prostanoid DP_2, PPAR-γ agonist	12.2±8.1

Each value represents the means±S.E.M. (n=8). Scratching behavior was measured for 24 h (Pre value), then for 24 h after application of each compound (Post value). Inhibition % represents (Pre value−Post value)×100 Pre value. $^{a}P<0.05$ and $^{b}P<0.01$ were compared between each Pre value and each Post value.

spontaneous scratching behavior by NC/Nga mice was also significantly suppressed by topical treatment of 0.1% of prostaglandin E_2 and prostaglandin I_2, but not by prostaglandin $F_{2\alpha}$ and U46619 (stable analogues of thromboxane A2) (Fig. 2A). Dose-related responses are shown and the order of inhibitory activities of these prostaglandins is as follows; prostaglandin $D_2 \gg$ prostaglandin I_2>prostaglandin E_2=prostaglandin E_1 (Fig. 2B). We noted that the inhibitory activities of prostaglandin E_1, prostaglandin E_2 and prostaglandin I_2 abruptly disappeared in case of 0.01% (Fig. 2B).

3.2. Effects of prostaglandin D_2 metabolites on spontaneous scratching behavior by NC/Nga mice

Consistent with the above actions of prostaglandins, the spontaneous scratching behavior of NC/Nga mice was significantly suppressed by topical treatment of prostaglandin J_2, a metabolite of prostaglandin D_2. However, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (prostanoid DP_2 and PPAR-γ receptor agonist) and 13-14-dehydro-15-keto-prostaglandin D_2 (prostanoid DP_2 receptor agonist) did not have any significant effects (Table 1).

3.3. Effect of indomethacin on spontaneous scratching behavior by NC/Nga mice

We examined the effect of indomethacin, an inhibitor of prostaglandins synthesis, to confirm the involvement of prostanoids in the suppression of the scratching behavior by NC/Nga mice. The topical application of 0.1% indomethacin significantly increased the scratching behavior by NC/Nga mice, especially between the time from 19:00 to 06:00 of the following day (Fig. 3A). The total counts of scratching were significantly augmented by treatment with 0.1% indomethacin (563.3±92.2 counts/24 h) compared to the case of pre-treatment (349.6±27.0 counts/24 h) (Fig. 3B).

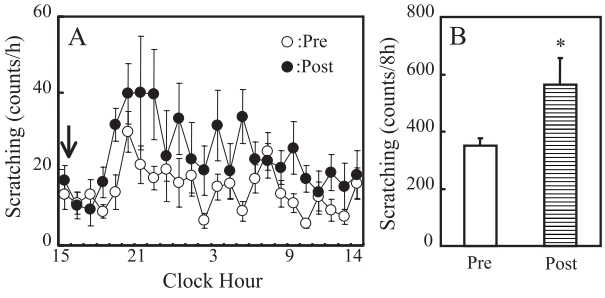

Fig. 3. Effect of indomethacin on spontaneous scratchings by NC/Nga mice. (A) Typical intra-day inhibition pattern of topically applied indomethacin on spontaneous scratchings by NC/Nga mice The number of scratchings by NC/Nga mice was measured for the first 24 h (○: Pre), followed by the next 24 h after 0.1% indomethacin application (●: Post). ↓: Application of drug. Data represents intra-day scratchings at each clock hour. (B) Total scratching counts for 8 h of pre- or post-treatment of 0.1% indomethacin. *P<0.05 when compared with Pre value (paired Student's t-test).

3.4. Effect of indomethacin on the skin prostaglandins contents in NC/Nga mice

Basal values for prostaglandin D_2, prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostaglandin I_2) and prostaglandin $F_{2\alpha}$ were 12.49±1.25, 8.82±2.62, 2.84±0.52 and 3.38±0.88 pg/mg tissue weight, respectively. Pretreatment with indomethacin significantly decreased these prostaglandins contents 0.07±0.07, 0.32±0.17, 0.56±0.16 and 0.30±0.15 pg/mg tissue weight, respectively (Table 2).

3.5. Effect of arachidonic acid on spontaneous scratching behavior by NC/Nga mice

We examined the effect of arachidonic acid, as an origin of prostanoids, on scratching behavior by NC/Nga mice. The topical application of 0.1% arachidonic acid significantly suppressed the scratching behavior in NC/Nga mice, especially between the time from 16:00 to 09:00 of the following day (Fig. 4A). The total counts of scratching were significantly suppressed by treatment with 0.1% arachidonic acid (431.1±113.8 counts/24 h) compared to pre-treatment (692.4±101.9 counts/24 h) (Fig. 4B).

3.6. Effect of arachidonic acid on skin prostaglandins contents in NC/Nga mice

Basal values for prostaglandin D_2, prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$ were 9.25±1.38, 3.54±0.50, 1.42±0.06, and 2.96±0.43 pg/mg tissue weight, respectively. Pretreatment with arachidonic acid significantly increased these prostaglandins contents 51.85±6.25, 27.97±3.73, 4.10±0.32, and 32.82±3.47 pg/mg tissue weight, respectively (Table 3).

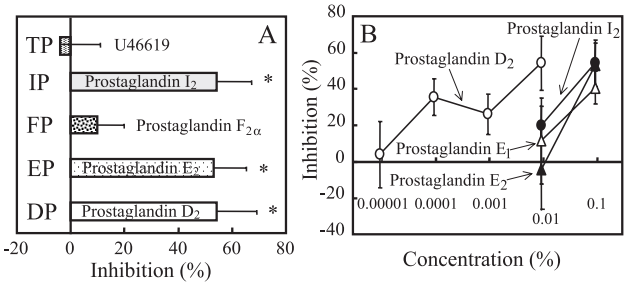

Fig. 2. Effects of several prostaglandins on spontaneous scratchings by skin-lesioned NC/Nga mice. (A) The number of scratchings by NC/Nga mice was measured for the first 24 h (Pre value) followed by the next 24 h after application of each prostanoid. Inhibition (%), (Pre−Post value)×100/Pre values shown as the marker of efficacy. *P<0.05 when compared each Pre value vs. each Post value (paired Student's t-test). (B) Dose-related inhibitory activities of topically applied prostaglandins on spontaneous scratchings by NC/Nga mice. Data evaluation was done as described above. Each column or symbol and bar represents the mean±S.E.M.

Table 2
Effect of indomethacin on skin prostaglandins contents in NC/Nga mice

Treatment	Prostaglandin D_2	Prostaglandin E_2	6-Keto-prostaglandin $F_{1\alpha}$	Prostaglandin $F_{2\alpha}$
Non-treatment	12.49±1.25	8.82±2.62	2.84±0.52	3.38±0.88
Indomethacin	0.07±0.07[c]	0.32±0.17[a]	0.56±0.16[b]	0.30±0.15[a]

Each value represents the means±S.E.M. pg/mg tissue weight (n=6). Indomethacin; 0.2 ml of 0.1% indomethacin was topically treated to mouse back skin, 1 h before the experiment. [a]P<0.05, [b]P<0.01 and [c]P<0.001 compared with non-treatment group of each prostaglandins.

3.7. Effect of prostaglandin D_2 and indomethacin on histamine-induced scratching by ICR mice

The number of short-duration (over 0.3 s) scratching counts for the saline-injected group was 90.94±4.6 counts/30 min. In the vehicle control group, histamine significantly increased the scratching counts 171.1±16.8 counts/30 min. Topical application of 0.01% prostaglandin D_2 (191.0±23.1 counts/30 min) and 0.1% indomethacin (178.0±18.5 counts/30 min) showed no significant effect on this histamine-induced scratching behavior (Fig. 5).

4. Discussion

In the present study, we discovered for the first time the innovative physiological function of prostanoids on the regulation of atopic like dermatitis, using a new method to research scratching behavior of mice with atopic dermatitis (NC/Nga mice). The outline of our strategy for evaluation of scratching behavior was as follows: We focused on the difference in spontaneous scratching behavior between SPF-NC/Nga mice (housed in SPF, normal skin) and Conventional-NC/Nga mice (housed under conventional circumstances, lesioned skin). The pattern of scratching behavior of normal NC/Nga mice as well as ICR and BALB/c mice was non-sustained (0.3–0.5 s/scratch) and of small-amplitude, which may relate to extension of locomotor activity. On the other hand, the pattern of scratching behavior by skin-lesioned NC/Nga mice was persistent (over 1.0 s/scratch) and of large amplitude. We considered the pattern of scratching with a persistent component (over 1.5 s/scratch) to be an atopy-like behavior and took the number of persistent components to evaluate pathological scratching. We consider that our newly developed methodology is useful for evaluating scratching behavior and can be used for evaluation of a drug for treating atopic dermatitis, which has applicability for itching in atopic dermatitis.

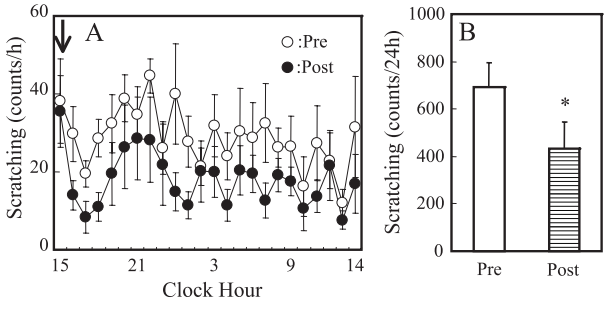

Fig. 4. Effect of arachidonic acid on spontaneous scratchings by NC/Nga mice. (A) Typical intra-day inhibition pattern of topically applied arachidonic acid on spontaneous scratching behavior of NC/Nga mice. The number of scratchings by NC/Nga mice was measured for the first 24 h (○: Pre), followed by the next 24 h after 0.1% arachidonic acid application (●: Post). ↓: Application of drug. Data represents intra-day scratchings at each clock hour. (B) Total scratchings for 24 h of pre- or post-treatment of 0.1% arachidonic acid. *P<0.05 when compared with Pre value (paired Student's t-test).

We examined the effects of several prostanoids and their related compounds on spontaneous scratching behavior in skin-lesioned NC/Nga mice using our evaluation system to elucidate their regulatory mechanisms governing itching. Topical application of high concentrations (0.1%) of prostaglandin D_2, prostaglandin E_1, prostaglandin E_2 and prostaglandin I_2, but neither of prostaglandin $F_{2\alpha}$ nor thromboxane A_2 agonist, significantly inhibited the spontaneous scratching behavior of skin-lesioned NC/Nga mice. The lower concentrations (0.01%) of prostaglandin E_1, prostaglandin E_2 and prostaglandin I_2 had no significant effect on the scratching behavior. Only prostaglandin D_2 showed remarkable inhibition on this scratching even with a low concentration (0.0001%), and the effect of prostaglandin D_2 had the most potent inhibitory effect on itching by NC/Nga mice. Our speculation regarding the inhibitory mechanism of high concentrations of prostaglandin E_1, prostaglandin E_2 and prostaglandin I_2 on itching might relate to pharmacological effects, as their respective receptors do crosstalk with the prostanoid DP_1 receptor.

PPAR-γ is a member of the nuclear receptor superfamily that includes receptors for steroid, thyroid, and retinoid hormones (Evans, 1988). PPAR-γ activation is mediated exclusively by metabolites of prostaglandin D_2, the most active of which is 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 as a natural PPAR-γ ligand (Barry et al., 1995). On the other hand, the prostanoid DP_2 receptor is a seven-transmembrane G protein-coupled receptor structurally related to members of the *N*-formyl peptide receptor subfamily (Nagata et al., 1999a). The prostanoid DP_2 receptor can mediate intercellular Ca^{2+} mobilization in response to a factor released from activated mast cells, suggesting that DP_2 may be closely involved in

Table 3
Effect of arachidonic acid on skin prostaglandins contents in NC/Nga mice

Treatment	Prostaglandin D_2	Prostaglandin E_2	6-Keto-prostaglandin $F_{1\alpha}$	Prostaglandin $F_{2\alpha}$
Non-treatment	9.25±1.38	3.54±0.50	1.42±0.06	2.96±0.43
Arachinonic acid	51.85±6.25[a]	27.97±3.73[a]	4.10±0.32[a]	32.82±3.47[a]

Each value represents the means±S.E.M. pg/mg tissue weight (n=8). Arachidonic acid; 0.2 ml of 0.1% arachnidonic acid was topically treated to mouse back skin, 1 h before the experiment. [a]P<0.05 compared with non-treatment group of each prostaglandins.

mast cell-mediated allergic inflammation (Nagata et al., 1999b). One of the prostaglandin D_2 metabolites, 13,14-dihydro-15-keto-prostaglandin D_2 is a highly selective agonist for the prostanoid DP_2 receptor (Hirai et al., 2001). In this study, PPARγ agonists (15-deoxy-$\Delta^{12,14}$-prostaglandin J_2) and a prostanoid DP2 receptor agonist (13,14-dihydro-15-keto-prostaglandin D_2) had no apparent effect on the scratching behavior, indicating that the suppressive effect of prostaglandin D_2 is mediated by the prostanoid DP_1 receptor.

Indomethacin, an endogenous prostaglandin biosynthesis inhibitor, significantly enhanced the scratching behavior by NC/Nga mice. Topical application of indomethacin decreased the endogenous prostaglandin D_2, prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostaglandin I_2) biosynthesis, followed by the cancellation of intrinsic anti-pruritic functions. On the other hand, arachidonic acid, the substrate of prostaglandins significantly suppressed the scratching behavior in like fashion with its metabolite, in which topical application of arachidonic acid increased endogenous prostaglandin D_2, prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ contents, followed by generation of anti-pruritic activities. These observations indicate that endogenous prostaglandins are physiological regulators of pruritic activities of NC/Nga mice.

Topical application of NSAID, aspirin, was reported to have antipruritic effects in histamine-induced itching in humans (Yosipovitch et al., 1997). On the contrary, Thomsen et al. (2002) reported that topically applied aspirin did not suppress it. These results indicated that role of endogenous prostaglandins on histamine-induced itching is not clear. In our present study, prostaglandin D_2 did not suppress the histamine-induced scratching, and indomethacin did not enhance it. From these results, we suggested that endogenous prostaglandins did not regulate the histamine-induced itching. Histamine is one of the most common mediators of itching particularly in urticaria-like skin symptoms. The scratching counts in ICR mice were significantly accelerated by the intra-dermal injection of histamine, but the pattern of scratching behavior was non-sustained and of small-amplitude (counted over 0.3 s duration of scratching), which is apparently different from that of persistent and spontaneous behavior (counted over 1.5 s duration of scratching) of NC/Nga mice. We attempted to confirm the participation of prostaglandin D_2 on histamine-induced scratching of ICR mice. We reported that histamine-induced scratching behavior of ICR mice was significantly inhibited by oral administration of anti-histamine drugs (chlorpheniramine and ketotifen), but not by dexamethasone and FK-506 (Takano et al., 2003), which suggested that histamine-induced scratching is induced through histamine receptors in peripheral sensory nerves. We found that prostaglandin D_2 and indomethacin did not affect histamine-induced scratching behavior nor did as dexamethasone and FK-506, which means that endogenous prostaglandins probably do not participate in the regulation of histamine-induced pruritic activities. In contrast, spontaneous scratching behavior by NC/Nga mice was significantly suppressed by dexamethasone, FK-506 and prostaglandin D_2, but not by anti-histamine. Hence, the mechanism of development of atopy-like spontaneous, persistent scratching behavior of NC/Nga mice is obviously different from that of histamine-induced urticaria-like scratching (Takano et al., 2003).

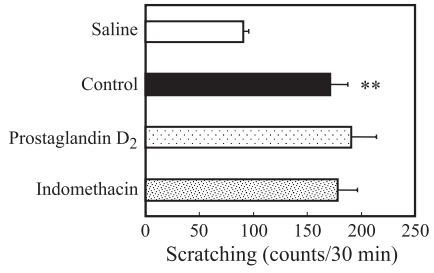

Fig. 5. Effects of prostaglandin D_2 or indomethacin on histamine-induced scratching of ICR mice. The number of scratching behaviors of ICR mice was measured for 30 min just after intra-dermal injection of saline or histamine. 0.01% prostaglandin D_2 or 0.1% indomethacin was topically applied 1 h before histamine injection. Data represents total scratching counts for 30 min of each group. Each bar and bar represents mean±S.E.M. **P<0.01 when compared with the saline-treated group.

In conclusion, we find evidence for the physiological function of prostaglandins in suppressing the spontaneous atopy-like scratching by NC/Nga mice via the prostanoid DP_1 receptor. These receptor agonists or their stable analogues may prove to be useful therapeutics for patients with atopic dermatitis.

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