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D1-like dopamine receptors antagonist inhibits cutaneous immune reactions mediated by Th2 and mast cells

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ABSTRACT

Background: Dopamine transduces signals via five subtypes of G protein-coupled receptors. Among these subtypes, the D1 and D5 receptors belong to the D1-like group. Although dopamine is known to mediate immune responses, its involvement in cutaneous immunity remains unclear.

Objective: The aim of this study is to determine the role of dopamine and its D1-like receptors in cutaneous immune responses.

Methods: By using the D1-like receptor antagonist SCH 23390, we examined the role of D1-like receptors in murine models of Th1-type contact hypersensitivity and Th2-type atopic dermatitis *in vivo*, and in mast cells and Th2 cell differentiation *in vitro*.

Results: Administration of SCH 23390 did not affect Th1-type contact hypersensitivity but suppressed the immediate-type reaction (ITR) and the late phase reaction (LPR) in the atopic dermatitis model. In addition, SCH 23390-treated mice showed higher IFN- γ and lower IL-4 mRNA levels in the ear skin of challenged mice than did non-treated mice as analyzed by real-time RT-PCR. Consistently, the passive cutaneous anaphylaxis reaction was significantly reduced in SCH 23390-treated mice. Moreover, dopamine enhanced mast cell degranulation and Th2 cell differentiation, and both activities were abrogated by SCH 23390.

Conclusion: These findings suggest that the D1-like receptors mediate immediate and late phase skin reactions by promoting Th2 induction and mast cell degranulation.

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1. Introduction

CD4⁺ helper T (Th) cells, comprising Th1 cells secreting interferon (IFN)- γ , Th2 cells secreting interleukin (IL)-4, IL-5 and IL-13, and Th17 cells secreting IL-17A and IL-22, play a central role

Abbreviations: Th, helper T; IFN, interferon; IL, interleukin; Tc, cytotoxic T; CHS, contact hypersensitivity; ITH, immediate type hypersensitivity; LPR, late phase reaction; TNBC, 2,4,6-trinitrochlorobenzene; BMDC, bone marrow-derived mast cells; FSC, fetal skin-derived cultured mast cells; PCA, passive cutaneous anaphylaxis; DNP, dinitrophenyl; HSA, human serum albumin; cRPMI, complete RPMI; SCF, stem cell factor.

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in modulating immune responses. Similarly, CD8⁺ cytotoxic T (Tc) cells differentiate into two subsets, Tc1 and Tc2 cells. Th1 and Tc1 cells mediate cellular immune reactions such as delayed-type hypersensitivity (DTH) and contact hypersensitivity (CHS), whereas Th2 cells elicit allergic/humoral immune responses such as atopic dermatitis and asthma. Th17 cells mediate host immune responses against extracellular bacteria and fungi [1].

In addition to T cells, mast cells produce a wide variety of proinflammatory mediators, including cytokines, growth factors, and chemokines, which contribute to the development of allergic or autoimmune diseases [2]. In general, mast cells are stimulated by cross-linking of the high-affinity IgE receptor, Fc ϵ RI. They can be stimulated by inflammatory mediators during inflammatory responses via an Fc ϵ RI-independent pathway [3]. It is therefore of value to explore such mediators that modulate mast cell functions.

Dopamine is one of important neurotransmitters in the central nervous system and controls locomotion, emotion, cognition, and neuroendocrine secretion. Dopamine mediates signals via five subtypes of seven-transmembrane G protein-coupled receptors, named D1–D5. The D1 and D5 receptors, termed the D1-like receptor group, are coupled to Gs proteins that increase intracellular cyclic AMP, while D2, D3 and D4 form the D2-like receptor group that suppress intracellular cyclic AMP through Gi proteins [4,5]. Thus, the effects of D1- and D2-like receptors counteract each other.

Schizophrenia is associated with excessive dopamine stimulation of D2-like receptors [6], and D2-like receptor antagonists, such as sulpiride and nemonapride, are widely used for treatment of this disease. In schizophrenic patients, the expression of D2-like receptors is elevated not only in the brain but also in immune cells [7–9], and the ratio of IFN- γ /IL-4 in the peripheral blood is high [10], suggesting that Th1/Tc1 functions are dominant in schizophrenic patients. Consistent with this idea, a cohort study revealed that schizophrenic patients have a reduced risk of cancer [11], presumably because of the enhanced anti-tumor immunity resulting from the increased number of Th1/Tc1 cells. Moreover, effective neuroleptic treatment [12] and D2-like receptor antagonists [13] attenuated the increased levels of IFN- γ /IL-4. It has been reported that dopamine receptors are expressed on T cells, monocytes, neutrophils, eosinophils, B cells and natural killer cells [14], and moreover, dopamine is produced by lymphocytes and dendritic cells [15]. Therefore, there is a possibility that dopamine is involved in cutaneous immune responses.

We have recently reported that D1-like receptor antagonists have therapeutic effects on a murine experimental autoimmune encephalomyelitis model by suppression of Th17 cell activation [13]. In this study, we examined the effect of dopamine-D1-like receptor signals in Th1/Tc1- and Th2-mediated cutaneous immune reactions. Our results suggest that dopamine is involved in cutaneous Th2-type immune responses through activation of both mast cells and Th2 cells via D1-like receptors.

2. Materials and methods

2.1. Mice and reagents

Seven- to ten-week-old female BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). These mice were maintained in the Laboratory Animal Research Center in the University of Occupational and Environmental Health under specific pathogen-free conditions. This study was carried out in strict accordance with the recommendations in the Guide for the Ethics Committee of Animal Care Experimentation, University of Occupational and Environmental Health. The protocol was specifically approved by the Ethics Committee of Animal Care Experimentation, University of Occupational and Environmental Health (protocol #AE03-069). All surgery was performed under anesthesia and all efforts were made to minimize suffering.

Recombinant mouse IL-3 and stem cell factor (SCF) were purchased from Peprotech (Rocky Hill, NJ). Mouse anti-dinitrophenyl (DNP) IgE (clone SPE7), DNP-human serum albumin (HSA), D1-like receptor antagonist, SCH 23390, and dopamine were obtained from Sigma (St. Louis, MO). The K_i values of SCH 23390 obtained by competition-binding isotherms to displace the radioligand binding to the respective dopamine receptor are 1400, 1450, 2910, 0.4, and 0.5 nM for D2, D3, D4, D1 and D5, respectively. 2,4,6-trinitrochlorobenzene (TNCB) was purchased from Tokyo Kasei (Tokyo, Japan). SCH 23390 was administered orally twice a week.

2.2. Preparation of bone marrow-derived mast cells (BMMC) and fetal skin-derived cultured mast cells (FSMC)

For BMMC, bone marrow cells obtained by flushing tibias and femurs of BALB/c mice were cultured at 37 °C in a humidified atmosphere containing 5% CO₂ at a density of 5×10^4 cells/ml in complete RPMI (cRPMI) containing 10% heat-inactivated fetal bovine serum (GIBCO, San Diego, CA), 10 mM nonessential amino acids (GIBCO), 1% penicillin and streptomycin (GIBCO) and 50 mM 2-mercaptoethanol in RPMI-1640 (Sigma, St. Louis, MO) supplemented with 5 ng/ml IL-3 and 5 ng/ml SCF. Four weeks later, the populations were composed of >95% mast cells as evaluated by morphology and by Fc ϵ RI and c-kit expression as determined by flow cytometry.

FSMC were prepared from day 14 to 16 fetal skin as described previously [16]. Briefly, single-cell suspensions were prepared by trypsinization of excised trunk skin and cultured in cRPMI supplemented with 10 ng/ml IL-3 and 10 ng/ml SCF. Non-adherent and loosely adherent cells were collected 21–28 days after this initial culture and enriched for mast cells by density gradient centrifugation in 1.052 g/ml Percoll (Amersham Life Science, Arlington Heights, IL). The resulting mast cell preparations contain >98% CD117⁺ MCs.

2.3. CHS, repeated hapten challenge-induced atopic dermatitis, passive cutaneous anaphylaxis (PCA), and histology

For induction of CHS to TNCB, mice were sensitized on the abdomens with 50 μ l of 1% TNCB in acetone and olive oil (3:1). Seven days after sensitization, mice were challenged by painting both sides of each earlobe with 20 μ l of 0.2% TNCB in acetone and olive oil (3:1).

Thereafter, these mice were repeatedly challenged with 20 μ l of 0.2% TNCB twice a week for 3 weeks (7 total challenges) to induce atopic dermatitis. The ear thickness was measured using a dial thickness gauge (Ozaki Co, Tokyo, Japan). The D1-like receptor antagonist SCH 23390 (Sigma) was orally administered to the mice at a dose of 0.3 mg/kg, 30 min before painting.

PCA was performed as previously described [17] with slight modifications. The right and left ears were injected with 50 μ l of 1 μ g/ml anti-DNP IgE in PBS or PBS alone, respectively. Forty-eight hours later, 200 μ l of 1 μ g/ml DNP-HSA in 1% Evans blue solution (Sigma) was injected intravenously. The ear thickness was measured 30 min after injection and the ears were then resected by 6 mm punch biopsy. The amount of dye extracted from the ear using formamide was determined by the absorbance at 620 nm.

The ears were fixed with formalin and embedded in paraffin. Sections 3 μ m in thickness were cut and stained with hematoxylin and eosin, or, for mast cells, with toluidine blue.

2.4. In vitro T cell differentiation

For *in vitro* Th1 and Tc1 differentiation assays, B6 CD4⁺ T cells or CD8⁺ T cells were stimulated for two days with plate-bound anti-CD3 (1, 10 μ g/ml) and anti-CD28 (0, 1, 10, 100 μ g/ml) Abs in combination with 10 ng/ml IL-12, 10 μ g/ml anti-IL-4 Ab and 20 ng/ml IL-2. Similarly, naïve BALB/c CD4⁺ T cells were incubated in the presence of 10 ng/ml IL-4, 10 μ g/ml anti-IFN- γ Ab, and 20 ng/ml IL-2 to induce Th2 differentiation. Forty-eight hours later, the cells were washed and cultured further under the same conditions except without anti-CD3 or anti-CD28 Ab stimulation. We examined the expression level of D1 and D5 receptor in BL/6J and BALB/c spleen unstimulated CD4⁺ T lymphocytes. Real-time PCR analysis revealed that there was no difference in D1 and D5 receptor expression between these mouse strains (data not shown). For measurement of cytokine production, B6 CD4⁺ T cell

were stimulated for seven days with CD3/28 beads and dopamine/SCH 23390. The culture supernatants were collected 72 h after incubation and assayed by ELISA (BD Biosciences and R&D Systems, Minneapolis, MN) according to the manufacture's protocol.

2.5. ELISA

For measurement of dopamine, the ears were resected by 6 mm punch biopsy and homogenized and cell-lyzed in 1 ml PBS, and sum level of secreted and intracellularly stored dopamine were examined by the ELISA kit (SRL, Tokyo, Japan).

2.6. β -Hexosaminidase assay

BMMC were sensitized by incubation with 1 μ g/ml anti-DNP IgE for 2 h at 37 °C in cRPMI. The cells were then washed, resuspended at 1×10^6 cells/ml and stimulated with 3 ng/ml DNP-HSA for 30 min at 37 °C. The β -hexosaminidase activity of culture supernatants and cell lysates prepared by lysis with 0.5% Triton-X (Sigma) was determined as previously reported [18,19] with some modifications. 50 μ l aliquots of supernatants or cell lysates were incubated in 96-well plates with 100 μ l of 2.5 mM *p*-nitrophenyl-*N*-acetyl β -D glucosaminide (Sigma) solubilized in 0.04 M citrate buffer (pH 4.5). After incubation for 90 min at 37 °C, the reactions were terminated by the addition of 50 μ l of 0.2 M glycine (pH 10.7; MP Biomedicals, Solon, OH). The colored product was measured using an HTS7000 plate reader (Perkin Elmer, Wellesley, MA) at 405 nm wavelength using a 570 nm reference filter. The percentage of β -hexaminidase release was calculated as supernatant/(supernatant + pellet) $\times$ 100 [19].

2.7. Real-time quantitative PCR

Total RNA was extracted using the Trizol RNA extraction kit (Invitrogen, San Diego, CA). RNA was reverse transcribed and amplified with random hexamer primers using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) with primers and a detection probe labeled with the reporter dye 6-carboxyfluorescein. Primers and probes were obtained from TaqMan Gene Expression Assays Inventories (Applied Biosystems). Using an ABI Prism 7000 sequence detection systems (Applied Biosystems), duplicate samples were reverse transcribed and amplified as follows: 2 min at 50 °C, 10 min at 95 °C, followed by 50 amplification cycles of 15 s at 95 °C and 1 min at 60 °C. Quantification of gene-specific message levels was determined by comparing the fluorescence intensity from unknown RNA samples to the fluorescence intensity of mouse β -actin mRNA.

2.8. Statistical analysis

Unless otherwise indicated, data are presented as the mean $\pm$ standard deviation (SD) and are representative of three independent experiments. *P*-values were calculated with the two-tailed Student's *t*-test. *P* values less than 0.05 are considered to be significantly different between the indicated groups and are indicated by * in the figures.

3. Results

3.1. Treatment with SCH 23390 reduces immediate type hypersensitivity (ITH) and late phase reaction (LPR) in mice

To evaluate the effect of D1-like receptor-mediated signaling on cutaneous immune responses, we used two different sensitivity models: the CHS response to TNCB as a Th1/Tc1-induced model

and the repeated TNCB application-evoked murine atopic dermatitis as a Th2-induced model [20,21]. CHS, a model of delayed-type hypersensitivity, peaks at 24 h after challenge [22]. In the atopic dermatitis model, the ITH, which is induced by mast cells via an IgE-dependent manner, occurs at 15 min to 1 h after challenge [23]. On the other hands, the LPR, which is thought to be mediated by Th2 cells and/or eosinophils, peaks at 4–8 h [24].

In CHS, the changes in ear thickness at 1, 4, 8, and 24 h after a single challenge of TNCB were unaffected by SCH 23390 treatment (Fig. 1A–D, left). After the repeated TNCB challenge, a significant degree of ear swelling was observed at 1, 4 and 8 h after challenge in mice receiving hapten exposure (Fig. 1A–C, right). This swelling was attenuated by treatment with SCH 23390. However, the change in ear thickness 24 h after challenge (Fig. 1D, right).

The intensity of dermal edema in the ear 1 h after the repetitive challenge was mild upon treatment with SCH 23390 (Fig. 1E). Twenty-four hours after the last challenge, SCH 23390-treated and PBS-treated mice exhibited a comparable degree of inflammatory infiltration mainly in the dermis (Fig. 1F). The number of dermal mast cells was increased by the repeated application of TNCB. The SCH 23390 treatment had no effect on the number of mast cells (Fig. 1G), suggesting that SCH 23390 did not alter the number of mast cells.

3.2. Treatment with SCH 23390 reduces IL-4 expression and augments IFN- γ expression in TNCB repetitive challenged skin

Given that SCH 23390 reduced both ITH and LPR, we next sought to determine cellular targets of this D1-like receptor antagonist. To this end, we focused on Th2 cells and mast cells, which are involved in ITH and LPR [23,24]. Ears were excised 3 h and 24 h after the last repetitive challenge, and then analyzed for the expression of Th1 and Th2 cytokines by real-time RT-PCR. The mRNA expression of both Th1 cytokine IFN- γ and Th2 cytokine IL-4 was elevated compared to that of the non-treated, control ears as measured by real-time PCR (Fig. 2A and B). Treatment with SCH 23390 increased the levels of IFN- γ (Fig. 2A) and decreased the levels of IL-4 (Fig. 2B) at 3 h and 24 h. The SCH 23390 treatment also increased IFN- γ and decreased IL-4 in the draining lymph node cells 24 h after the last challenge (Fig. 2C and D). On the other hand, the levels of IL-17 and IL-10, representative cytokines produced by Th17 cells and regulatory T cells, respectively, in the skin and the draining lymph nodes were not affected by SCH 23390 treatment (data not shown).

In addition, we measured the amount of dopamine in the ear of mice with hapten repeated application. The amount of dopamine in the skin after hapten application was 5 times greater than that of non-treated mice (Fig. 2E), suggesting that dopamine is produced in the skin during the immune responses.

These results suggest that the inhibition of D1-like receptor signaling shifts the Th1/Th2 balance toward Th1 cells, thus downregulating Th2-type cutaneous immune responses, ITH and LPR that are induced by mast cells and Th2 cells.

3.3. Expression and function of D1 and D5 receptors in mast cells

To dissect the mechanism underlying the alterations of immune responses upon inhibition of D1-like receptors, we first examined whether D1-like receptors are expressed on mast cells. Using real-time PCR, we found that the levels of D1 and D5 receptors expressed on BMMCs and FSMCs were higher than those in keratinocytes (Fig. 3A). To investigate the functional role of mast cell D1-like receptors in an *in vivo* system, we exploited PCA, which is mediated at least by mast cells [21]. PCA response was assessed by measuring Evans blue dye exudation into ears, and administration of SCH 23390 suppressed this reaction (Fig. 3B and C).

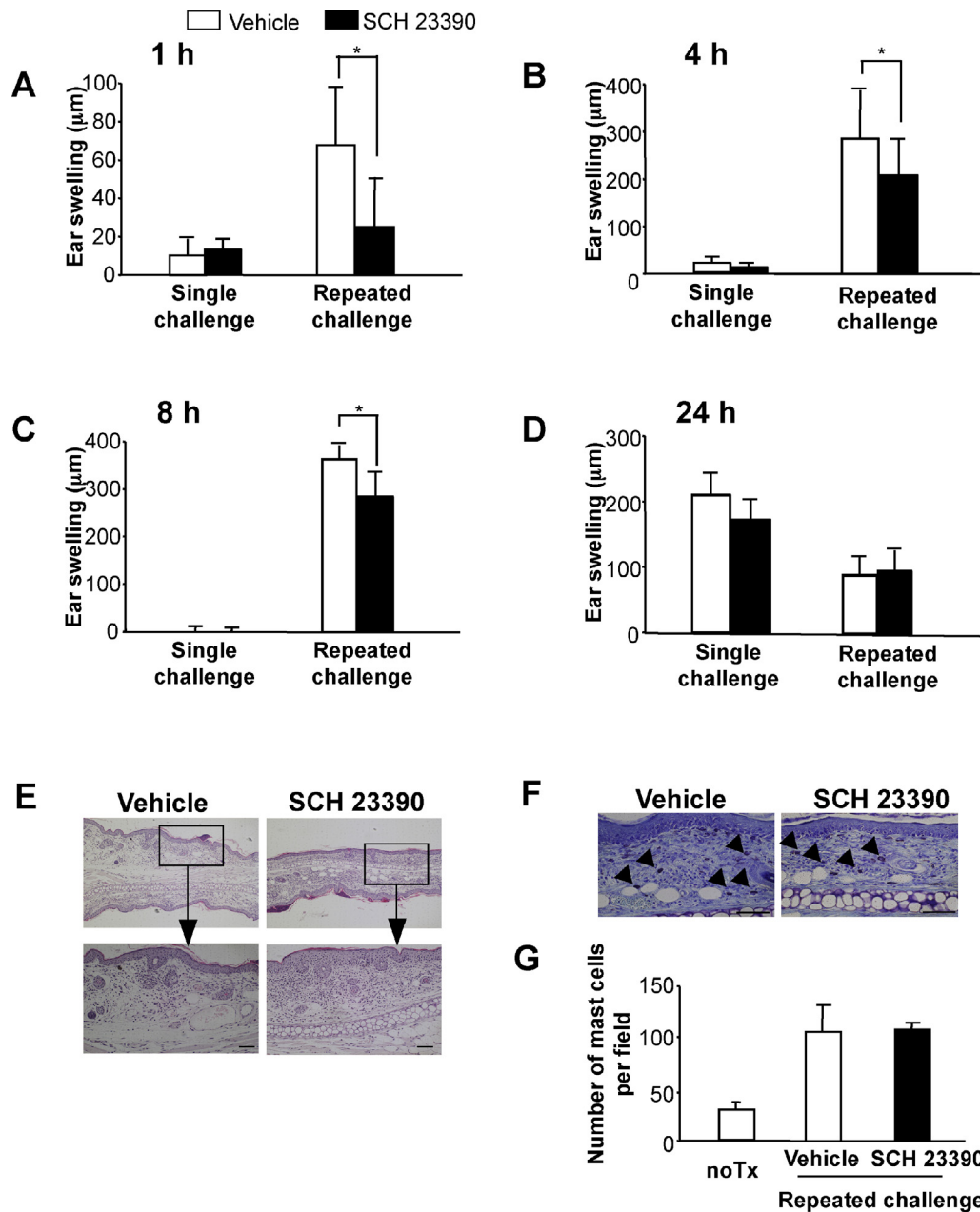


Fig. 1. Effects of SCH 23390 on cutaneous immune responses. Mice were sensitized and challenged with a single application (left bars) or repeated applications (right bars) of TNCB with or without treatment with SCH 23390. The ear swelling responses were evaluated at 1 h (A), 4 h (B), 8 h (C), and 24 h (D) after the challenge. Skin samples were prepared 1 h after repeated applications of hapten and stained with hematoxylin and eosin (E) and were prepared 24 h after treated and stained with toluidine blue (F). Bar, 100 μm. Arrows indicate mast cells (F), and the number of dermal mast cells was enumerated (G).

Consistent with these findings, the ear swelling 30 min after injection of DNP-HSA was significantly lower in SCH 23390-treated mice than in vehicle-treated mice (Fig. 3D). Therefore, the results suggest that signaling via D1-like receptors enhances mast cell functions.

3.4. Expression of D1 and D5 receptors in mast cells and Th2 cells

We next examined the distribution of D1 and D5 receptor expression in Th subset, Th1/Th2. To this end, we induced Th1 and Th2 cells from naïve mouse splenic T cells *in vitro*, and then analyzed receptor expression. The expression of mRNA for the D1-like receptors D1 and D5 in Th2 cells was much higher than that in Th1 cells (Fig. 4A).

We next incubated T cells with dopamine in the presence or absence of SCH 23390, and measured IL-4 and IFN-γ mRNA levels. The ratio of IL-4 to IFN-γ in mRNA level was elevated upon addition of dopamine, and this increase was canceled by further addition of SCH 23390 (Fig. 4B). These results suggest that D1-like receptors are expressed on Th2 cells in a comparison with Th1 cells, and that dopamine-D1-like receptor signaling seems to skew immune responses to Th2 responses.

3.5. Mast cell degranulation by the dopamine-D1-like receptor pathway

Degranulation is one of the hallmarks of inflammation mediated by mast cells. The addition of dopamine elevated β-

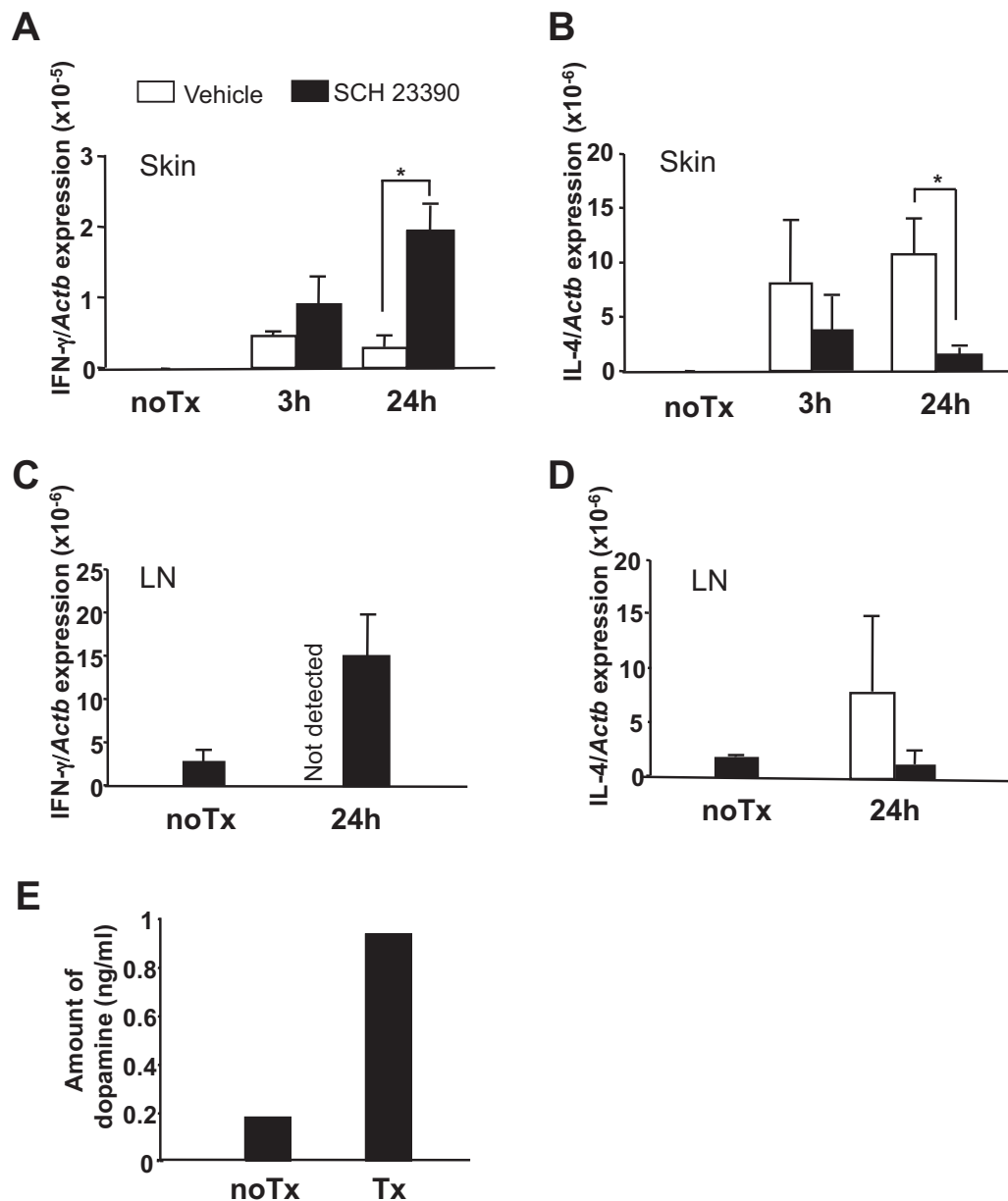


Fig. 2. IFN- γ and IL-4 mRNA expression in skin and draining lymph nodes, and dopamine production. Ears were excised 3 h and 24 hours after repeated application with TNCB, and mRNA for IFN- γ (A) and IL-4 (B) were evaluated by quantitative RT-PCR. Along with the skin specimens, the draining lymph nodes were taken from the mice 24 h after challenge, and the levels of mRNA for IFN- γ (C) and IL-4 (D) were measured. (E) Dopamine production in the skin. Mice were treated with or without TNCB repeated exposure.

hexosaminidase release in a dose-dependent manner (Fig. 5A). Enhanced degranulation by dopamine was abrogated by pretreatment for 30 min with SCH 23390 (Fig. 5A). We also evaluated the effect of dopamine-D1-like receptor signals on mast cell degranulation in response to Fc ϵ RI activation triggered by IgE plus multivalent antigen. The addition of dopamine significantly elevated β -hexosaminidase release in a dose-dependent manner (Fig. 5B). These results suggest that signaling through the dopamine-D1-like receptors mediates mast cell degranulation.

4. Discussion

In this study, we have demonstrated that a D1-like receptor antagonist did not suppress conventional CHS but did suppress ITH and LPR with reduced IL-4 mRNA levels in a repeated hapten application-induced atopic dermatitis model. The D1-like receptor antagonist also suppressed PCA and mast cell degranulation

induced by dopamine stimulation. In addition, D1-like receptors were selectively expressed in Th2 cells, suggesting that dopamine is capable of promoting Th2 cell differentiation through D1-like receptors. These results indicate that D1-like dopamine receptor signals induce mast cell degranulation and Th2 cell induction to develop ITH and LPR in cutaneous immune responses.

One of the longstanding questions in immunology is whether D1-like receptor signals are involved in the Th1/Th2 balance in cutaneous immune responses. In this study, we found that the D1-like receptor antagonist, SCH 23390, decreased IL-4 and increased IFN- γ expression levels, and suppressed ITH and LPR in the repeated-hapten application model. Since ITH and LPR are thought to be induced by mast cells and Th2 cells, our results suggest that D1-like dopamine receptor signals skew immune responses to Th2 domination. Inversely, but in accord with our finding, D2-like receptor antagonists have been reported to exert a Th17-skewing action in the experimental autoimmune encephalomyelitis model

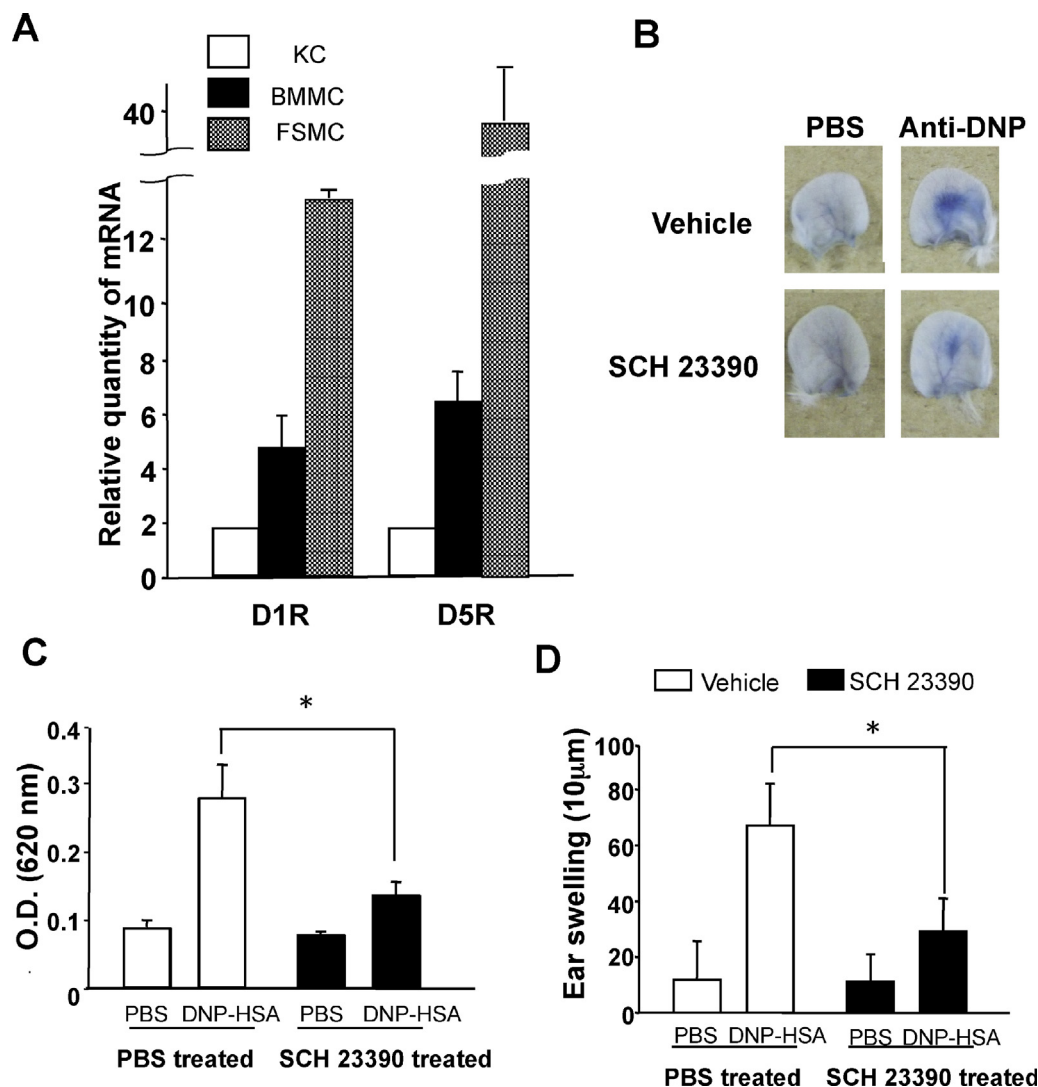


Fig. 3. D1-like receptor expression on mast cells and effect of SCH 23390 on PCA (A) Keratinocytes were prepared from the epidermis of mice. Expression levels for D1 and D5 in BMMC, FSMC and keratinocytes were evaluated by quantitative RT-PCR. (B) Mice were injected with anti-DNP IgE in the skin of the left ear or with PBS alone in the skin of the right ear. After 48 h, mice were treated intravenously with DNP-HSA in 1% Evans blue solution and the ears photographed. (C) The amounts of the dye extracted from the ears prepared in (B) were measured. (D) Ear thickness was measured 30 min after the injection of DNP-HSA.

[13]. Nakano et al. reported that high levels of dopamine released by DCs polarized Th2 differentiation [15]. There have been several clinical observations regarding the effects of dopamine and its receptors on Th1/Th2 balance. The functions of Th1 and Tc1 cells are enhanced in D2-dominant schizophrenic patients [10]. L-dopa applied to patients suffering from Parkinson's disease and psoriasis attenuated both conditions [25]. It has also been reported that the number of IFN- γ -producing, but not IL-4-producing, cells was decreased by treatment with either L-dopa or dopamine [26]. These intriguing reports collectively support the concept that D1- and D2-like receptor signals induce Th2 and Th1 immunological states, respectively. Furthermore, antagonists of each dopamine receptor inhibit their respective actions.

Despite this abundance of circumstantial evidence, the mechanism underlying the Th2-skewing action of dopamine has not clearly been defined, especially in terms of D1-like receptor functions. In the present study, we demonstrated that Th2 cells, but not Th1 cells, bear the D1-like receptors D1 and D5. This finding suggests that dopamine, when it binds to D1-like receptors, stimulates Th2 cells without Th1 activation, resulting in a Th2-polarized immune reaction. Given this concept, the D1-like

receptor antagonist may suppress Th2 cells, leading to the inhibition of LPR.

Of particular interest is the observation that degranulation of mast cells is promoted by dopamine and inhibited by a D1-like receptor antagonist, suggesting that endogenous dopamine enhances mast cell degranulation *via* D1-like receptors. Accordingly, BMMCs and FSMCs expressed high levels of D1-like receptors, and the PCA reaction was impaired by the addition of a D1-like receptor antagonist. It is suggested that the SCH 23390-induced attenuation of ITH and LPR in response to hapten is attributable to its effect on mast cells as well as on T cell subset balance.

In this study, dopamine levels were increased in the dermatitis skin lesions. While dopamine is produced by lymphocytes and dendritic cells [13]. It is reported that a high level of dopamine is also stored in BMMCs and released upon stimulation with ionomycin [27]. Therefore, it is possible that mast cells are exposed to dopamine not only *via* paracrine but also in an autocrine manner.

It is well known that allergic diseases, such as urticaria and atopic dermatitis, are aggravated by stressful events. It is also

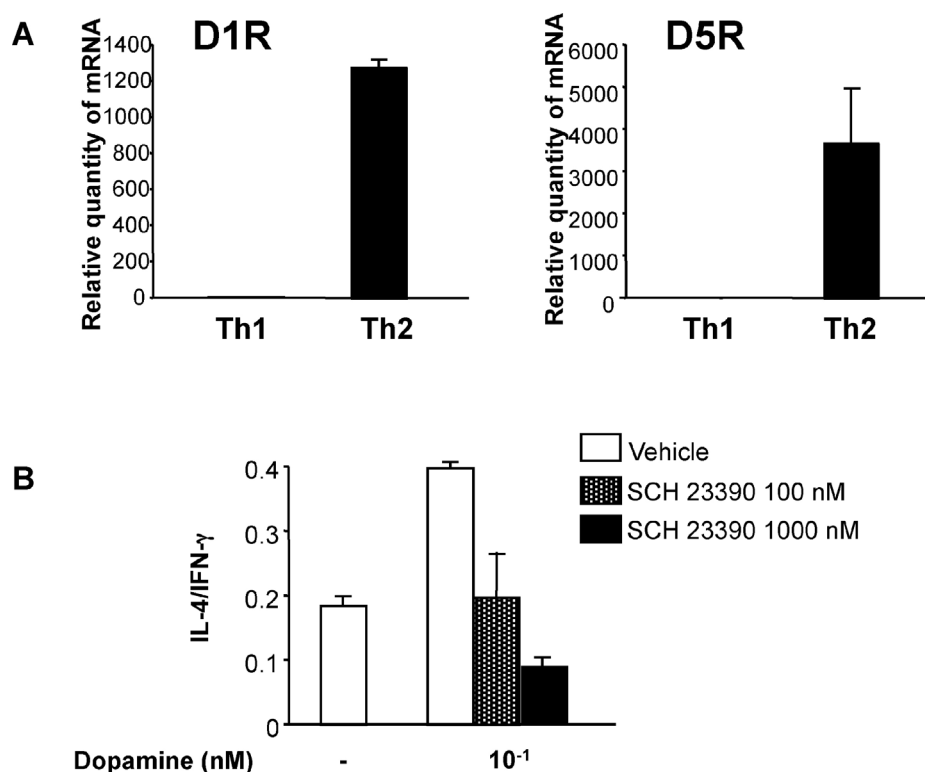


Fig. 4. Expression of D1 and D5 in BMMCs/FSMCs and in Th2 cells (A) Th1 and Th2 cells were induced from naive T cells and subjected to real-time PCR analysis to detect the mRNA expression levels of D1 and D5. (B) T cells were incubated with or without dopamine in the presence or absence of SCH 23390. The levels of IL-4 and IFN-γ mRNA were determined and the ratio of IL-4 to IFN-γ mRNA levels is indicated.

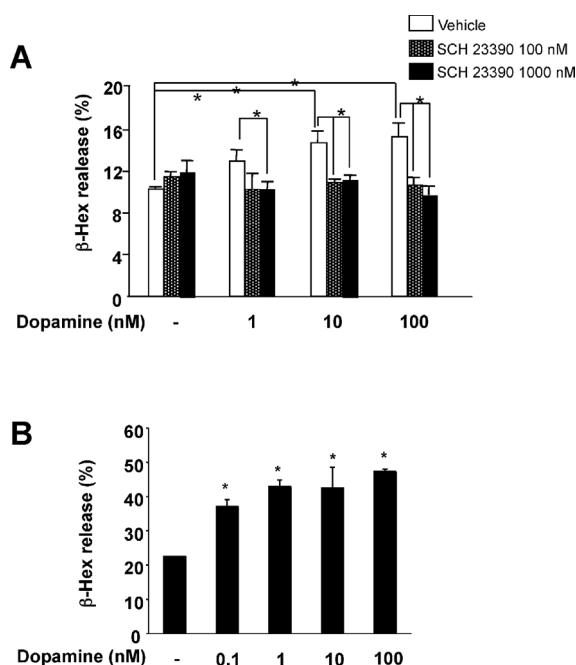


Fig. 5. Effect of SCH 23390 on degranulation of BMMC and expression of dopamine receptors in BMMC (A) BMMC were isolated and pretreated for 30 min with or without SCH 23390 at 100 or 1000 nM, then incubated for 30 min in the presence or absence of dopamine at the indicated concentrations. The release of β-hexosaminidase was then evaluated. (B) BMMC were sensitized with 1 μg/ml anti-DNP IgE for 2 h and stimulated with 3 ng/ml DNP-HSA for 30 min. Dopamine at the indicated concentrations was added for 30 min prior to the addition of DNP-HSA. **P* < 0.05 vs. respective control values observed in the absence of dopamine.

known that dopamine is involved in stress configuration. Herein we show that ITH and LPR in an atopic dermatitis model were attenuated by treatment with a D1-like receptor antagonist. Our new findings indicate a new therapeutic strategy to control Th2-mediated and mast cell-evoked allergic immune responses by modulating signals via dopamine receptors.

Clinical implications

Inhibition of the dopamine-D1-like receptor pathway may be a therapeutic target for Th2 and mast cell mediated-immEDIATE and late phase skin reactions of atopic dermatitis.

Capsule summary: D1-like receptors mediate the activation of mast cells as well as Th2 cells, thus enhancing immediate and late phase skin reactions.

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