



Involvement of cannabinoid CB₂ receptors in the IgE-mediated triphasic cutaneous reaction in mice

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Abstract

Involvement of cannabinoid CB₂ receptors in the IgE-mediated cutaneous reaction was investigated. Epicutaneous challenge with 2,4-dinitrofluorobenzene caused a triphasic swelling in the ear of BALB/c and C57BL/6 mice passively sensitized with anti-dinitrophenol IgE. Peak responses of the ear swelling appeared at 1 h, 24 h, and 8 days after the challenge in both strains of mice. In contrast, cannabinoid CB₂ receptor-deficient mice failed to exhibit the obvious triphasic ear swelling observed in wild-type mice. Oral administration of cannabinoid CB₂ receptor antagonist/inverse agonists [*N*-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide] (JTE-907) and [*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2,2,1]heptan-2-yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide} (SR144528) at doses of 0.1–10 mg/kg significantly and dose-dependently suppressed all three phases of ear swelling in BALB/c mice. Interestingly, epicutaneous treatment with an ether-linked analogue of endogenous cannabinoids, 2-arachidonoylglycerol, caused an ear swelling that could be detected at 1 h, 24 h, and 8 days after treatment of both BALB/c and C57BL/6 mice. These results suggest that cannabinoid CB₂ receptors are involved in induction of the triphasic cutaneous reaction mediated by IgE, and that cannabinoid CB₂ receptor antagonist/inverse agonists may serve as anti-allergic agents in the treatment of allergic dermatitis.

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Introduction

Cannabinoids exert a wide variety of effects on the central nervous system and the immune system (Pertwee, 2000; Piomelli et al., 2000; Porter and Felder, 2001). Two types of cannabinoid receptors, a central nervous type (CB₁) and a peripheral type (CB₂), have been cloned and found to be abundant in the central nervous system and peripheral tissues, respectively (Facci et al., 1995; Matsuda et al., 1990; Munro et al., 1993; Pettit et al., 1996; Schatz et al., 1997). The presence of cannabinoid CB₂ receptors (and cannabinoid CB₁ receptors) in immune system cells strongly suggests that endogenous cannabinoids could act as immunomodulators (Berdyshev, 2000; De Petrocellis et al., 2000; Hollister, 1992; Klein et al., 1998). Two arachidonic acid derivatives, 2-arachidonoylglycerol (2-AG) and anandamide, have been proposed as endogenous ligands for cannabinoid CB₁ and CB₂

receptors (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995). Notably, 2-AG is more likely a physiological ligand for cannabinoid receptors, because it acts as a full agonist at cannabinoid receptors (Gonsiorek et al., 2000; Hillard, 2000; Sugiura et al., 1999, 2000). 2-AG stimulates the migration of inflammatory and leukemia cells through a cannabinoid CB₂ receptor-dependent mechanism in vitro (Jorda et al., 2002; Kishimoto et al., 2003; Oka et al., 2004). Furthermore, 2-AG modulates acute inflammation in the mouse ear induced by a topical application of 12-*O*-tetradecanoylphorbol-13-acetate, and the topical application of 2-AG and its ether-linked analog, 2-AG-E, to the mouse ear induces swelling (Oka et al., 2005). We also demonstrated that mouse ears topically exposed to 2-AG-E and a cannabinoid CB₂ receptor selective agonist, HU-308 (Hanus et al., 1999), exhibit swelling, and that ear swelling induced by 2-AG-E is suppressed by treatment with cannabinoid CB₂ receptor selective antagonist/inverse agonists, JTE-907 (Iwamura et al., 2001) and SR144528 (Rinaldi-Carmona et al., 1998) in BALB/c mice (Ueda et al., 2005).

Repeated application of 2,4-dinitrofluorobenzene (DNFB) to the ear of BALB/c mice results in a typical allergic dermatitis

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accompanied by an elevation of serum-specific IgE against DNFB. We recently reported that the allergic dermatitis was inhibited by cannabinoid CB₂ receptor selective antagonist/inverse agonists, which suggests that the cannabinoid CB₂ receptor is involved in the induction and development of allergic dermatitis (Ueda et al., 2005). Although dermatitis seems to involve an IgE-mediated response (Nagai et al., 1997a,b, 2000), little is known about the role of cannabinoid CB₂ receptors in the IgE-mediated cutaneous reaction *in vivo*.

In the present study, therefore, to clarify the involvement of cannabinoid CB₂ receptors in the IgE-mediated cutaneous reaction, we examined the triphasic cutaneous reaction induced by intravenous injection of monoclonal anti-dinitrophenol (DNP) IgE and subsequent topical treatment by DNFB in cannabinoid CB₂ receptor-deficient mice. We also investigated the effects of cannabinoid CB₂ receptor selective antagonist/inverse agonists on the triphasic cutaneous reaction. Furthermore, we studied whether 2-AG-E applied epicutaneously would induce an ear swelling similar to that seen in the triphasic cutaneous reaction.

Materials and methods

Animals

Female BALB/c and C57BL/6 mice were obtained from Japan SLC Inc. (Hamamatsu, Japan), and female cannabinoid CB₂ receptor-deficient mice and their age-matched wild-type littermates were provided by Prof. A. Zimmer, (Bonn University, Bonn, Germany) (Buckley et al., 2000). They were housed in plastic cages in a room illuminated on a 12 h-cycle (8:00–20:00), with controlled temperature (25±3 °C) and humidity (55±15%), and fed a standard laboratory diet and given water *ad libitum*. All procedures related to the use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Japan Tobacco Inc.

Reagents

JTE-907 [*N*-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide], SR144528 [*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2,2,1]heptan-2yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide] and 2-AG-E were synthesized at Japan Tobacco Inc. (Osaka, Japan). Prednisolone and acetone were obtained from Sigma–Aldrich (St. Louis, MO, USA). DNFB was purchased from Nacalai Tesque Inc. (Kyoto, Japan). 2-AG-E was dissolved in acetone. JTE-907, SR144528, and prednisolone were suspended in 0.5% methylcellulose, and administered orally at a volume of 10 ml/kg of body weight.

IgE-dependent triphasic cutaneous reaction in the mouse ear

The IgE-dependent triphasic cutaneous reaction in the mouse ear was induced as reported previously (Ray et al., 1983; Nagai et al., 1995; Tahara et al., 1999). In brief, mice were passively sensitized by the intravenous injection of 1 µg of mouse anti-DNP monoclonal IgE (Yamasa Shoyu, Chiba, Japan). Non-

sensitized-animals were similarly injected with saline. Twenty-four hours later, the cutaneous reaction was evoked by the painting of each surface of both ear lobes with 25 µl of a 0.15% DNFB solution in acetone–olive oil (3:1). Ear thickness was measured in a blind-manner with a thickness gauge (Mitutoyo Corporation, Kawasaki, Japan) just before and after the DNFB challenge and was expressed as the increase in ear thickness relative to the value measured immediately before exposure to DNFB. JTE-907, SR144528, and prednisolone were orally administered 1 h before the DNFB challenge (day 0) and once a day for 8 days from day 1 after the challenge.

Cannabinoid agonist-induced mouse ear swelling

Experiments were carried out by a method reported previously (Ueda et al., 2005). In brief, both surfaces of the left ears of mice were topically exposed to 2 mg/ear 2-AG-E (10 µl/site). Control mice were exposed to the vehicle (acetone) alone. The time-course change of ear thickness after exposure was measured with a thickness gauge just before and 1 h, 24 h, and 8 days after 2-AG-E treatment.

Statistics

Data are represented as means±S.E.M. Inter-group data for the IgE-mediated cutaneous reaction were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test or Student's *t*-test. The change in ear thickness between pre- and post-2-AG-E-exposed mice was assessed by a paired *t*-test with the Bonferroni correction. A probability of *p*<0.05 was considered to be significant.

Results

IgE-mediated cutaneous reaction in cannabinoid CB₂ receptor-deficient mice

Involvement of cannabinoid CB₂ receptors in the triphasic cutaneous reaction was investigated by using cannabinoid CB₂ receptor-deficient mice and their wild-type littermates. As shown in Fig. 1, a single epicutaneous challenge with DNFB elicited triphasic ear swelling in sensitized wild-type (C57BL/6) mice. Peak responses of the immediate phase reaction (IPR) and late phase reaction (LPR) were observed at 1 h and 24 h after DNFB challenge, respectively. The following very late-phase reaction (vLPR) starting on day 4 peaked on day 8. Importantly, no significant increases in ear thickness corresponding to IPR, LPR, and vLPR in the wild-type mice were observed in sensitized and challenged cannabinoid CB₂ receptor-deficient mice. Nonsensitized mice, which received an epicutaneous DNFB challenge without prior sensitization, did not exhibit any cutaneous reaction.

Effects of cannabinoid CB₂ receptor antagonist/inverse agonists on triphasic cutaneous reaction

To confirm the involvement of cannabinoid CB₂ receptors in the IgE-mediated cutaneous reaction in BALB/c mice, the

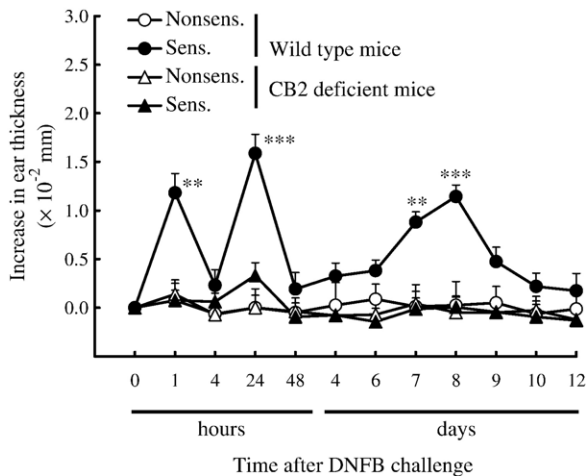


Fig. 1. Time-course study for the IgE-mediated cutaneous reaction in cannabinoid CB₂ receptor-deficient mice. Mice received an intravenous injection of 1 μ g of anti-DNP IgE 24 h before challenge with 0.15% DNFB. Results are presented as the increase in ear thickness relative to the value measured immediately before treatment. Data are expressed as means $\pm$ S.E.M. ($n=4$ or 8). **, $p<0.01$, ***, $p<0.001$ vs. Nonsensitized mice (Student's t -test). Sens.: sensitized, Nonsens.: nonsensitized.

effects of cannabinoid CB₂ receptor antagonist/inverse agonists, JTE-907 and SR144528, on the triphasic cutaneous reaction were examined. Results are shown in Fig. 2. Upon challenge with DNFB, ear swelling appeared triphasically with peak responses at 1 h, 24 h, and 8 days after the challenge in BALB/c mice; results were similar to those for C57BL/6 mice. Orally administered JTE-907 (0.01–10 mg/kg) showed a significant and dose-dependent inhibition of IPR, LPR, and vLPR. Similar results were obtained for SR144528 at 0.1–10 mg/kg.

Table 1

Changes in ear thickness caused by 2-AG-E in BALB/c and C57BL/6 mice

Time after treatment	BALB/c mice ($\times 10^{-2}$ mm)	C57BL/6 mice ($\times 10^{-2}$ mm)
Pre	18.91 $\pm$ 0.18	17.76 $\pm$ 0.12
1 h	20.46 $\pm$ 0.37**	21.67 $\pm$ 0.31***
24 h	22.31 $\pm$ 0.37***	19.56 $\pm$ 0.27***
8 days	21.05 $\pm$ 0.32***	19.40 $\pm$ 0.36**

2-AG-E (2 mg/ear) was topically applied to both surfaces of the left ear of mice. Data are expressed as means $\pm$ S.E.M. ($n=8$). **, $p<0.01$, ***, $p<0.001$ vs. Pre (paired t -test with Bonferroni correction).

Prednisolone, a reference drug, significantly inhibited all three phases of the response at 5 mg/kg of body weight.

Cutaneous reaction induced by 2-AG-E in BALB/c and C57BL/6 mice

Next, we examined the ear swelling induced by topical application of 2-AG-E in BALB/c and C57BL/6 mice. As IPR, LPR, and vLPR in the IgE-mediated cutaneous reaction appeared at 1 h, 24 h, and 8 days after the DNFB challenge, ear thickness was measured at the corresponding times after 2-AG-E treatment. For the BALB/c mice, apparent ear swelling was observed at 1 h, 24 h, and 8 days after the treatment, with values of 1.54 ± 0.33 , 3.39 ± 0.32 , and $2.14 \pm 0.26 \times 10^{-2}$ mm, respectively (Table 1). For the C57BL/6 mice, apparent ear swelling was also observed, and the values at 1 h, 24 h, and 8 days were 3.91 ± 0.27 , 1.80 ± 0.22 , and $1.64 \pm 0.32 \times 10^{-2}$ mm, respectively. No significant increase in ear thickness was observed in vehicle (acetone) treated BALB/c and C57BL/6 mice (data not shown).

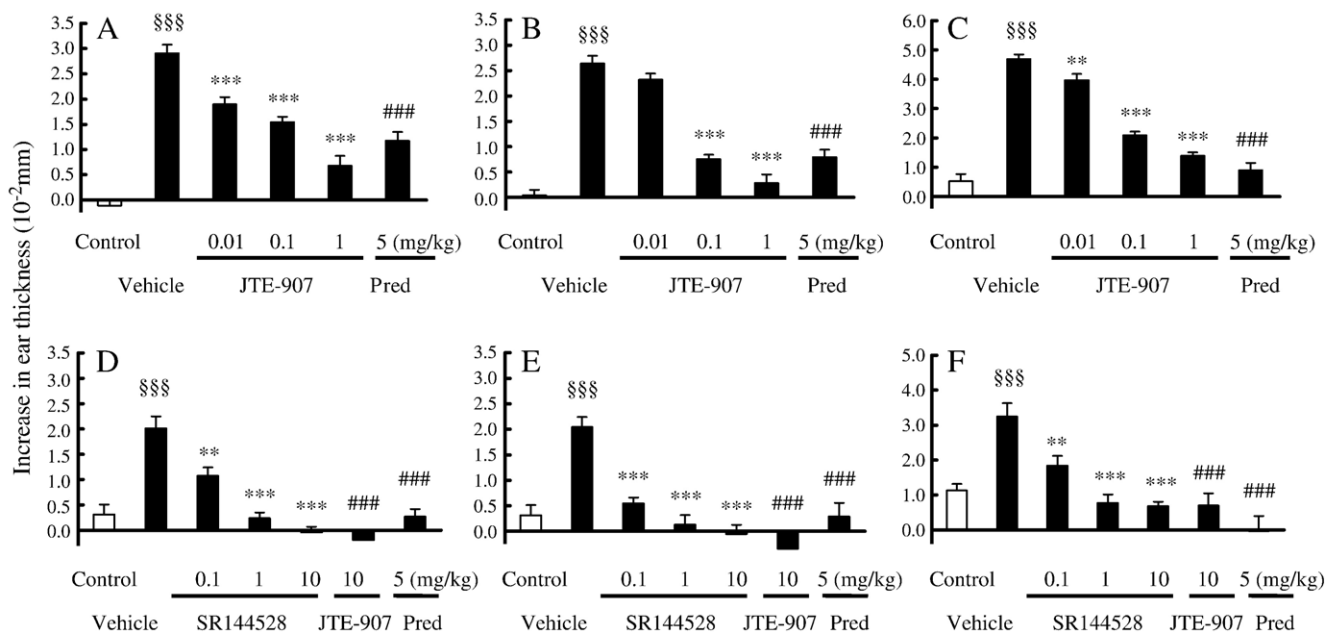


Fig. 2. Effects of cannabinoid CB₂ receptor selective antagonist/inverse agonists, JTE-907 and SR144528, and prednisolone (Pred) on the triphasic cutaneous reaction in BALB/c mice. Mice received an intravenous injection of 1 μ g of anti-DNP IgE 24 h before challenge with 0.15% DNFB. Vehicle, JTE-907, SR144528, and Pred were orally administered 1 h before the DNFB challenge (day 0), and once a day for 8 days from day 1 after exposure to DNFB. Ear thickness was measured in a blind manner before, 1 h (IPR: A and D), 24 h (LPR: B and E), and 8 days (vLPR: C and F) after the challenge by using a thickness gauge. Data are expressed as means $\pm$ S.E.M. ($n=8$). \$\$\$, $p<0.001$ vs. Control (Student's t -test). ###, $p<0.001$ vs. Vehicle (Student's t -test). **, $p<0.01$, ***, $p<0.001$ vs. Vehicle (Dunnett's test).

Discussion

In the present study, we demonstrated that the IgE-mediated triphasic cutaneous reaction, which was induced by passive sensitization with anti-DNP IgE following DNFB challenge, could be induced in C57BL/6 as well as in BALB/c mice, and that the triphasic reaction was drastically attenuated in cannabinoid CB₂ receptor-deficient mice with the C57BL/6 background. Furthermore, we confirmed that cannabinoid CB₂ receptor selective antagonist/inverse agonists, JTE-907 and SR144528, significantly inhibited the three phases of the cutaneous response mediated by IgE in BALB/c mice. These results indicate that cannabinoid CB₂ receptors are involved in the IgE-mediated cutaneous reaction in both C57BL/6 and BALB/c mice.

Single and topical application of DNFB to the ears of mice systemically sensitized with anti-DNP IgE is known to induce a triphasic cutaneous reaction in BALB/c mice (Tahara et al., 1999; Inagaki et al., 2005). In the present study, we demonstrated that the IgE-mediated triphasic cutaneous reaction was also evoked in C57BL/6 mice. The first phase reaction, IPR is absent in mast cell-deficient WBB6F1-W/W^v mice (Ray et al., 1983; Nagai et al., 1995) and inhibited by antihistamines (Nagai et al., 1995; Inagaki et al., 1998) and platelet-activating factor (PAF) receptor antagonists (Yamaguchi et al., 1998; Yamada et al., 2000). This demonstrates that the IPR is dependent on mast cells and mast cell-derived mediators, histamine and PAF. PAF induces the rapid generation of endogenous cannabinoids, such as 2-AG, from mouse macrophages via a PAF receptor-dependent mechanism (Berdyshev et al., 2001; Liu et al., 2003). Endogenous cannabinoids are also rapidly produced from IgE-sensitized mast cells by antigen stimulation (Di Marzo et al., 2000). Therefore, it may be possible that endogenous cannabinoids are rapidly produced in the ear of sensitized mice upon challenge with DNFB, and that they may participate in the induction of all phases of the IgE-mediated triphasic cutaneous reaction in both BALB/c and C57BL/6 mice. In fact, a single application of 2-AG-E to the ears of C57BL/6 mice as well as BALB/c mice resulted in an apparent ear swelling observed at 1 h, 24 h, and 8 days after the treatment, which corresponded to the IPR, LPR, and vLPR, respectively, in the triphasic cutaneous reaction. A similar result has also been reported for ICR mice, although measurements of the cutaneous reaction induced by 2-AG and 2-AG-E were not performed at 24 h and 8 days after the application (Oka et al., 2005). Moreover, some investigators, including our own group, have previously suggested that cannabinoid CB₂ receptors are involved in the 2-AG-E-induced cutaneous reaction (Oka et al., 2005; Ueda et al., 2005). In this study, we also found that cannabinoid CB₂ receptor antagonist/inverse agonists, JTE-907 and SR144528, potently suppressed all three phases of the cutaneous response. The mechanism of the cannabinoid CB₂ receptor antagonist/inverse agonists' suppressive action may relate to their antagonist action against endogenous cannabinoids.

The IPR reaction peaked at 1 h after the challenge in the triphasic cutaneous reaction for both BALB/c and C57BL/6 mice. Nitric oxide (NO) seems to be involved in the IPR, and the mouse ear treated with an NO donor exhibits a potent edematous reaction

(Musoh et al., 1998). On the other hand, Oka et al. (2005) reported the involvement of NO in 2-AG-induced ear swelling that peaked at 1 h after the treatment. Furthermore, 2-AG is reported to induce NO in certain types of cells in vitro (Stefano et al., 2000; Bisogno et al., 2001). In addition, the cannabinoid CB₂ receptor is widely expressed in several cell types in the skin, such as epidermal keratinocytes, and macrophages, and mast cells (Facci et al., 1995; Casanova et al., 2003; Samson et al., 2003; Stander et al., 2005). Therefore, the effect of selective antagonist/inverse agonists of the cannabinoid CB₂ receptor to suppress the IPR may result from reduced NO production by skin cells stimulated with endogenous cannabinoids.

The IPR is characterized by a rapid increase in capillary permeability; whereas, the LPR and vLPR are represented by skin thickening with significant infiltration of mononuclear cells, eosinophils, and other inflammatory cells (Nagai et al., 1995; Tahara et al., 1999). 2-AG induces the migration of human macrophage-like cell lines and peripheral blood monocytes. The 2-AG-induced migration is involved chemotaxis and chemokinesis, and is inhibited by pre-treatment of the cells with SR144528, which suggests the involvement of cannabinoid CB₂ receptors in the 2-AG-induced migration (Kishimoto et al., 2003; Walter et al., 2003). 2-AG induces the migration of mouse splenocytes and eosinophilic leukemia cells as well as monocytes, which is also blocked by SR144528 pre-treatment (Jorda et al., 2002; Oka et al., 2004). Lunn et al. (2006) recently reported that cannabinoid CB₂ receptor-selective inverse agonists suppress cell recruitment against cannabinoid agonists in vivo, and that they reduced the number of eosinophils in the bronchoalveolar lavage fluid in a murine model of allergic asthma. It is therefore possible that the recruitment of inflammatory cells involves cannabinoid CB₂ receptor-mediated cell migration caused by endogenous cannabinoids produced in the mouse ear by the LPR and vLPR. These findings suggest that the suppressive effects of cannabinoid CB₂ receptor antagonist/inverse agonists on the LPR and vLPR may be related to the inhibitory effects of inflammatory cell trafficking mediated through cannabinoid CB₂ receptors.

In conclusion, the present study revealed that cannabinoid CB₂ receptors participate in the induction of three phases of the IgE-dependent triphasic cutaneous reaction in mice, and that cannabinoid CB₂ receptor selective antagonist/inverse agonists, JTE-907 and SR144528, potently inhibited these three phases. These results suggest that cannabinoid CB₂ receptor selective antagonist/inverse agonists such as JTE-907 and SR144528 are potentially useful for the treatment of IgE-mediated allergic dermatitis represented by atopic dermatitis.

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