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Short communication

Intradermal leukotriene B_4, but not prostaglandin E_2, induces itch-associated responses in mice

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Abstract

The itch-associated responses induced by intradermal injection of leukotriene B_4 and prostaglandin E_2 were studied in mice. Leukotriene B_4 (0.001–1 nmol/site) elicited scratching of the injected site; the dose–response curve was bell-shaped with a peak effect at 0.03 nmol/site. The effect of leukotriene B_4 (0.03 nmol/site) started within 3 min, peaked in the second 10-min period, had almost subsided by 30 min, and was inhibited by the simultaneous injection of the leukotriene B_4 receptor antagonist ONO-4057, 5-[2-(2-carboxyethyl)-3-(6-(*p*-methoxyphenyl)-5*E*-hexenyl) oxyphenyoxy] valeric acid. Prostaglandin E_2 (0.003–300 nmol/site) did not significantly elicit scratching. The results raise the possibility that leukotriene B_4 is an endogenous itch mediator in the skin.

Keywords: Leukotriene B_4; Prostaglandin E_2; Itch; Scratching; (Mouse); Intradermal injection; Leukotriene B_4 receptor antagonist

1. Introduction

Although pruritus is a common complaint and severe pruritus is an important issue related to the quality of life, the details of mechanisms and endogenous mediators of pruritus are unclear. Concerning the involvement of the arachidonic acid cascade in pruritus, an intradermal injection of prostaglandin E_2 is pruritogenic (Hägermark and Strandberg, 1977) and prolongs experimentally induced itching (Hägermark and Strandberg, 1977; Fjellner and Hägermark, 1979). However, although aspirin was reported to be effective against pruritus in polycythemia vera (Fjellner and Hägermark, 1979), non-steroidal antiinflammatory drugs, that is, inhibitors of cyclooxygenase, do not generally affect itching (Daly and Shuster, 1986; Fjellner and Hägermark, 1982). With regard to lipoxygenase products, there have been few reports on the pruritogenic activity of leukotrienes (Camp et al., 1983). Leukotriene B_4 was reported to be increased in the skin of pruritus patients (Brain et al., 1984; Ruzicka et al., 1986), and pruritus was shown to be inhibited by azelastin (Kanai et al., 1995; Matsui et al., 1994), an agent which inhibits the production and action of leukotriene B_4. These findings suggest the possibility that leukotriene B_4 is pruritogenic and/or enhances pruritus. Thus, in the present experiments, we examined whether the intradermal injection of prostaglandin E_2 and leukotriene B_4 would be pruritogenic in mice.

2. Materials and methods

2.1. Materials

Leukotriene B_4 and ONO-4057 (5-[2-(2-carboxyethyl)-3-(6-(*p*-methoxyphenyl)-5*E*-hexenyl) oxyphenoxy] valeric acid), an leukotriene B_4 receptor antagonist (Kishikawa et al., 1991; Yokomizo et al., 1997), were gifts from Ono Pharmaceutical (Osaka, Japan). Prostaglandin E_2 was purchased from Sigma (St. Louis, MO, USA). ONO-4057 and prostaglandin E_2 were dissolved in dimethylsulfoxide and ethanol, respectively, and then diluted with physiological saline. The sodium salt of leukotriene B_4 was dissolved in physiological saline. These agents were injected intradermally in a volume of 50 μl into the rostral part of the back.

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2.2. Behavioral observation

Male ICR mice 5–6 weeks of age were used in the experiments. They were housed under controlled temperature (23–25°C) and light (lights on from 08:00 to 20:00). Food and water were freely available. The hair was clipped over the rostral part of the mouse back. Before the experiments, the mice were put into an acrylic cage ($26 \times 18 \times 30$ cm) composed of 4 cells for at least 1 h for acclimation. Immediately after intradermal injection, they were put back into the same cell and videotaped with no one present. Scratching of the injected site with the hind paws was counted as an index of itch response (Kuraishi et al., 1995).

2.3. Data processing

All data are presented as means and S.E. Statistical significance was analyzed using the one-way analysis of variance followed by Dunnett's multiple comparisons; $P < 0.05$ was considered significant.

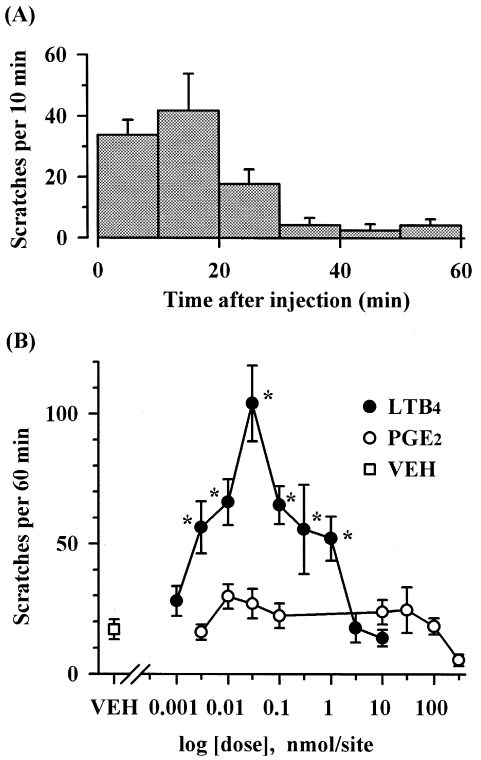

Fig. 1. Scratching following intradermal injection of leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) in mice. (A) Time-course of scratching induced by LTB_4 (0.03 nmol/site). (B) Dose–response curves for the scratch-inducing effect of LTB_4 and PGE_2. The mice were given an intradermal injection of LTB_4 ($n = 8$–16), PGE_2 ($n = 7$–8) or physiological saline (VEH, $n = 8$). Values represent the means and S.E. $* P <$ 0.05 when compared with VEH.

3. Results

3.1. Effects of leukotriene B_4 and prostaglandin E_2

Fig. 1A shows the time-course of scratching behavior for 60 min after the injection of leukotriene B_4 (0.03 nmol/site). Scratching was first observed within 3 min after injection in all mice examined and then appeared intermittently. The effect of leukotriene B_4 peaked in the second 10-min period and had almost subsided by 30 min. Leukotriene B_4 at intradermal doses of 0.001–1 nmol/site elicited significant scratching; the dose–response curve was bell-shaped with a peak effect after 0.03 nmol/site (Fig. 1B). Higher doses of 3 and 10 nmol/site were without significant effects. Intradermal injections of prostaglandin E_2 at doses of 0.003–300 nmol/site did not significantly elicit scratching (Fig. 1B). No changes in gross behaviors other than scratching were observed after these doses of leukotriene B_4 and prostaglandin E_2.

3.2. Effect of leukotriene B_4 receptor antagonist on leukotriene B_4-induced scratching

To determine whether leukotriene B_4-induced scratching was mediated by leukotriene B_4 receptors, the leukotriene B_4 receptor antagonist, ONO-4057, was injected intradermally together with leukotriene B_4 at the most effective dose, 0.03 nmol/site. ONO-4057 at doses of 0.003–0.03 nmol/site dose dependently ($F(3,31) = 23.1$, $P < 0.01$) suppressed the leukotriene B_4-induced scratching (Fig. 2).

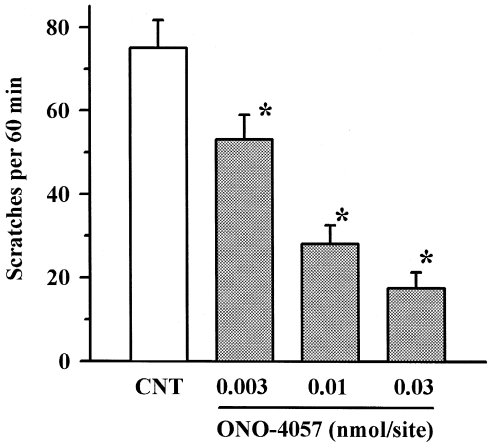

Fig. 2. Suppressive effects of the leukotriene B_4 (LTB_4) receptor antagonist ONO-4057 on LTB_4-induced scratching. LTB_4 (0.03 nmol/site) was injected intradermally alone (CNT) or together with ONO-4057. Values are the means and S.E. for eight animals. $* P < 0.05$ when compared with CNT.

4. Discussion

Intradermal injections of leukotriene B_4 into mice clearly elicited scratching, which may be an itch-associated response (Kuraishi et al., 1995). The action of leukotriene B_4 (0.03 nmol/site) was inhibited dose dependently by the leukotriene B_4 receptor antagonist, ONO-4057 (0.003–0.03 nmol/site), suggesting the mediation of leukotriene B_4 receptors. Intradermal injection of leukotriene B_4 (0.15–1.5 nmol/site) was reported to produce persistent itching in one of four healthy human subjects (Camp et al., 1983). Leukotriene B_4 (30 nmol/site) applied to the conjunctiva does not elicit scratching in the guinea-pig (Woodward et al., 1995). Although these findings apparently contradict the view that leukotriene B_4 is pruritogenic, it should be noted that, in the present experiments, the effective dose (0.001–1 nmol/site with a peak dose of 0.03 nmol/site) was relatively low and that the dose–response curve was bell-shaped. Thus, it is possible that, at lower doses, leukotriene B_4 is pruritogenic in human subjects and in other animals also.

The precise mechanisms of the scratch-inducing action of leukotriene B_4 remain to be settled. One possible explanation is that leukotriene B_4 acts directly on primary afferent terminals. Itch signals may be mediated mainly by C fibers (Schmelz et al., 1997) and leukotriene B_4 sensitizes cutaneous C-fiber nociceptors of the rat (Martin et al., 1988). Another explanation is that leukotriene B_4 acts on leukocytes or cutaneous cells (Camp et al., 1984; Iwamoto et al., 1993) to release pruritic mediators. Leukotriene B_4 is a potent leukocyte chemoattractant, but this type of action may not be the main cause of scratching, because the onset of the leukotriene B_4-induced scratching response was relatively rapid.

As mentioned in Section 1, leukotriene B_4 is increased in the skin of patients with pruritus such as in atopic dermatitis and psoriasis (Brain et al., 1984; Ruzicka et al., 1986). Azelastin, an inhibitor of leukotriene B_4 production and leukotriene B_4 receptors, alleviates pruritus of chronic hemodialysis patients (Kanai et al., 1995; Matsui et al., 1994). With these findings taken into account, the present results raise the possibility that leukotriene B_4 is involved in itching of some pruritic diseases.

In the present experiments, intradermal injection of prostaglandin E_2 did not significantly elicit scratching, suggesting that prostaglandin E_2 itself does not produce itching in mice. In human subjects, prostaglandin E_2 itself elicits itch and prolongs the itching induced by histamine and serotonin (Fjellner and Hägermark, 1979; Hägermark and Strandberg, 1977). As part of this prostaglandin E_2 action is claimed to be mediated by histamine release (Hägermark and Strandberg, 1977), pruritogenic activity might be partly dependent on the histamine-releasing capacity and/or histamine sensitivity of the skin. In this context, mouse skin is less sensitive to histamine than is human skin (Kuraishi et al., 1995).

In conclusion, intradermal leukotriene B_4 induced scratching at relatively low doses via leukotriene B_4 receptors, suggesting that it is an endogenous itch mediator in the skin. Leukotriene B_4 may be involved at least partly in the itching of some pruritic diseases.

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