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Vascular permeability in allergic conjunctivitis in mice lacking histamine H_1 receptors

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Abstract

To clarify the role of histamine H_1 receptors in allergic conjunctivitis, changes in vascular permeability of the conjunctiva were measured in histamine H_1 receptor deficient mice. Wild-type mice showed a significant increase in vascular permeability of the conjunctiva induced by histamine. However, no such increase was found in histamine H_1 receptor deficient mice. On the other hand, no differences were observed between wild-type and histamine H_1 receptor deficient mice in response to serotonin. A significant increase in vascular permeability was observed in actively sensitized wild-type mice, whereas no increase was observed in histamine H_1 receptor deficient mice. Similar findings were noted in passively sensitized animals. Histamine contents of the conjunctiva were significantly decreased by topical application of antigen in both wild-type and histamine H_1 receptor deficient mice after active sensitization with antigen. These findings suggested that vascular permeability in the conjunctiva in allergic conjunctivitis is entirely regulated through histamine H_1 receptor.

Keywords: Allergic conjunctivitis; Vascular permeability; Histamine H_1 receptors; 5-HT (5-hydroxytryptamine, serotonin); Histamine

1. Introduction

Recently, we developed new experimental models for allergic conjunctivitis by topical application of histamine in non-sensitized guinea pigs and of antigen in sensitized guinea pigs (Kamei et al., 1991a, 1995; Takada et al., 2000). Histamine was found to play a dominant role in the process leading to allergic conjunctivitis as histamine was released from the conjunctival tissues of sensitized animals when conjunctivitis was induced by topical application of specific antigen, and this allergic conjunctivitis was significantly inhibited by various histamine H_1 receptor antagonists (Woodward et al., 1986a, 1989; Calonge et al., 1990; Kamei et al., 1991a, 1995). Histamine H_1 receptor antagonists including chlorpheniramine are widely used for the clinical treatment of allergic conjunctivitis. On the other hand, it has been reported that microvascular permeability in allergic conjunctivitis was inhibited by pretreatment with histamine H_1 receptor antagonists, but complete inhibition was not observed (Woodward et al., 1986a, 1989; Calonge et al., 1990; Kamei et al., 1991a, 1995). These results suggested that histamine H_2 receptors are also involved in histamine-induced changes in microvascular permeability, because combinations of histamine H_1 and H_2 receptor antagonists completely inhibited vascular permeability (Mortillaro et al., 1981; Woodward and Ledgard, 1986; Woodward et al., 1986b). However, receptor antagonists have many pharmacological actions due to interaction with a wide variety of molecules, including non-target receptors.

In the present study, therefore, to clarify the role of histamine H_1 receptors in allergic conjunctivitis, we used histamine H_1 receptor deficient mice.

2. Materials and methods

2.1. Animals

Histamine H_1 receptor deficient mice were generated by homologous recombination as described (Inoue et al., 1996). Male histamine H_1 receptor deficient and wild-type

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mice weighing 20–25 g were used. Both strains were bred in our laboratory and housed in a temperature-controlled room at 24 ± 2°C with 55 ± 15% humidity and were given food and water ad libitum.

2.2. Reagents

Ovalbumin was obtained from Sigma (St. Louis, MO, USA). Pertussis toxin was purchased from Research Biochemicals International (Natick, MA, USA). Mouse anti-dinitrophenol immunoglobulin E (IgE) and dinitrophenol-ovalbumin were obtained from Seikagaku Kogyo (Tokyo, Japan). All other chemicals used in the study were of the highest quality commercially available.

2.3. Antigen and immunization

The histamine H_1 receptor deficient and wild-type mice were given an intraperitoneal injection of ovalbumin (100 μg), aluminum hydroxide gel (alum; 1 mg) and pertussis toxin (300 ng) (Oettgen et al., 1994). Five days later, they received a booster injection of 50 μg of ovalbumin alone subcutaneously in the back. Eighteen days after the first immunization, the mice were used as actively sensitized animals. To prepare IgE-dependent passive anaphylaxis in the conjunctiva, phosphate buffered saline with or without 2 μg of mouse anti-dinitrophenol IgE was injected into the tail vein of each mouse. Twenty-four hours later, these mice were used as passively sensitized animals.

2.4. Measurement of vascular permeability

Vascular permeability in the conjunctiva was estimated using Evans blue dye as previously described (Yamaji et al., 1997). Animals were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). Ten minutes after intravenous injection of 1% Evans blue dye solution (0.05 ml/kg), 5 μl of ovalbumin solution (150 mg/ml in phosphate buffered saline, for actively sensitized animals), dinitrophenol-ovalbumin solution (100 mg/ml in phosphate buffered saline, for passively sensitized animals), histamine or serotonin solution (20 mg/ml in phosphate buffered saline) were applied to the eyes. Thirty minutes after topical instillation, the conjunctiva (bulbar and palpebral conjunctiva) was carefully removed and weighed. Evans blue dye contents in the conjunctiva were extracted with 0.1 ml of 1 N KOH for 12 h at 37°C. Then, 0.9 ml of H_3PO_4-acetone mixture (H_3PO_4: acetone = 5:13) was added and mixed well. After centrifugation at 400 × *g* for 20 min, the amounts of extracted dye in the supernatant were determined with a spectrophotometer (Model U-2000, Hitachi, Tokyo, Japan) at 620 nm. The results are indicated as ng of Evans blue dye per mg wet tissue of the conjunctiva and expressed as the value obtained by subtracting the dye content before challenge.

2.5. Passive cutaneous anaphylaxis reaction

The IgE titer in the serum was determined by passive cutaneous anaphylaxis reaction. Blood specimens were obtained from the tail vein. The serum samples were stored at −20°C until use. Serial dilutions of serum obtained from sensitized rats were injected intradermally in volumes of 0.1 ml into the shaved backs of normal rats (200–220 g). After 48 h, the rats were challenged with intravenous injection of 1 ml/animal of physiological saline containing 2 mg of ovalbumin and 10 mg of Evans blue into the tail vein. After 30 min, the rats were sacrificed, the dorsal back skin was peeled off, and the diameter of the blue spot on the underside of the skin was measured. The passive cutaneous anaphylaxis titer was expressed as the reciprocal of the maximum dilution of the antiserum that gave a positive reaction of more than 5 mm in diameter on the dorsal skin.

2.6. Measurement of histamine contents in the conjunctiva

Thirty minutes after antigen application, the mice were sacrificed under ether anesthesia, the conjunctiva was carefully excised, weighed as described above and washed twice with ice-cold phosphate buffered saline. The tissues were homogenized in 1 ml of 0.4 N perchloric acid with a Polytron® (Kinematica, Lucerne, Switzerland) on ice, and the homogenates were placed in an ice-bath for 1 h. After centrifugation at 10,000 × *g* for 30 min at 4°C, the histamine contents of the supernatant were measured by high-performance liquid chromatography (HPLC) system (CCP and 8010, Tosoh, Tokyo, Japan) with a fluorometric detector (Model FT-1050, Hitachi, Tokyo, Japan) as previously described (Yamaji et al., 1997). The results are expressed as ng of histamine per mg wet tissue of the conjunctiva.

2.7. Data analysis

Data are expressed as means ± S.E.M. The significance of differences between the means of the test and control groups was evaluated using analysis of variance with Dunnett's test. Probability values less than 0.05 were considered significant.

3. Results

3.1. Absence of vascular permeability induced by histamine in histamine H_1 receptor deficient mice

At first, we investigated whether histamine H_1 receptor deficient mice caused an increase in vascular permeability by serotonin contained in rat mast cells. As a result, topical application of serotonin caused a significant increase in

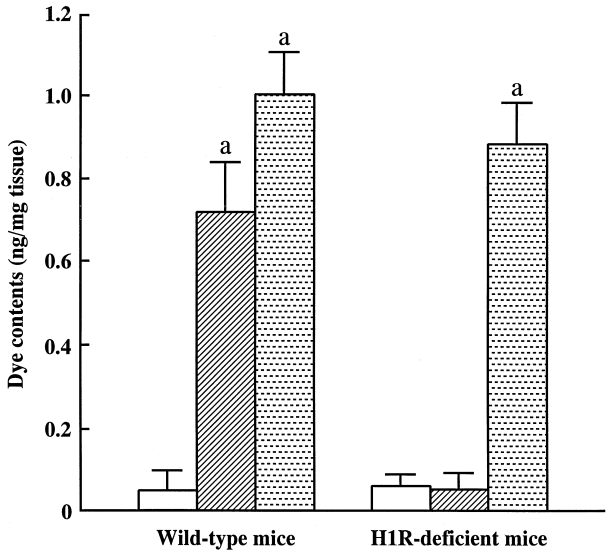

Fig. 1. Effects of histamine and serotonin on conjunctival dye content in the conjunctiva of wild-type and histamine H_1 receptor deficient mice. After intravenous injection of Evans blue, conjunctivitis was induced by topical instillation of phosphate buffered saline (open columns), histamine (hatched columns) or serotonin (stippled columns). Thirty minutes after instillation, the leaked conjunctival dye contents in both wild-type and histamine H_1 receptor deficient mice were determined. Each value represents the mean ± S.E.M. for five mice. [a]: Significantly different from control at $P < 0.01$.

dye content in the conjunctiva in both wild-type and histamine H_1 receptor deficient mice.

However, no increase in dye content was observed in histamine H_1 receptor deficient mice when histamine was applied topically, although a significant increase was noted in wild-type animals (Fig. 1).

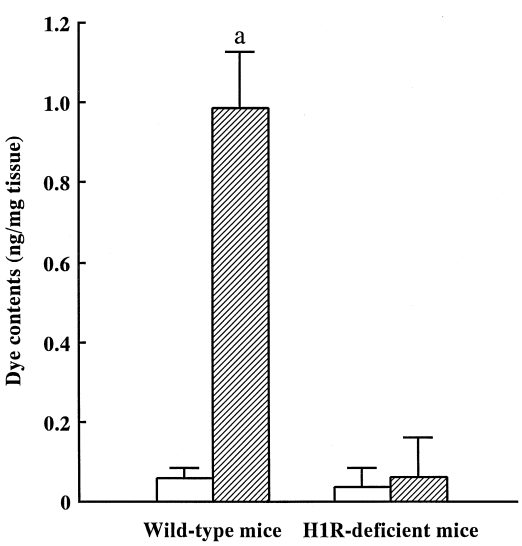

Fig. 2. Antigen-induced increase in dye content in the conjunctiva of actively sensitized wild-type and histamine H_1 receptor deficient mice. Mice were actively immunized with ovalbumin, conjunctivitis was induced by topical instillation of ovalbumin. Thirty minutes after instillation of phosphate buffered saline (open columns) or ovalbumin (hatched columns), the leaked dye content was measured. Each value represents the mean ± S.E.M. for five mice. [a]: Significantly different from control at $P < 0.01$.

Table 1
Changes in histamine content in the conjunctiva after instillation of antigen (ovalbumin) in actively sensitized mice

Animals	*N*	Histamine contents (μg/mg) tissue)		Histamine release
		Control	Ovalbumin (750 μg/site)	
Wild-type mice	5	15.9 ± 0.7	10.5 ± 0.8[a]	33.6%
Histamine H_1 receptor deficient mice	5	21.4 ± 0.4	14.6 ± 1.8[a]	31.7%

Mice were actively sensitized with ovalbumin and conjunctivitis was induced by application of ovalbumin. Each value represents mean ± S.E.M. for five mice.
[a] Significantly different from control at $P < 0.05$.

3.2. Absence of vascular permeability induced by antigen in actively sensitized histamine H_1 receptor deficient mice

Topical installation of specific antigen (ovalbumin) caused a significant increase in dye content in the conjunctiva of actively sensitized wild-type mice, whereas no such increase was detected in actively sensitized histamine H_1 receptor deficient mice (Fig. 2).

3.3. Histamine release in the conjunctiva of actively sensitized histamine h_1 receptor deficient mice

To determine whether topical instillation of ovalbumin causes histamine release from mast cells in conjunctival tissue, histamine contents in the conjunctiva were deter-

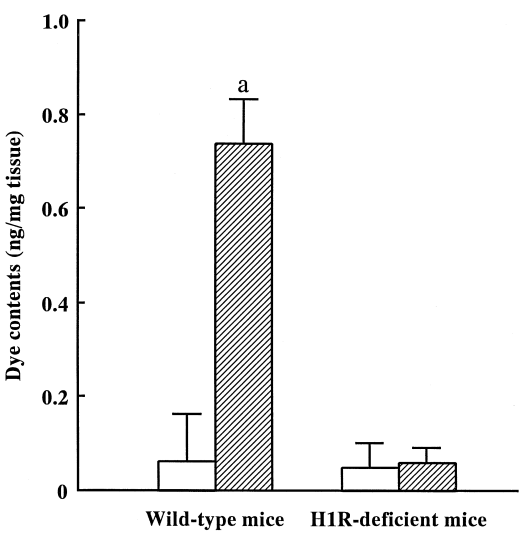

Fig. 3. Antigen-induced increase in dye content of passively sensitized wild-type and histamine H_1 receptor deficient mice. Mice were sensitized by injection of anti-dinitrophenol-IgE and vascular permeability was induced by instillation of dinitrophenol-ovalbumin. Each value represents the mean ± S.E.M. for five mice. [a]: Significantly different from control at $P < 0.01$.

mined as shown in Table 1. Approximately 30% of histamine in the conjunctiva was released after instillation of specific antigen in both wild-type and histamine H_1 receptor deficient mice.

3.4. Absence of vascular permeability induced by specific antigen in passively sensitized histamine h_1 receptor deficient mice

To clarify the role of histamine H_1 receptors in the IgE-dependent passive anaphylaxis in this conjunctivitis model, the mice were administered mouse anti-dinitrophenol IgE. As shown in Fig. 3, the increase in dye content induced by specific antigen dinitrophenol-ovalbumin was significantly increased in wild-type mice, but no observable changes were noted in histamine H_1 receptor deficient mice.

4. Discussion

An increase in vascular permeability by vasoactive mediators released from mast cells is a hallmark of anaphylaxis reaction (Holgate, 1991; Stevens and Austen, 1989). Various histamine H_1 receptor antagonists have been shown to partially inhibit the vascular permeability in conjunctival immediate hypersensitivity induced by specific antigen (Woodward et al., 1986a, 1989; Calonge et al., 1990; Kamei et al., 1991a, 1995). In rodent mast cells, not only histamine but also serotonin is stored in mast cell granules and released by specific IgE- and non-IgE-stimuli (Maling et al., 1974). In this study, we first compared the effects of histamine and serotonin on vascular permeability in the conjunctiva of histamine H_1 receptor deficient mice. As shown in Fig. 1, histamine H_1 receptor deficient mice showed a significant increase in vascular permeability induced by serotonin, but no such increase was found when histamine was applied topically. This result strongly indicated that histamine-induced vascular permeability in the conjunctiva occurred through activation of histamine H_1 receptor.

Next, we tested the changes in vascular permeability of the conjunctiva in actively sensitized mice. In this study, alum and pertussis toxin were used as adjuvants, because in rodents both compounds have been shown to induce high levels of production of specific IgE by enhancement of interleukin-4 production (Munoz and Peacock, 1990; Mu and Sewell, 1993). Animals were challenged by topical application of ovalbumin after 18 days to induce active sensitization. At the same time, the serum IgE titers of wild-type and histamine H_1 receptor deficient mice were estimated by the 48-h heterologous passive cutaneous anaphylaxis reaction in normal rats (Kamei et al., 1991b). The serum IgE titers were increased by this immunization and no differences were observed between wild-type and histamine H_1 receptor deficient mice (data not shown). The topical instillation of specific antigen (ovalbumin) caused no apparent changes in vascular permeability in the conjunctiva of actively sensitized histamine H_1 receptor deficient mice (Fig. 2). To determine whether the topical instillation of ovalbumin causes histamine release from mast cells in the conjunctival tissue, histamine contents in the conjunctiva were determined as shown in Table 1. The instillation of specific antigen caused a significant decrease in histamine contents in the conjunctiva not only in wild-type but also in histamine H_1 receptor deficient mice. Total histamine content of histamine H_1 receptor deficient mice before antigen challenge was higher than that of wild-type mice. At present, we have no explanation for this finding. These results clearly indicated that the topical instillation of specific antigen caused histamine release from conjunctival mast cells of both actively sensitized wild-type and histamine H_1 receptor deficient mice, and subsequent microvascular permeability of the conjunctiva was evidently increased in wild-type but not in histamine H_1 receptor deficient mice. In addition, to confirm the role of histamine H_1 receptor in IgE-dependent passive anaphylaxis in conjunctivitis, the mice were sensitized by administration of mouse anti-dinitrophenol IgE. As a result, similar to actively sensitized mice, the increase in the vascular permeability induced by specific antigen dinitrophenol-ovalbumin was not observed in histamine H_1 receptor deficient mice.

On the other hand, chemical mediators other than histamine such as platelet activating factor, prostaglandins, leukotrienes and substance P have also been suggested to participate on antigen-induced conjunctivitis (Gray et al., 1988; Proud et al., 1990; Holgate, 1991; Yamaji et al., 1997). However, the roles of these chemical mediators were almost negligible. Although prostacyclin content in the tears was increased by application of antigen this prostacyclin release was thought to be mediated by histamine H_1 receptor (Helleboid et al., 1991). Taken together, the above findings and the results of the present study clearly indicated that the changes in vascular permeability in allergic conjunctivitis are completely regulated by histamine released from activated mast cells through histamine H_1 receptor.

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