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ORIGINAL ARTICLE

Role of the Hypothalamo-Pituitary-Adrenal Axis in the Modulation of Pollinosis Induced by Pollen Antigens

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ABSTRACT

Background: To clarify the mechanism of stress-induced modification of allergic diseases, we studied the effect of restraint stress on plasma levels of cytokines and the symptoms of pollinosis in mice.

Methods: The effects of restraint stress and the role of the hypothalamo-pituitary-adrenal axis (HPA-axis) in the development of pollen antigen-induced pollinosis were studied in control, hypophysectomized, adrenalectomized or ACTH-administered mice. Twenty days after sensitization, animals were subjected to mild restraint stress for 3 hours, and plasma levels of IFN-γ, IL-10, and IgE were measured. We analyzed the incidence of sneezing and nasal rubbing in the sensitized animals.

Results: Plasma levels of IL-10 and IgE increased in the sensitized animals with a concomitant increase in the incidence of sneezing and nasal rubbing. The increases in plasma IgE, IL-10 and the incidence of sneezing and nasal rubbing were suppressed by restraint stress. Adrenalectomy increased IFN-γ, inhibited the increase in plasma IL-10 and IgE, and suppressed the incidence of sneezing. In contrast, hypophysectomy increased plasma levels of IL-10, IFN-γ, and IgE and the incidence of sneezing. Intraperitoneal administration of ACTH decreased IL-10 in plasma but increased IFN-γ and suppressed the incidence of nasal rubbing.

Conclusions: The present findings show that the HPA-axis and ACTH play important roles in the regulation of plasma cytokines and IgE thereby modulating symptoms of pollinosis. The results also suggest that a mild restraint stress suppresses the increase in Th2-dependent cytokines and IgE to reduce the symptoms of pollinosis.

KEY WORDS

ACTH, HPA axis, IgE, pollinosis, stress

INTRODUCTION

The clinical symptoms of allergic diseases including pollinosis and asthma decrease the quality of life, affecting areas such as work productivity and school performance.[1] The prevalence of allergic diseases including pollinosis and asthma has been increasing in various countries.[2] Pollen from the Japanese cedar (Cryptomeria japonica) is one of the major allergens; more than 10% of Japanese have been suffering from pollinosis.[2,3] Pollinosis is a type I allergic disease mainly induced by Japanese cedar or Japanese cypress pollens and is characterized by an enhanced production of IgE, release of histamine and leukotrienes from mast cells, and infiltration of eosinophils in the nasal mucosa.

Various stresses have been reported to affect the symptomatic manifestations of allergic diseases, such as atopic dermatitis, asthma, and allergic rhinitis, by activating the HPA-axis that modulates the balance between Th1- and Th2-type immune responses.[4-6] Both ACTH and glucocorticoids have been reported to play important roles in the modulation of stress responses and immunological reactions.[7] ACTH has been known to inhibit INF-γ thereby promoting Th2-type immune reactions.[8-12] Glucocorticoids have been reported to increase Th2-type cytokines but suppress Th1-type cytokines and enhance the Th2-type immune reactions including IgE production.[13,14] However, various stresses have been known to have dif-

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ferent effects on symptoms of allergic diseases depending on their types, strength and timing.[15-26] In fact, the symptoms of allergic dermatitis can be aggravated by a strong stress but suppressed by a mild stress.[27,28] To clarify the mechanism of stress-induced modification of allergic diseases, we studied the effect of restraint stress on plasma levels of various cytokines and the symptom of rhinitis observed in mice sensitized with pollen antigens.

METHODS

ANIMALS

Eight-week-old male BDF-1 mice (control) and hypophysectmized, adrenalectomized or sham-operated mice were purchased from Japan SLC (Shizuoka, Japan). These mice were housed under standard environmental conditions (3-6 mice per cage) at 22-24℃ under a 12 : 12 hour light-dark cycle, and fed laboratory chow and water *ad libitum*. The adrenalectomized mice were given a half-saline solution. Animal experiments were carried out according to the Animal Care and Use Committee of the Osaka City University Medical School.

SENSITIZATION OF MICE

Mice were intraperitoneally injected with 1 μg of pollen allergen (Cry j 1, Hayashibara, Okayama, Japan) mixed with 0.3 ml of aluminum hydroxide gel (Sigma Chemical, St. Louis, MO, USA) on days 0 (first day) and 4. Then, 5 μg of pollen antigen was administered into the bilateral nasal cavities once per day (from day 10 to 16).

ADMINISTRATION OF ACTH

From day 0 to 21, mice were injected intraperitoneally with 0.3 ml of physiological saline containing 57 ng of ACTH 1-24 (Wako Pure Chemical Industries, Osaka, Japan) three times a week as described previously.[29]

STRESS EXPERIMENTS

On day 21, each mouse was placed for 3 hours in a 50 ml conical tube with multiple ventilation holes as described previously.[30] The restraint stress allowed mice to rotate from a supine to prone position, but not to turn their heads toward their tails, not to take food and water. Control mice were not exposed to the restraint stress but deprived of food and water for the same duration.

ANALYSIS OF ANIMAL SYMPTOMS

Nasal rubbing and sneezing behaviors were observed on day 21 as described previously.[31] Five μl of antigen (0.1 μg/mice) was bilaterally instilled into the nasal cavities. After challenging antigen, animals were immediately placed into the observation cage (one animal/cage, φ 11 × 6 cm), and the incidence of nasal rubbing and sneezing was counted for 5 minutes. To evaluate nasal rubbing, each time the animal rubbed or touched the area near the nose with their forepaws, it was counted as one event. Touches around the eyes and the mouth were disregarded.

BLOOD SAMPLES

On day 21, mice were anesthetized with ether (Wako Pure Chemical Industries), and blood samples were collected from the heart in heparinized tubes. Blood samples were centrifuged at 15000 g for 10 minutes. Then, plasma samples were stored at -20℃ until use for the analysis.

ANALYSIS OF CYTOKINES, IgE AND HORMONES

Plasma levels of IFN-γ, IL-10, IgE, cortisol and ACTH were determined using IFN-γ and IL-10 ELISA kit (Pierce Biotechnology, Rockford, IL, USA), IgE EIA kit (Yamasa, Ciba, Japan), corisol EIA kit (Oxford Biomedical Research, Oxford, MI, USA) and ACTH ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA, USA), respectively, according to the manufacture's instructions.

STATISTICAL ANALYSIS

The data were expressed as mean ± SD derived from 3-29 animals. A *p*-value of less than 0.05 was considered significant. The differences between the groups were analyzed by Student's t-test.

Results

EFFECT OF SENSITIZATION ON PLASMA CYTOKINES, IgE AND CLINICAL SYMPTOMS

Number of sneezing and nasal rubbing increased markedly after sensitization of animals with pollen allergen. Sensitization significantly increased plasma levels of IL-10 and IgE without affecting IFN-γ levels (Table 1). Thus, sensitization with pollen antigen enhanced the production of IL-10 to shift the Th1/Th2 balance to Th2-dominant type reactions and enhanced the secretion of IgE from B cells, and finally induced clinical symptoms of rhinitis, such as sneezing and nasal rubbing.

EFFECT OF RESTRAINT STRESS ON PLASMA CYTOKINES, IgE AND CLINICAL SYMPTOMS

The restraint stress decreased the plasma levels of IgE and IL-10 without affecting levels of IFN-γ. Although plasma levels of both ACTH and cortisol remained unchanged after sensitization, they increased after receiving restraint stress both in control and sensitized groups. Under identical conditions, the incidence of both sneezing and nasal rubbing in the sensitized mice was decreased by restraint stress, suggesting that pollinosis was suppressed by a mild stress (Table 1).

Table 1 Effects of mild stress on plasma factors and clinical symptoms

	IgE (mg/ml)	Cytokines (pg/ml)		ACTH (ng/ml)	Cortisol (ng/ml)	Sneezing	Nasal rubbing
		IL-10	IFN-γ				
Control	0.03 ± 0.04 (8)	31.94 ± 30.50 (20)	4.92 ± 4.94 (12)	0.48 ± 0.55 (5)	1.43 ± 0.35 (5)	0.93 ± 2.16 (14)	32.40 ± 11.81 (15)
Sensitized	1.60 ± 0.72** (8)	161.66 ± 63.85* (20)	8.22 ± 6.50 (21)	0.57 ± 0.25 (5)	1.23 ± 0.34 (5)	3.07 ± 3.01* (15)	55.00 ± 15.46* (15)
Control + stress	0.00 ± 0.01 (5)	6.34 ± 10.64 (5)	11.70 ± 26.16 (5)	2.47 ± 3.00 (3)	2.68 ± 0.90* (3)	1.00 ± 1.00 (3)	17.67 ± 2.52 (3)
Sensitized + stress	0.39 ± 0.14 †‡ (4)	16.38 ± 31.54 § (5)	5.12 ± 7.16 (5)	1.76 ± 0.92 ‡ (3)	1.93 ± 0.45 ‡ (3)	0.33 ± 0.58 ‡ (3)	23.33 ± 7.51 § (3)

The mice were intraperitoneally injected with 1 μg of pollen allergen mixed with 0.3 ml of alminium hydroxide gel on days 0 and 4. Then, 5 μg of pollen allergen was administrated bilateraly into the nasal cavity once per day (from day 10 to 16). On day 21 after the sensitization, the mice were subjected to restraint stress for 3 hr between 9:00 am to 12:00 am. The plasma levels of cytokines, IgE, ACTH and cortisol were measured as descrived in the text. Data are mean ± S.D. (*n*). * $p < 0.05$, ** $p < 0.01$ vs. Control, † $p < 0.05$ vs. Control + stress, ‡ $p < 0.05$, § $p < 0.01$ vs. Sensitized.

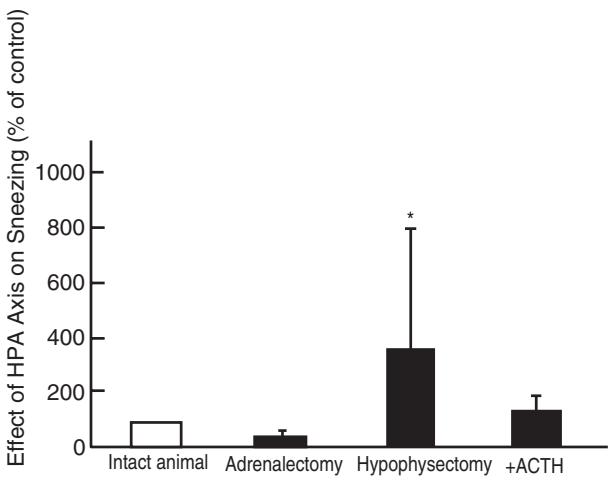

Fig. 1 Effect of the HPA axis on sneezing. Effects of sensitization were observed with animals that had received either adrenalectomy or hypophysectomy as described in the text. In some experiments, 57 ng of ACTH was administrated intraperitoneally three times a week from day 0 to 21. On day 21, the number of sneezing was counted for 5 minutes. Values are expressed as the percent of control group. Data are expressed as mean ± S.D. (Intact animal, *n* = 15; Adrenalectomy, *n* = 5; Hypophysectomy, *n* = 4; +ACTH, *n* = 5).
*$p < 0.05$.

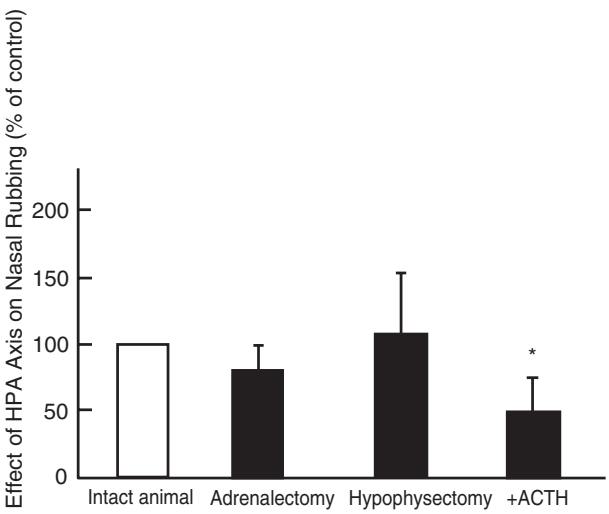

Fig. 2 Effect of the HPA axis on nasal rubbing. Experimental conditions were the same as in Figure 1. On day 21, the number of nasal rubbing was counted for 5 minutes. Values are expressed as the percent of control group. Data are expressed as mean ± S.D. (Intact animal, *n* = 15; Adrenalectomy, *n* = 5; Hypophysectomy, *n* = 4; +ACTH, *n* = 5).
*$p < 0.01$.

EFFECT OF THE HPA AXIS ON THE SENSITIZED MICE

To elucidate the possible involvement of the HPA axis, we studied the effects of adrenalectomy and hypophysectomy on both the clinical status and plasma cytokines. The incidence of sneezing was decreased by adrenalectomy but increased by hypophysectomy. When mice were injected intraperitoneally with ACTH during sensitization, the incidence of sneezing remained unchanged (Fig. 1). Under the identical conditions, the incidence of nasal rubbing was not affected by either adrenalectomy or hypophysectomy but decreased significantly by ACTH administration (Fig. 2). The increase in plasma levels of IL-10 in the sensitized animals was enhanced significantly by hypophysectomy but suppressed by either adrenalectomy or the administration of ACTH (Fig. 3). The increase in plasma levels of IFN-γ in the sensitized animals was significantly enhanced by either adrenalectomy, hypophysectomy or the administration of ACTH (Fig. 4). The increase in plasma levels of IgE after sensitization was significantly decreased by adrenalectomy but enhanced by hypophysectomy. Under identical conditions, ACTH had no appreciable

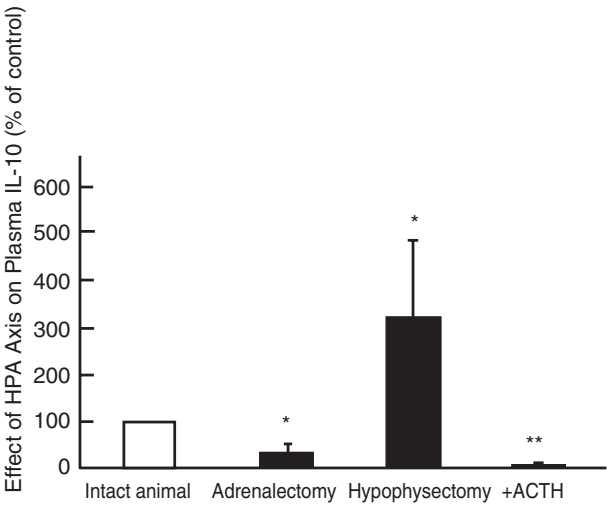

Fig. 3 Effect of the HPA axis on plasma IL-10. Experimental conditions were the same as in Figure 1. The levels of plasma IL-10 were measured on day 21 after the first sensitization. Values are expressed as the percent of control group. Data are expressed as mean ± S.D. (Intact animal, $n = 6$; Adrenalectomy, $n = 3$; Hypophysectomy, $n = 5$; +ACTH, $n = 4$) . $^{*}p < 0.05$, $^{**}p < 0.01$.

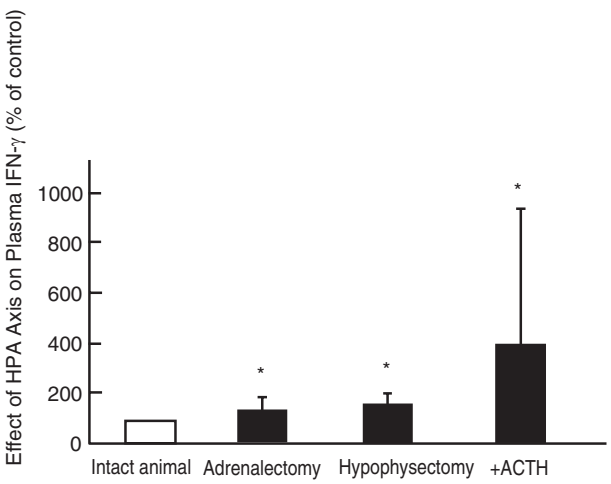

Fig. 4 Effect of the HPA axis on plasma IFN-γ. Experimental conditions were the same as in Figure 1. The levels of plasma IFN-γ were measured on day 21 after the first sensitization. Values are expressed as the percent of control group. Data are expressed as mean ± S.D. (Intact animal, $n = 29$; Adrenalectomy, $n = 3$; Hypophysectomy, $n = 5$; +ACTH, $n = 4$). $^{*}p < 0.05$.

effects on IgE levels in plasma (Fig. 5).

DISCUSSION

Stress responses in animals can be categorized into three stages, eustress, resilience and distress, and immune reactions in each stage may be different depending on the stage.[23] It is well known that symptoms of inflammatory diseases are affected by a variety of stressors. In fact, Chida *et al.*[16] and Amano *et al.*[28] reported that strong stress accelerated allergic diseases. On the other hand, Nishioka *et al.*[19] reported that allergic diseases could be suppressed by exposure of animals to restraint stress. Although strong stress has been known to accelerate allergic diseases, it is not clear whether the aggravation depends on the increase or decrease of glucocorticoids. Therefore, biological effects of stress should be analyzed depending on the type, strength, and duration of exposure to stressors.

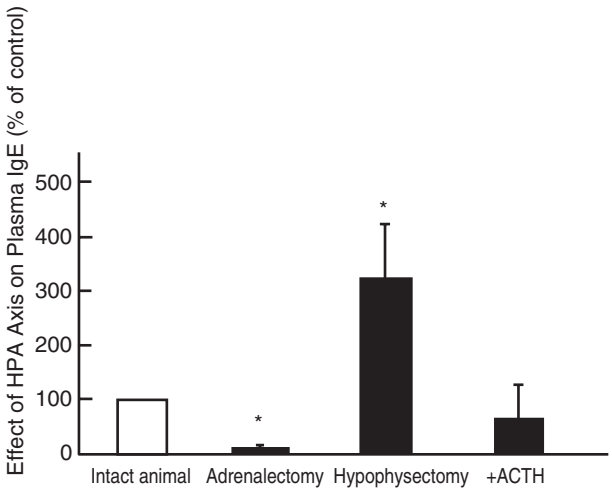

Fig. 5 Effect of the HPA axis on plasma IgE. Experimental conditions were the same as in Figure 1. The levels of plasma IgE were measured on day 21 after the first sensitization. Values are expressed as the percent of control group. Data are expressed as mean ± S.D. (Intact animal, $n = 20$; Adrenalectomy, $n = 3$; Hypophysectomy, $n = 3$; +ACTH, $n = 4$). $^{*}p < 0.05$.

The present work shows that mild restraint stress elevated the plasma levels of ACTH and cortisol but decreased IL-10 and IgE levels without changing IFN-γ, and suppressed the symptoms of pollinosis as determined by the incidence of sneezing and nasal rubbing. Since the stress response could be mediated predominantly via the hypothalamo-pituitary-adrenal axis that involves secretion of ACTH and cortisol, the effect of ACTH on the symptoms of pollinosis was analyzed. We found that physiologically low levels of ACTH suppressed the symptoms of pollinosis. To study the mechanism by which ACTH suppressed the symptoms of pollinosis, we used animals that had received either hypophysectomy or adrenalectomy. The symptoms of pollinosis were suppressed by adrenalectomy but enhanced by hypophysectomy, suggesting the pivotal action of the two organs on modulation inflammatory reactions.

The present work also showed that adrenalectomy

decreased the plasma levels of IL-10 and IgE, thereby suppressing the symptoms of pollinosis. These changes induced by adrenalectomy seem to reflect the decrease in plasma cortisol, a potent stimulant for the Th2-type immune reactions including pollinosis.[5,13] Preliminary experiments revealed that adrenalectomy decreased plasma levels of cortisol from 1.4 to 0.5 ng/ml. This observation is consistent with our hypothesis that plasma cortisol plays important roles in the enhanced secretion of IL-10 and IgE to aggravate symptoms of pollinosis.

It has been well documented that pituitary secretion of ACTH is enhanced in adrenalectomized animals by a feed-back mechanism that stimulates synthesis and secretion of POMC-related peptides.[32-34] Consistent with this hypothesis, preliminary experiments in this laboratory showed that adrenalectomy increased the plasma levels of ACTH from 1.0 to 5.9 ng/ml. These observations suggest that the elevation of ACTH in plasma might be responsible for the mechanism by which adrenalectomy suppressed the symptoms of pollinosis.

To our surprise, the symptoms of pollinosis were aggravated by hypophysectomy that decreased plasma levels of both ACTH and glucocorticoids. This observation suggests that POMC-derived peptides, such as ACTH, and cortisol secreted from the adrenal gland seem to modulate the balance between Th1- and Th2-type immune reactions in a pivotal manner. To test this hypothesis, we analyzed the effect of ACTH administration on the plasma levels of Th1- and Th2-related cytokines and IgE. Immunological analysis revealed that administration of ACTH increased the plasma levels of IFN-γ but decreased IL-10 and the incidence of nasal rubbing without affecting IgE levels in plasma.

It has been postulated that both nasal rubbing and sneezing are induced predominantly by IgE and histamine-dependent type I reactions. However, not only histamine but also other ligands, such as serotonine, leukotriens, substance P and opiate peptides released from inflammatory and/or neuronal pathways, have been shown to induce nasal rubbing.[28] Thus, it is not surprising that administration of ACTH decreased nasal rubbing without affecting the incidence of sneezing.

ACTH and/or POMC-related peptides have been known to stimulate melanocortin receptors expressed on plasma membranes of macrophages, lymphocytes and other immunocytes, thereby modulating immunological reactions.[35-40] In fact, the present work shows that administration of ACTH stimulated Th1-type reactions (as judged from the elevation of IFN-γ) with concomitant suppression of both Th2-type reactions (as determined from the decrease in IL-10) and the symptoms of pollinosis presumably by interacting with melanocortin receptors on immunocytes. In this context, Johnson *et al.*[12] reported that administration of 1 to 3 μM of ACTH suppressed the Th1-type reactions. However, a recent study showed that physiological levels of ACTH (1-100 nM) stimulated Th1-type reactions (as judged from the elevation of IFN-γ).[41] This observation is consistent with the results obtained from animals administered with physiologically low levels of ACTH (65 nM). It has been well documented that Th1- and Th2-type immunocytes interact with each other to modulate immunological reactions. These observations suggest that ACTH directly stimulates Th1-type cells but inhibits Th2-type cells, thereby increasing IFN-γ secretion and decreasing IL-10 to suppress the symptoms of pollinosis. Since the secretion of glucocorticoids from the adrenal gland is one of the major responses to ACTH, the effect of cortisol might also play an important role in the modulation of allergic reactions. In this context, glucocorticoids have been known to inhibit Th1-type immunocytes but stimulate Th2-type cells.[13] Thus, ACTH secreted from the pituitary gland and glucocorticoids derived from the adrenal gland might regulate the Th1/Th2 balance in a pivotal manner thereby modulating stress responses including allergic reactions. The pathophysiological significance of the pivotal modulation of immunological reactions by ACTH and glucocorticoids (and other ligands secreted from the pituitary and adrenal glands) should be studied further to elucidate the full scope of the effects of various stressors on the clinical symptoms of allergic diseases including pollinosis.

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