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TYPE II VANILLOID RECEPTOR SIGNALING SYSTEM: ONE OF THE POSSIBLE MECHANISMS FOR THE RISE IN ASTHMA CASES

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1. ABSTRACT

The prevalence of asthma keeps on increasing worldwide, especially in western societies over last 40 years. The mechanism of asthma is unclear. Recently, concern about indoor air pollution as a risk factor for asthma has been arisen. In present study, 25 Kun Ming male mice were placed in an air chamber containing respective formaldehyde (FA) concentration of 0, 0.5, 1.0, 3.0 mg/m^3,and 3.0 mg/m^3 with Capsazepine (CPZ, a specific antagonist of vanilloid receptor)-pretreatment in five testing groups (n=5 per group) for inhale experiments. The inhaled groups were exposed to gaseous FA for 6 hours each day in 10 successive days. After exposure, the concentrations of IL4 in blood serum and broncho alveolar lavage fluid (BALF) were measured. Experimental results showed that the IL4 level in serum was too low to be detected; and the concentrations of IL4 in BALF increased in a dose-dependent manner. However, for the CPZ-pretreated group the IL4 level in BALF decreased significantly (compared with 3.0 mg/m3 FA inhaled group, $p<0.01$). This paper describes experimental animal methods to probe IL4 level, an important indicator for IgE response. The studies in this paper indicated that gaseous FA might induce acquired atopy by type II VR1 signaling system. These findings suggested that indoor air pollutants such as FA might be key risk factors for the rise in asthma cases, and type II VR1 signaling system might be one of the mechanisms for the rise.

2. INTRODUCTION

Asthma, in which more than 50% of affected adults and at least 80% of affected children were caused from allergens, occurs in around 5-15% of the paediatric population (1). World Health Organization (WHO) reported that asthma affects about 150 million people world wide, and is a major incidence causing hospitalizations for chronic diseases in children in the western world (1). Asthma has three epidemiologic characters: First, the disease rates keep on increasing allover the world, especially in western societies over last 40 years. Gretchen Vogel described that asthma cases have skyrocketed – doubled in the United States since 1980 (2). Second, asthma is more prevalent in developed countries. As we know, asthma is more severe in western countries than in the remaining world. For example, the reported prevalence is about 5-30% for children in the United States while it is only 1-5% in China. Interestingly, two studies suggested that migrants from underdeveloped to developed countries acquired more atopic diseases including asthma (3). Third, prevalence and incidence of asthma is greater in both upper class of society(4) and urban residence (5).Thus, discovery of risk factors for the rise in asthma cases is essentially important. The most popular theory is that asthma has increased partly because of greater exposure to allergens such as house dust mites or cockroaches. However, allergens from the described sources have not been increased but significantly decreased over last 40 years in developed countries. It seems unlikely that changes in exposure to allergens are sufficient for the prevalent of asthma in developed areas. An increased level of atopy could be another hypothesis for the rise of the disease. Nevertheless, the increase of the asthma cases has been much faster than the alteration of the genetic constitution in any population. A genetic cause is not persuadable. This has led many investigators to investigate other factors in western world for the causes of rises of asthma.

Studies in laboratory animals have indicated that FA might enhance animal sensitization to inhaled allergens and it might be an underlying factor for the increase of asthma. In earlier report, we demonstrated that the tested mices were sensitized to ovalbumin, leading the serum titre of ovalbumin specific IgE antibodies to increase by approximately 3-fold after exposing the mices to FA (6). Similarly, exposure to a low concentration of FA enhanced the guinea-pigs' sensitization to allergens (7). A recent discovery showed that repeated exposure to FA worsens allergic bronchoconstriction through enhancing the antigen sensitization of guinea-pigs (8). Although some studies have indicated that indoor FA might worsen allergies and be an underlying factor for increasing incidence and severity of asthma, the mechanism was not clear and needs to be further investigated.

In order to explore the mechanism of sensitization enhancement of inhaled allergens from FA, this paper describes experimental animal methods that were employed to probe levels of IL4, an important cytokine protein in IgE response, in bronchoalveolar lavage fluid (BALF) and blood sera of mice after exposure to different levels of FA. In our experiments, an electrochemical FA sensor (Interscan Inc., USA) was used to monitor the exposure concentrations of gaseous FA, and IL4 was measured by Enzyme-Linked ImmunoSorbent Assay (ELISA) method. Capsazepine (CPZ), a vanilloid receptor (VR)-1 specific antagonist, was also used to evaluate the distribution of type II VR signaling system (FA/VR1/Ca^{2+}/IL4/IgE or FA/?/VR1/Ca^{2+}/IL4/IgE) in the formation of acquired atopy.

3. MATERIAL AND METHODS

3.1. Animals

25 Kun Ming male mice were supplied by the Experimental Animal Center of Hubei, PRC. Animals weighing between 18 and 20g were divided into 5 testing groups (n=5 each) to expose to different concentrations of FA - 0, 0.5, 1.0, 3.0 mg/m^3 and 3.0 mg/m^3 with CPZ (sigma, USA)-pretreated mice by tail-vein injection.

3.2. Exposure of mice to FA

The inhaled groups were exposed to different concentrations of gaseous FA for 6 hours per day in consecutive 10 days. During the exposure animals were not allowed to drink and eat.

FA inhaled groups were placed into varied glass inhalation chambers, where different concentrations of gaseous FA were generated by a small environmental chamber with formalin (Sigma, USA). The chamber temperature was controlled in the range from 26□ to 30□ and the humidity 45% ± 5% was kept for all testing time. The gas flux was metered at 1±0.03 L/min.

A 4160 type digital electrochemical analyzer (Interscan Inc., USA) was used to measure the concentrations of gaseous FA. The sensitivity was 0.012 mg/m^3, and the variation was ±0.024 mg/m^3.

3.3. Bronchoalveolar lavage and collection of serum

Bronchoalveolar lavage (BAL) was performed for 24 h after the last exposure. Mice were anesthetized and the tracheas were cannulated while gently massaging the thorax. The lungs were lavaged with 0.6 ml of 0.9% NaCl. The BALF samples were collected and the recovery of the total lavage volume exceeded 95%. Then the samples were centrifuged (at 600 g for 10 min at 4□) and the fluid phase of BALF was frozen at –80□ until the IL4 levels were assessed. Blood was collected from the retro-orbital plexus of anesthetized mice. The collected bloods were centrifuged to produce the sera , and were stored at –80□ until measurement.

3.4. Measurement of IL4 level

The levels of IL4 in the BALF and blood serum were determined by ELISA. ELISA kit from R&D Systems (USA) was used for the measurement of IL4. The ELISA assay employed an antibody specific for mouse IL-4 and it was coated on the well surfaces of a 96-well plate. Samples were pipetted into the wells and IL-4 present in a sample was bound to the immobilized antibody in the wells. Then biotinylated anti-mouse IL-4 antibody was added. After

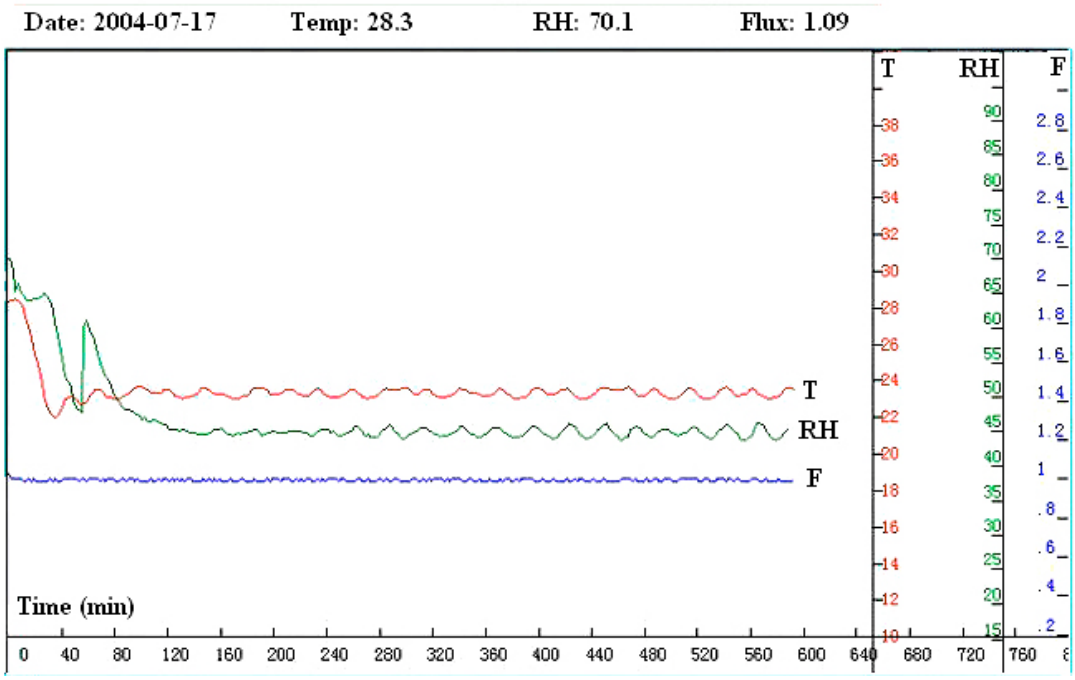

Figure 1. The measurement results of gaseous FA concentrations. Gaseous FA concentrations generated by a small environmental chamber were measured 3 times per day for 10 days. Each point indicates mean±standard error of the mean (SEM).

washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed following by addition of TMB substrate solution into the wells. The color developed was proportional to the amount of IL-4 bound.

3.5. Statistical analysis

All data are presented as mean±standard error of the mean (SEM). Student's t-test was applied to evaluate the significance of the differences in the concentrations of IL4 between two groups. A level of $P<0.05$ was considered to be statistically significant.

4. RESULTS AND DISCUSSION

4.1. The parameters of chamber and the concentrations of gaseous FA

The concentrations of gaseous FA generated by small environmental chamber were measured 3 times per day for consecutive 10 days. The measurement results (0.03±0.03, 0.49±0.03, 1.03±0.04 and 3.03±0.08 mg/m^3) were very close to the anticipative concentrations (0, 0.5, 1.0 and 3.0 mg/m^3). It indicated that the gaseous FA from the chamber effluent was quite stable and reliable (Figure 1). In the previous studies (9), gaseous FA was generated from paraformaldehyde. Then high level of FA was diluted with clean, filtered air to achieve the desired gas concentrations. In this way, it was difficult to control the temperature (T), the humidity (RH) and the gas flux (F) of gaseous FA. In addition, it is well-known that the alterations of temperature and humidity are allergic factors and the gas flux of FA is important to the inhalational quality of mice. In the present study, different concentrations of gaseous FA were generated by small environmental chamber with formalin. With this method the temperature, the humidity and the gas flux in the testing chambers could be well controlled before FA exposure to the testing animals. The operational conditions of the chamber were very stable after 3-hour operation. The results shown in figure 2 demonstrate the great reliability and reproducibility.

4.2. IL4 levels in blood serum

In the serum samples, the concentrations of IL4 were too low to be detected using ELISA kit. The readings were all below the minimum detectable dose of this kit (the detection limit of the ELISA kit is 10 pg/ml). The low level of IL4 was possibly due to the short exposure duration of FA (6 hours per day for consecutive 10 days). In the short exposure, the increase of IL4 in lung might be too low to produce detectable concentrations in serum.

4.3. IL4 levels in BALF

Figure 3 shows the effect of gaseous FA exposure on IL4 levels in BALF. There was no significant difference between the IL4 levels of 0 and 0.5 mg/m^3 FA inhaled groups. The IL4 levels at 1.0 and 3.0 mg/m^3 groups were significantly ($P<0.05$) higher than that in the 0 mg/m^3 control group, and increased with increase of the inhaled gaseous FA concentrations. D. Nowak *et al.* proposed that the most likely explanation for the increase of asthma is the changes in environmental exposures (10). The rise of indoor entertainment time was also suspected to relate with the rise of asthma (11). FA is an important indoor pollutant. Some studies suggested that FA may enhance the sensitization to inhaled allergens and it may be an underlying factor for the increase of asthma incidences (6-8). Xu *et al* have reported that FA induced the long-lasting

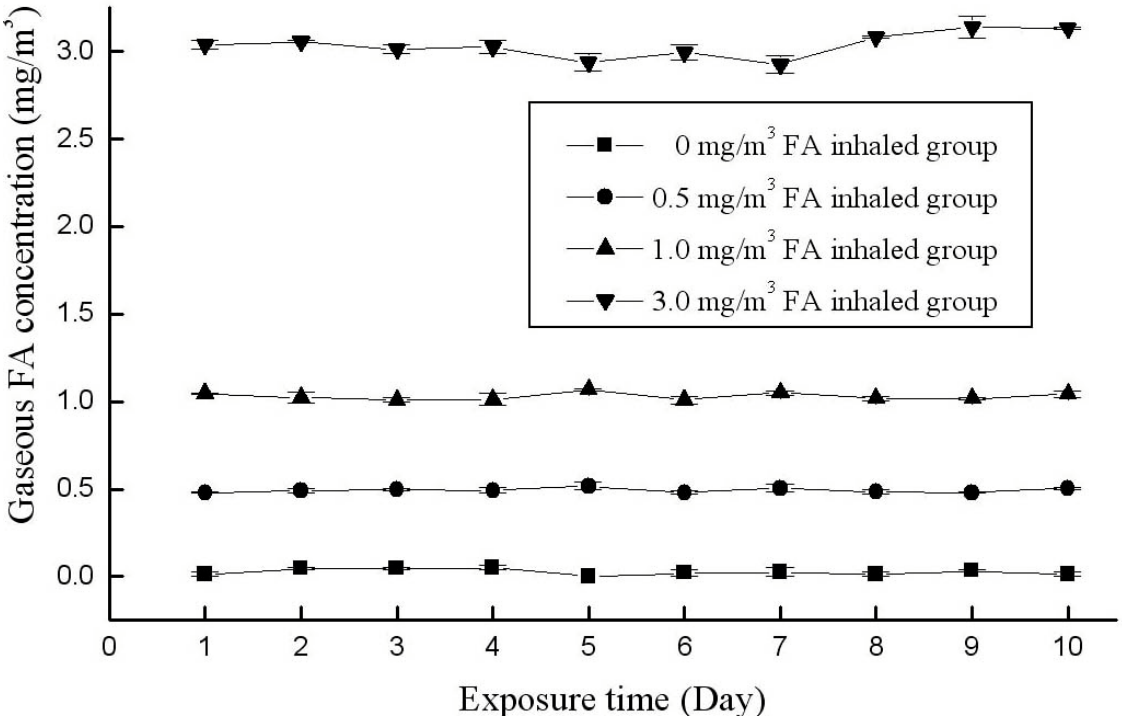

Figure 2. The Operational contiditions of a small environmental chamber. The curves show the alterations of the temperature (T), the humidity (RH) and the gas flux (F) of gaseous FA, respectively.

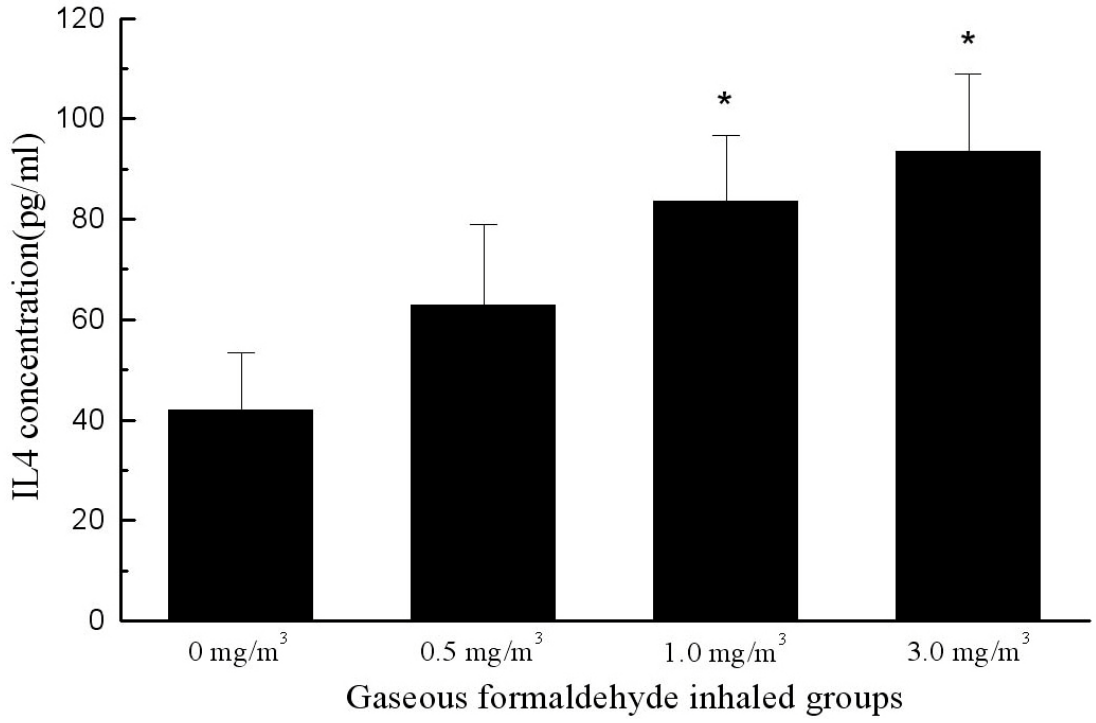

Figure 3. The histograms showing the effect of the mice exposure to FA by the IL4 levels in the mice BALF . BALF was collected for 24 h after varied concentration of FA exposure to the different mice groups; the bars indicate the responses of IL4 levels in BALF after exposure of different concentrations of FA to the mice and each one represents mean±standard error of the mean (SEM).

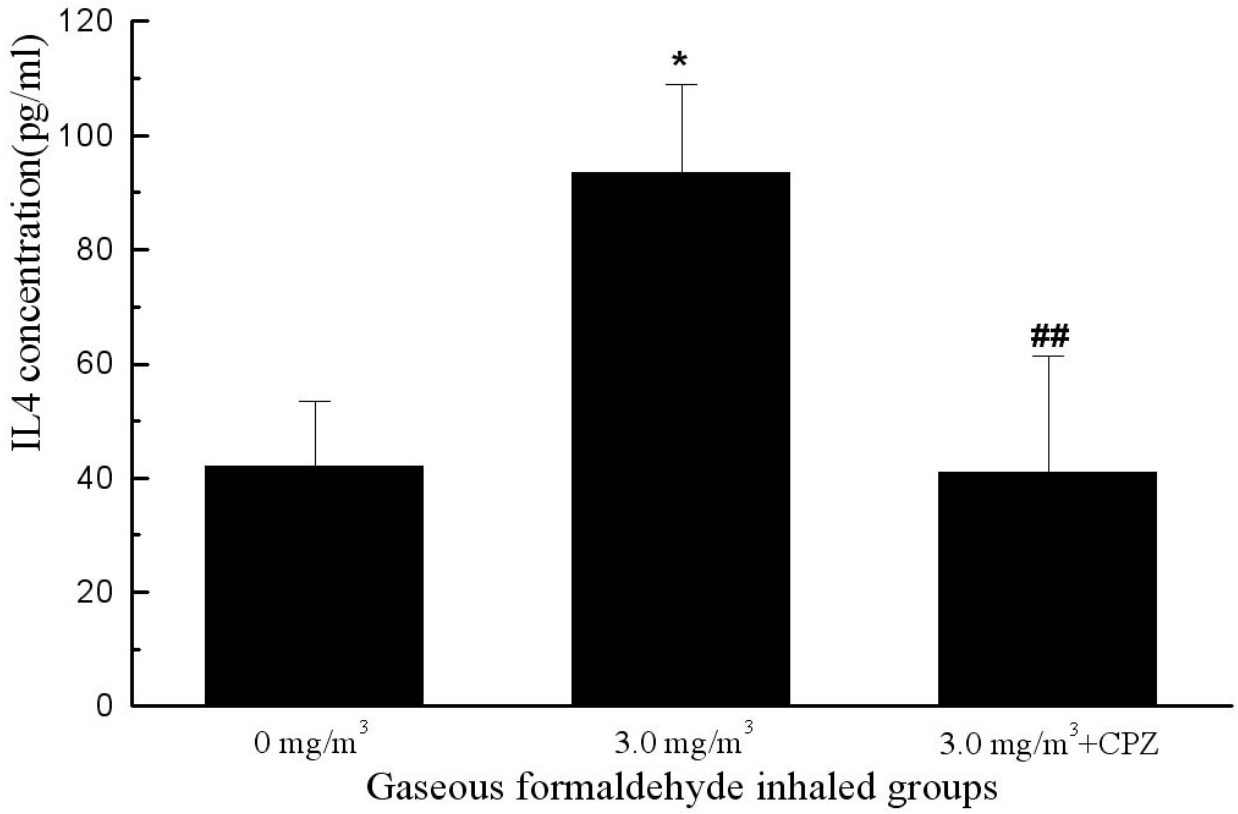

Figure 4. The histograms showing the effect of capsazepine (CPZ) pretreatment on the IL4 levels in mice BALF after 0 and 3.0mg/m^3 formaldehyde (FA) exposure to the three testing groups. BALF was collected for 24 h after varied amount of FA exposure to the testing mice; the bars indicate the responses of IL4 levels in BALF with/without the CPZ treatment and each one represents mean±standard error of the mean (SEM)..

expression of IL-4 in mouse spleen and draining lymph nodes (12). The present results further indicated that gaseous FA exposure increased the IL4 levels in the mice BALF in a dose-dependent manner. It is known that IL4 is essential for the development of airway inflammation present in asthma. It can induce the synthesis of IgE in B cells and differentiation of T cells to a Th2 phenotype by binding to its receptor (IL4 R) in the target cells (13-14). Our experimental results showed that the IL-4 levels were increased with the increase of the inhaled gaseous FA concentration, demonstrating that FA exposure could cause the enhancement in allergen sensitivity and then FA-induced asthma. Thus, FA could be regarded as a model chemical to explore the effects of indoor pollutants on the rise of asthma cases.

4.4. Effect of CPZ on IL4 levels in BALF

The effect of CPZ pretreatment on IL4 levels after 3.0 mg/m^3 FA exposure is illustrated in figure 4. The figure exhibited that the IL4 level of BALF in the group with CPZ pretreatment was significantly ($P<0.01$) decreased in comparison to that in 3.0 mg/m^3 group without CPZ pretreatment, and the level of IL4 after CPZ pretreatment was actually as same as the baseline ($P>0.05$, compared with control group). The results showed that CPZ, a specific antagonist of vanilloid receptor (VR) 1, could block the increase of IL4 in BALF . Because VR1 is a molecular integrator of physical and chemical stimuli in the peripheral nociceptor terminals and an ionotropic channel, it plays a critical role in both thermal nociception and inflammatory hyperalgesia (15). Many gaseous chemicals such as residual oil, flying ash, other urban and industrial particulate matter (PM) could induce airway neurogenic inflammation through activating type I VR1 signaling system (CA/VR1/Ca^{2+}/SP/NKR) (16-18). In our former study, FA exposure caused the increase of VR1 mRNA expression in rat brain stem too (19). Thus, the above result also indicated that FA might enhance the immune sensitivity to inhaled allergens via a vanilloid receptor subunit 1 (VR1) mediate pathway.

Biro recently reported that capsaicin, a specific agonist of VR1, evoked the selective IL4 release in mast cell without the degranulation of histamine (20). Our results showed that FA inhalation increased the level of IL4 in a VR1 dependent way. This possibly indicates that FA might activate VR1 and then induce the release of IL4 from mast cells via type II VR1 signaling system (FA/VR1/Ca^{2+}/IL4/IgE). We named this phenomenon as "acquired atopy" (Figure 5). Acquired atopy induced by the type II VR1 signaling system may be one of the mechanisms of FA-induced asthma. This mechanism could

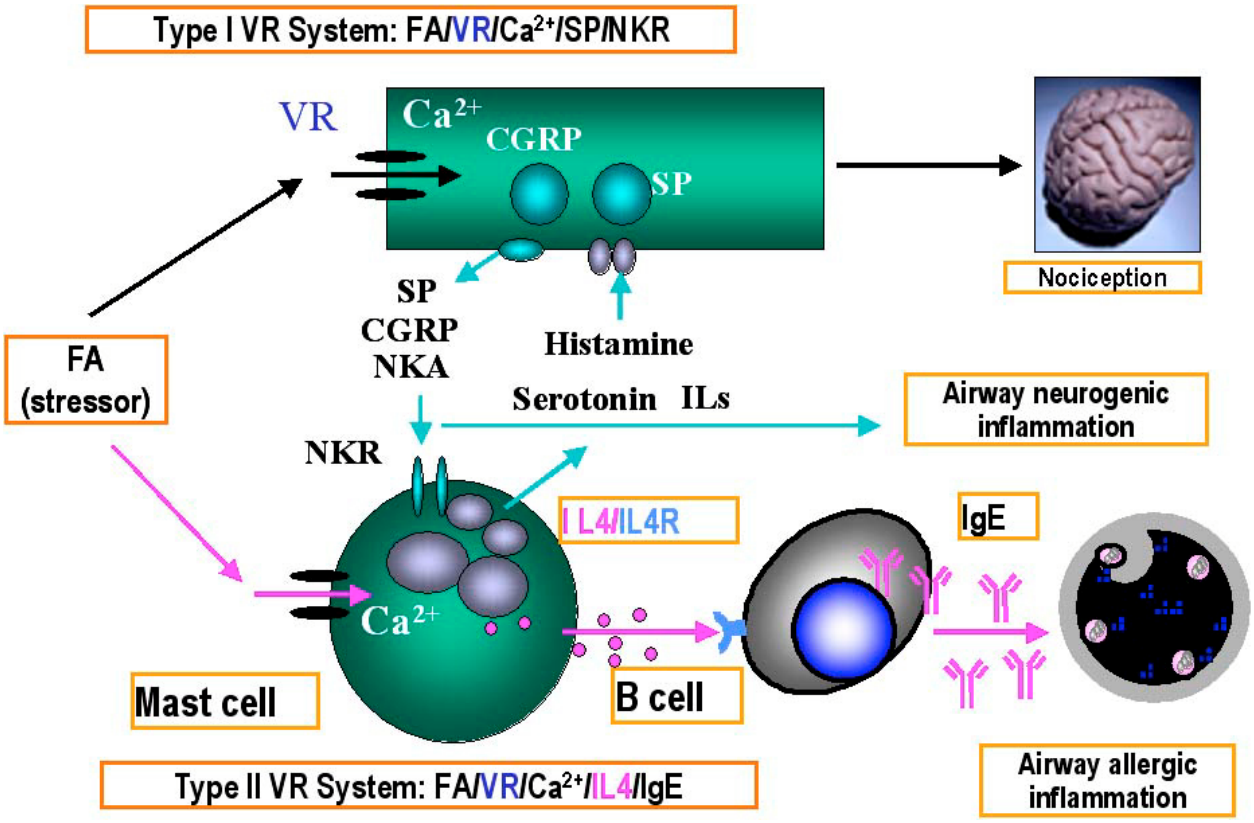

Figure 5. The schematic of the pathways causing asthma via the two types of VR1 signaling system.

also be used to explain the rise of asthma cases in western worlds. Environment pollutants, such as FA, may be a key factor for the growing prevalence of asthma in developed countries. The functional VR1 has been found out to exist in many kinds of non-neuronal cells such as mast cells, glial cells, keratinocytes, liver, polymorphonuclear granulocytes, macrophages, bladder urothelium and smooth muscle (20, 21). Thus, type II VR1 signaling system might widely exist in different cell types for mediating different biological functions.

5. CONCLUSION

Although some studies suggested that FA may enhance the sensitization to inhaled allergens and it may be an underlying factor for the rise of asthma, the suggested mechanism was unclear. The present studies employed ELISA to detecting IL4 levels in blood serum and broncho alveolar lavage fluid (BALF) of the tested mice after exposure to FA. Experimental results showed that the concentrations of IL4 in BALF increased in a dose-dependent manner, and the IL4 level in BALF decreased significantly for the CPZ-pretreated animal group, indicating that FA could enhance the immune sensitivity of animals to inhaled allergens. Study of the results also proposed that the phenomenon was mediated by type II VR1 signaling system (FA/VR1/Ca^{2+}/IL4/IgE) and this system could be a possible mechanism of FA-induced asthma and the rise of asthma cases. These findings suggested that indoor air pollutants such as FA might be the key risk factors for the rise in asthma cases, and type II VR1 signaling system might be one of the mechanisms for the rise. FA could be regarded as a model chemical to explore the effects of indoor pollutants on the rise of asthma cases.

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