

Pharmacological Manipulation of Immune-Induced Food Aversion in Rats

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Key Words

Food aversion · Psychoneuroimmunology · Mast cells · IgE-mediated food allergy

Abstract

Background: Mice allergic to ovalbumin (OVA) avoid drinking a solution containing this antigen. This was interpreted as related to IgE-dependent mast cell degranulation and sensory C fiber activation. **Methods:** We employed pharmacological manipulation to further investigate the mediators involved in immune-induced food aversion. **Results:** While nonimmunized rats preferred a sweetened OVA solution, immunized rats avoided it. We also employed a paradigm in which rats are conditioned to drink water for two 10-min sessions a day. Tolerant rats presented lower IgE titers, and this manipulation abrogated food aversion. Dexamethasone (1.0 mg/kg) prevented the aversion of OVA-immunized rats to the antigen-containing solution. Combined blockade of H₁ and 5-hydroxytryptamine (5-HT)₂ receptors by promethazine (3.0 mg/kg) plus methysergide (5.0 mg/kg) was unable to alter food aversion. Blockade of 5-HT₃ receptors by ondansetron (1.0 mg/kg) caused a twofold increase in the ingestion of the sweetened OVA solution by immunized rats, suggesting the involvement of 5-HT₃ receptors in food aversion. Finally, we showed that dexamethasone or promethazine plus methysergide, but not ondansetron, effectively prevented the IgE-dependent mast-cell-degranulation-induced increase in vascular permeability in rats. **Conclusion:**

We suggest that regardless of whether or not they cause edema, IgE-mediated mast cell degranulation and consequent 5-HT₃ signaling are involved in the process that triggers avoidance to the source of the allergen in allergic rats.

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Introduction

Food allergy and other allergic diseases have emerged as major public health problems on account of their dramatic increase over the past 2–3 decades. It is estimated that approximately 8% of children and 2% of the adult population suffer from food allergy [1, 2]. Allergic reactions are responsible for a myriad of symptoms that involve the airways, the gastrointestinal tract and the skin, among other systems [3–6]. In allergic patients, the allergic reaction progresses in two distinct phases representing parts of the same phenomenon: an early response mediated by IgE, mast cells and autacoids and a late phase, during which Th2 lymphocytes, cytokines and other cells build an inflammatory milieu that plays a major role in the disease [7]. Mast cell activation and degranulation are the first phenomena observed during the course of the inflammatory component of the allergic process. During this process, mast cells release several inflammatory mediators including histamine and 5-hydroxytryptamine (5-HT; serotonin), among many others [8, 9].

Peripheral inflammation has long been implicated in changes in brain activity and behavior, thus influencing the outcome of several biological phenomena [10]. We previously reported some neural and behavioral correlates of allergic reactions using murine models of ovalbumin (OVA)-induced food allergy [3, 4] and asthma [11, 12]. Mice allergic to OVA avoided drinking an antigen-containing solution or entering a box compartment previously associated with OVA nebulization. Furthermore, OVA-sensitized animals displayed an increase in the activity of the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala shortly after OVA challenge [4, 5, 11], as measured by neurons stained for the Fos protein, a product of the immediate early gene and transcription factor *c-fos* [13]. These areas, the major collections of corticotropin-releasing-factor-expressing neurons in the central nervous system [14], are involved in affective and emotional responses compatible with those of anxiety and avoidance in rodents [15, 16].

Mast cell degranulation is thought to play a fundamental role in food allergy signaling to the brain. In fact, we have previously shown that IgE-dependent mechanisms are necessary for allergy-induced behavioral changes and brain activation [3, 5]. Additionally, we have provided evidence supporting the idea that sensory C fiber nerve terminals adjacent to mast cells in the gastrointestinal mucosa are partially responsible for sensing the release of mediators following IgE-dependent mast cell degranulation and further conveying this information to the brain [3, 17].

Our previous findings pointing to the participation of mast cell and sensory C fiber interactions in the neural correlates of allergic responses prompted us to further investigate the chemical mediators involved in immune-induced food aversion. In order to do that, we employed pharmacological manipulation to block different histamine and serotonin receptors, and questioned whether this blockade would interfere with the aversion to an antigen-containing solution observed in OVA-sensitized animals.

Methods

Animals

Adult male Wistar rats (250–350 g) were obtained from the animal breeding unit at the Department of Pathology, School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil. The animals were housed in plastic cages (41 × 36 × 16 cm, 5 per cage) at a controlled temperature (22–26°C) in artificially lighted rooms on a 12-hour light/dark cycle (lights on at 7.00 a.m.) with

free access to rodent chow and water during the first 8 days of the experiment. Animals were housed and used in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, based on the *Guide for the Care and Use of Laboratory Animals* by the National Research Council, USA. Each animal was used once.

Sensitization Protocol

Sensitization consisted of 2 intraperitoneal injections on experimental days (ED) 0 and ED7 with 10 µg of OVA (grade V, Sigma) adsorbed to 10 mg of aluminum hydroxide gel (alum) in 0.5 ml of 0.9% NaCl. The OVA suspensions were prepared immediately before use.

Food Aversion Test: Two-Bottle Preference Test

The method used to induce food aversion was similar to that described elsewhere for mice [3]. Briefly, immediately after the second OVA injection (ED7), rats were housed individually. Each individual cage was supplied with two identical bottles (both filled with water) on opposite sides. Seven days later (ED14), one of the bottles was filled with a sweetened (0.5% saccharin) solution of 20% OVA in water, while the other bottle remained filled with normal water. Every bottle was weighed immediately before the beginning of the aversive test and after 24 h. The total fluid intake (water plus sweetened OVA solution) was expressed as milligrams of ingested solution per kilogram of body weight. The position of the bottles in the cage was switched every 4 h during the first 12 h to avoid a possible preference by the animal for one of the sides of the cage. To evaluate the preference of the rats for the sweetened solution, we used the following index: percentage of preference = (consumption of sweetened solution of OVA/total fluid intake) × 100.

Water Deprivation and New Food Aversion Test: One-Bottle Preference Test

Since pharmacological manipulation of immune-induced food aversion was part of our experimental design, a new aversive test was developed. In this protocol, the amount of sweetened OVA solution ingested was assessed for two periods of 10 min. Thus, on ED9, animals were housed individually and deprived of water. The methodology consisted of conditioning the rats to drink water for two 10-min periods a day (from 10.00 to 10.10 a.m. and from 5.00 to 5.10 p.m.). This procedure was repeated for 5 consecutive days, i.e. from ED9 to ED13. On ED14, the aversive test was performed. Water was removed from the drinking bottles and replaced by a sweetened (0.5% saccharin) solution of 20% OVA in water. To measure liquid consumption, the bottles were weighed before and after each 10-min drinking period, and the difference in weight was quantified. The data were presented as the sum of the sweetened OVA solution intake per kilogram of body weight in the morning and afternoon test sessions.

Drug Treatments

Drugs were used in an attempt to abrogate or inhibit immune-induced food aversion in rats. Thus, the H₁ receptor antagonist promethazine (Fenergan®, Aventis, Brazil; 3.0 mg/kg), the 5-HT₂ receptor antagonist methysergide (Sigma, USA; 5.0 mg/kg) and the 5-HT₃ receptor antagonist ondansetron (Ansentrone®, Biosintética, Brazil; 0.5 mg/kg) were employed. Dexamethasone (Decadron®, Merck, Sharp and Dohme, Brazil; 2 doses of 1.0 mg/

kg) was also employed. Drugs were dissolved in 0.9% NaCl solution and injected intraperitoneally 30 min before the 1st (morning) aversive test. The two dexamethasone doses were given 48 and 24 h before the 1st aversive test, respectively. The drugs, doses and time intervals chosen agree with data described elsewhere for similar purposes [18–21].

Induction of Oral Tolerance to OVA

Oral tolerance to OVA was induced as described elsewhere [22]. Briefly, the drinking bottles were filled with a 1% aqueous OVA solution (grade V, Sigma) and offered to the rats as the only fluid available in the cage, for 5 consecutive days before the 1st sensitization (ED0). This solution was freshly prepared each 24 h. This procedure was reported to suppress both the immediate and late phases of the allergic response [22].

Passive Cutaneous Anaphylaxis

Passive cutaneous anaphylaxis (PCA) was employed here to serve two purposes: titration of rat IgE antibodies in mice (experiment 3, see below) and the confirmation of the efficacy of the drugs used here in rats (experiment 5, see below) as described elsewhere [23]. Basically, the recipient animals' backs were shaved, and intradermal injections (100 μ l) of serial dilutions (1/20, 1/40, 1/80, 1/160, 1/320 and 1/640) of sera harvested from OVA-sensitized animals were given on each side of the dorsal midline skin. After 24 h, i.e. the time required for mast cell sensitization by IgE present in the serum [23], the animals were challenged intravenously with 0.2 ml (mice) or 1.0 ml (rats) of 2.5% Evans blue dye (Sigma, USA) aqueous solution containing 500 μ g of OVA. Thirty minutes after the challenge, the animals were euthanized, and dye leakage in the back skin was measured at each injection point. All tests were performed in duplicate or triplicate, and PCA titers were expressed as the reciprocal of the highest dilution that produced a reaction >5 mm in diameter.

Experimental Design

We carried out five experiments, which were performed in accordance with protocols and quality assurance methods.

In the 1st experiment, 10 rats were randomly and equally divided into 1 control and 1 experimental group. Rats of the experimental group were sensitized to OVA on ED0 and ED7, whereas those of the control group received identical volumes (1.0 ml/kg) of 0.9% NaCl. On ED14, animals of both groups were submitted to the food aversion test described above.

The 2nd experiment was similar to the 1st, except that rats were submitted to a new aversive test. For that purpose, animals were first submitted to a 5-day period of water deprivation conditioning (ED9–ED13). On ED14, they were submitted to the new test for consumption of OVA-sweetened solution, as described above.

In the 3rd experiment, 30 rats were randomly and equally divided into 3 groups: one control (C), one experimental (E) and one tolerant (Tol). From ED -5 to ED -1, rats of the Tol group were made tolerant to OVA as described above; those of groups C and E remained undisturbed and drank filtered water during this period. On ED0 and ED7, the OVA sensitization protocol was performed on rats of the E and Tol groups; on those days, animals of group C received similar volumes of 0.9% NaCl solution intraperitoneally. All rats were submitted to the water deprivation conditioning protocol, and we analyzed their aversion to the sweet-

ened OVA solution on ED14. Immediately after the aversion test, the rats of all groups were euthanized, and their sera were submitted to titration of IgE antibodies by PCA, performed using mice as recipients, as described above.

In the 4th experiment, we performed pharmacological manipulations of these phenomena. This experiment was performed 3 times, once for each drug treatment. The data obtained for each drug treatment were analyzed independently. In each replication, 40 rats were randomly divided into 4 equal-sized groups: C1, C2, E1 and E2. Animals of groups E1 and E2 were sensitized to OVA on ED0 and ED7, whereas those of groups C1 and C2 received similar intraperitoneal volumes of 0.9% NaCl solution instead. Rats of groups C2 and E2 received one of the following treatments before the aversion test: 1.0 mg/kg dexamethasone (1st replication), 3.0 mg/kg promethazine combined with 5.0 mg/kg methysergide (2nd replication) or 1.0 mg/kg ondansetron (3rd replication). Rats of groups C1 and E1 received similar amounts (1.0 ml/kg) of 0.9% NaCl. All rats were submitted to the water deprivation conditioning protocol used for OVA aversion on ED14.

In the 5th experiment, 5 mice were sensitized to OVA as described elsewhere [3, 17], in order to produce anti-OVA mouse serum with high levels of IgE, to be used later for PCA in rats. On ED14, the mice were sacrificed, and their sera were collected. PCA reactions were induced as described above, except that rats were used as recipients. Rats were treated with either two injections of dexamethasone (1.0 mg/kg, 48 and 24 h before PCA), a single injection with a combination of promethazine and methysergide or a single injection of ondansetron, 30 min before PCA. The positive control group received similar intraperitoneal volumes of 0.9% NaCl solution.

Statistical Analysis

Statistical analyses were performed using the software package (SPSS Systat, version 7.0). Bartlett's test was used to evaluate whether data should be analyzed by parametric or nonparametric tests. The differences in preference were determined by a nonparametric Mann-Whitney U test for two samples, or by a Kruskal-Wallis analysis of variance followed by its proper resolution. Data are presented as medians, with superior and inferior limits. A probability of $p < 0.05$ was considered to show significant differences for all measures.

Results

Allergic Rats Develop Food Aversion

Our data show that an immune reaction was capable of altering the behavior of rats with regard to their food preference, thus suggesting that the immune system influences diet selection (ultimately a behavioral change) also in this animal species. Figure 1 shows that, although there were no differences in total fluid intake (fig. 1a), nonimmunized rats showed a significant preference ($p < 0.01$) for the sweetened OVA solution, while immunized rats avoided it, drinking more water instead (fig. 1b).

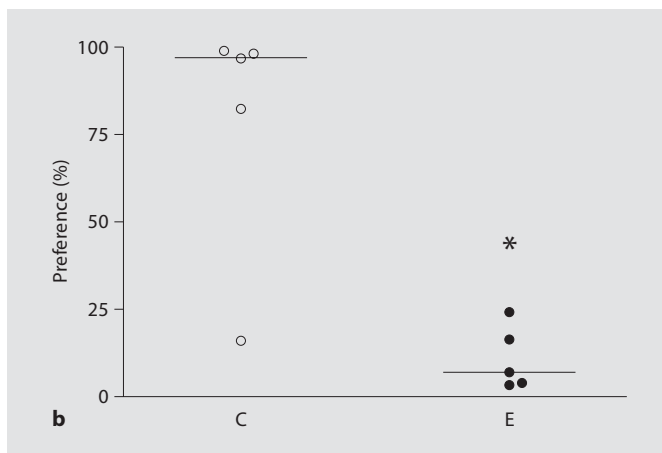
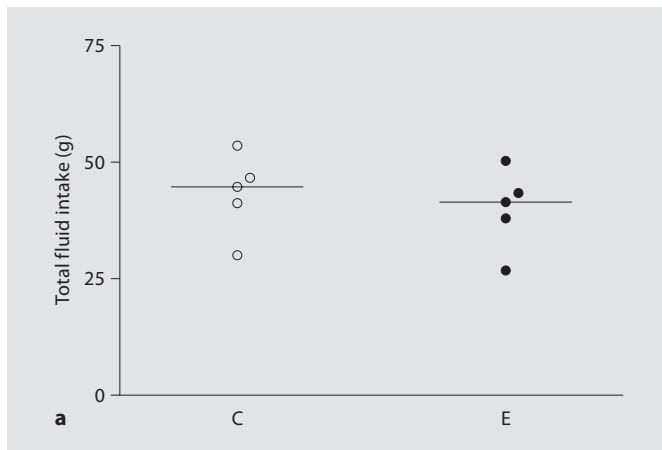


Fig. 1. Allergy-induced food aversion with the two-bottle preference test. **a** Total fluid intake for individual rats shows a similar distribution of values for C (nonimmunized) and E (OVA-immunized) groups. **b** Evaluation of preference for the sweetened OVA-containing solution by individual rats shows that while nonimmunized rats (group C) preferred drinking the antigen-containing solution, OVA-immunized rats (group E) developed food aversion, choosing water over the sweetened OVA solution. Horizontal bars represent medians (n = 5 in each group). * $p < 0.05$ compared to group C. The experiment was performed 3 times.

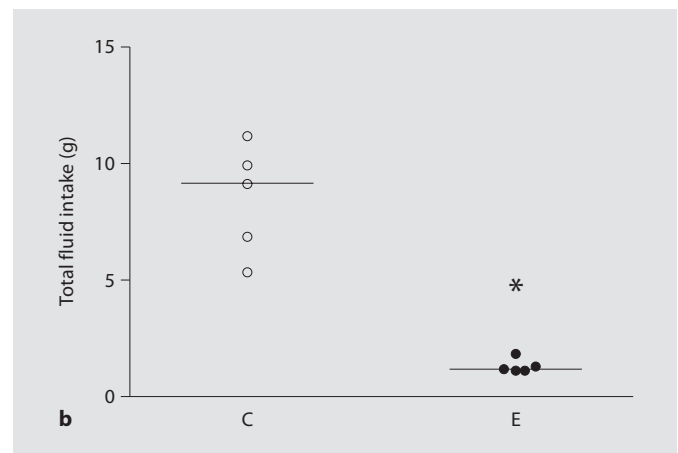
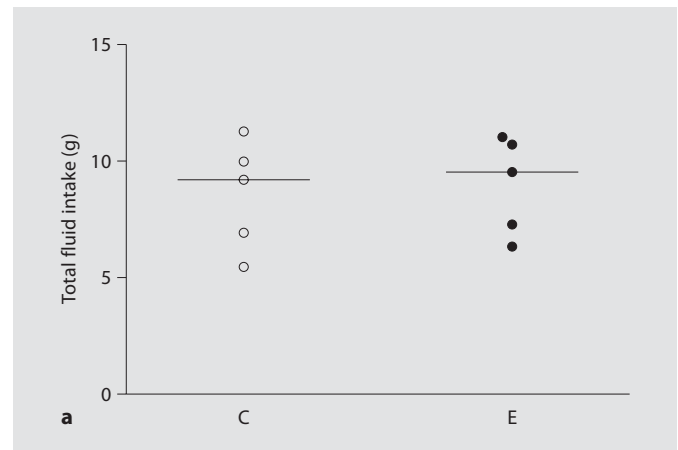


Fig. 2. Allergy-induced food aversion with the one-bottle preference test. **a** Total fluid intake for individual rats shows a similar distribution of values for C (nonimmunized) and E (OVA-immunized) groups on ED13. **b** On ED14, the bottles were filled with OVA solution instead of water. Total fluid intake on ED14 for individual rats shows allergy-induced avoidance to the antigen present in the diet as measured by a reduction in the consumption of the OVA-containing solution in immunized rats (group E) compared to nonimmunized animals (group C). Horizontal bars represent medians (n = 5 in each group). * $p < 0.05$ compared to group C. The experiment was performed 3 times.

Similar findings were observed with our new aversive test. Thus, no differences in total liquid consumption were found between nonimmunized and OVA-immunized rats (fig. 2a). However, figure 2b shows that OVA-immunized rats ingested less sweetened OVA solution than those of the control nonimmunized group ($p < 0.01$) on ED14, i.e. the day the food aversion test was carried out. As a matter of fact, an 80% decrease in fluid consumption was observed in OVA-immunized rats on ED14.

Oral Tolerance Prevents Food Aversion

It appears that food aversion is triggered by OVA-specific antibodies [4, 24]. Therefore, we next examined whether the induction of immunological tolerance to OVA would block food aversion. As previously shown, continuous feeding with 1% OVA for 5 consecutive days renders mice tolerant to subsequent immunization with the antigen. We found that rats fed with 1% OVA dissolved in the drinking water for 5 consecutive days (group

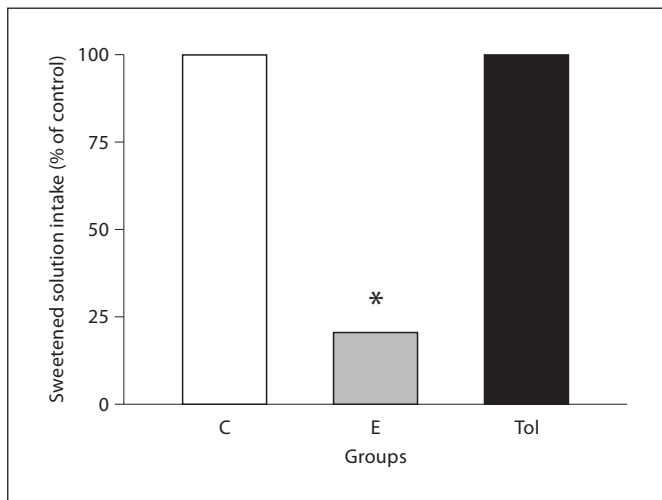


Fig. 3. Oral tolerance to OVA abrogates allergy-induced food aversion. While OVA-immunized rats (group E) developed allergy-induced avoidance to the antigen present in the diet as measured by a reduction in the consumption of OVA-containing solution, tolerant rats (Tol) behaved exactly as did the nonimmunized animals (group C), drinking large amounts of OVA solution. * $p < 0.05$ compared to groups C and Tol. The experiment was performed twice.

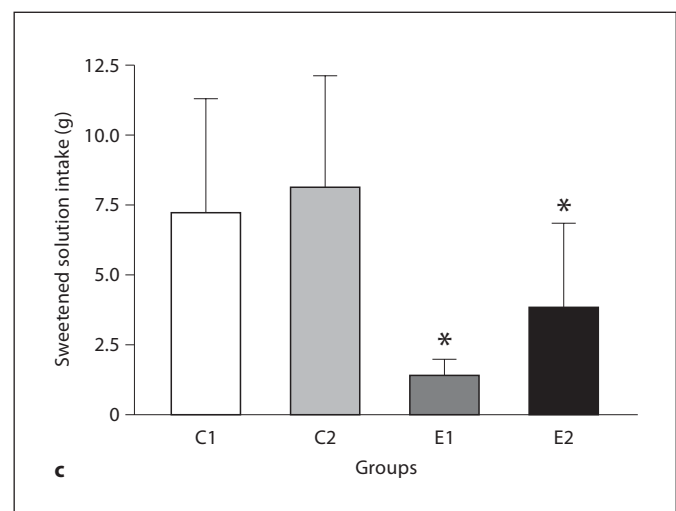
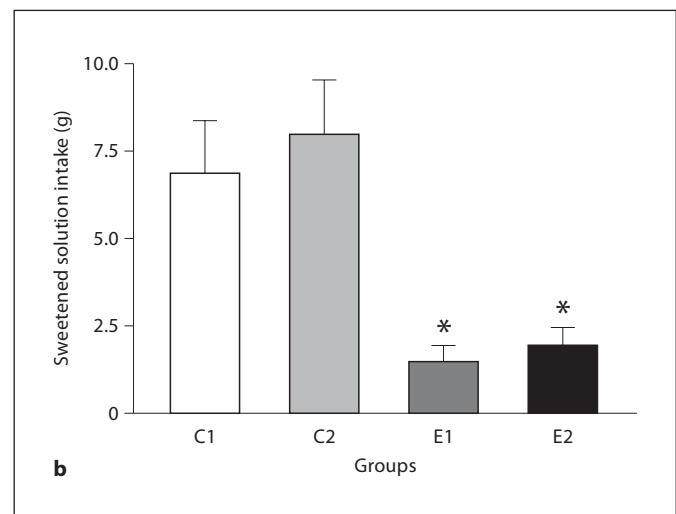
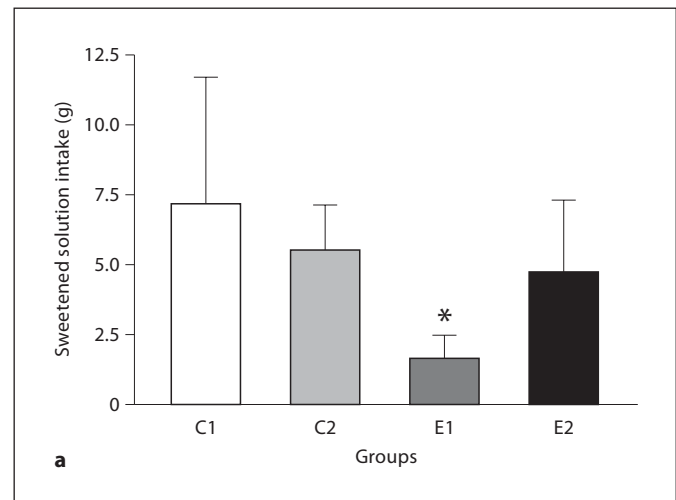


Fig. 4. Pharmacological manipulation of allergy-induced food aversion. **a** Treatment with dexamethasone abrogates allergy-induced food aversion. While nontreated immunized rats (group E1) showed a reduction in the consumption of OVA solution compared to nonimmunized animals (groups C1 and C2), allergic animals treated with dexamethasone (group E2) drank as much OVA solution as nonimmunized rats (group C2) and significantly more than group E1. * $p < 0.05$ compared to group C1. **b** H_1 and $5-HT_2$ receptor signaling is not involved in allergy-induced food aversion. Immunized rats treated (group E2) or not (group E1) with H_1 and $5-HT_2$ blockers developed immune-induced food aversion and drank less OVA solution than nonimmunized animals (groups C1 and C2). * $p < 0.05$ compared to respective controls. **c** $5-HT_3$ signaling is partially involved in allergy-induced food aversion. Although immunized animals treated with a $5-HT_3$ antagonist (group E2) drank less OVA solution than nonimmunized rats (groups C1 and C2), the magnitude of the aversion was reduced and the amount of antigen-containing solution consumed was about 100% higher compared to nontreated allergic animals (group E1). * $p < 0.05$ compared to respective controls. Six animals per group were used for both the dexamethasone and ondansetron treatments and 8 per group for promethazine plus methysergide. The experiment was performed twice.

Table 1. PCA in mice using rat sera

	Source of sera		
	control rats	OVA-immunized rats	tolerant rats
Reciprocal of highest PCA dilution	<20	≥80	<20

Serum from tolerant animals leads to a pattern and magnitude of reaction similar to that with sera from nonsensitized (control) rats, while serum from OVA-sensitized animals causes a strong passive cutaneous response to OVA. The experiment was performed 3 times.

Tol) did not develop food aversion. Instead, as shown in figure 3, while OVA-immunized rats (group E) showed a significant reduction in fluid consumption ($p < 0.01$), tolerant rats presented the same pattern of preference for the sweetened OVA-containing solution displayed by non-immunized animals (group C). No differences in fluid consumption were found between groups Tol and C.

Tolerance induction in rats was confirmed by PCA tests. Thus, injection of serum from OVA-immunized rats into the back skin of naïve recipient rats led to increased vascular permeability following intravenous challenge with the antigen plus Evans blue compared to recipients injected with serum from tolerant animals (table 1). Confirming previous findings, the results indicate a clear correlation between OVA-specific antibody titers in the serum and the development of food aversion.

Pharmacological Manipulation

During allergic responses, mast cells release several mediators, including histamine, 5-HT, platelet-aggregating factor, leukotrienes and a variety of cytokines that can elicit many events associated with allergic inflammation, such as edema and cellular infiltration [9]. Thus, we first sought to examine whether treatment with dexamethasone would be able to prevent immune-induced food aversion. Exogenous glucocorticoids are known to exert potent anti-inflammatory effects, including modulation of cytokine production and mast cell degranulation [25, 26]. As depicted in figure 4a, two doses of dexamethasone (1.0 mg/kg/dose) given 48 and 24 h before the aversion test were able to prevent the aversion of OVA-immunized rats to the sweetened OVA solution. Indeed, the amount of sweetened OVA solution ingested by OVA-immunized rats treated with dexamethasone (group E2) did not differ from that recorded for nonimmunized animals (groups C1 and C2). Further analyses showed a significant difference ($p < 0.05$)

between data of OVA-immunized rats treated with dexamethasone and those of the similarly immunized but untreated group (E2 and E1, respectively; fig. 4a).

We next investigated whether specific histamine and 5-HT receptors were involved in food allergy-induced aversion. As shown in figure 4b, blockade of H_1 and 5-HT₂ receptors, which are involved in edema formation following mast cell degranulation, did not abrogate food aversion in OVA-immunized rats. Indeed, although differences were found between OVA-sensitized and nonsensitized animals ($p < 0.05$), no differences in fluid consumption were found between OVA-immunized rats treated with promethazine + methysergide and the similarly immunized but untreated rats (E2 and E1, respectively; fig. 4b). In contrast, the effects of treatment with ondansetron suggest that 5-HT₃ receptors, which are present in sensory C fiber nerve terminals, might play a role in signaling food allergy to the brain. Indeed, although not statistically different, a 100% increase in the consumption of the sweetened OVA solution was observed in ondansetron-treated OVA-sensitized rats compared to that of OVA-sensitized rats that received no treatment (E2 and E1, respectively; fig. 4c). However, OVA-immunized rats treated with the 5-HT₃ receptor antagonist still drank significantly less OVA solution than nonimmunized animals (E2 compared to groups C1 or C2; fig. 4c). Again, the consumption of OVA-sensitized animals differed to that of control, nonsensitized rats ($p < 0.05$).

Food Aversion Is Not Associated with Allergy-Associated Symptoms Such as Edema

Finally, we investigated whether there is any direct relationship between food aversion and allergic symptoms such as increased vascular permeability or edema formation. To answer this question, we injected serum from OVA-immunized mice into the back skin of recipient rats treated with dexamethasone, a combination of H_1 and 5-HT₂ blockers or an antagonist of 5-HT₃ receptors. The recipient rats were further challenged intravenously with the antigen plus Evans blue in order to evaluate antibody-dependent mast-cell-degranulation-induced vascular permeability. We found that treatment with dexamethasone or with H_1 /5-HT₂ blockers was able to reduce edema formation compared to nontreated recipient rats (table 2). In contrast, although the 5-HT₃ blocker ondansetron interfered with food aversion, it was not able to prevent the allergy-induced increase in vascular permeability. In fact, there were no differences in edema formation when comparing ondansetron-treated and nontreated recipients (table 2).

Table 2. PCA in rats using mouse sera

	Treatment			
	untreated	dexamethasone	promethazine + methysergide	ondansetron
Reciprocal of highest PCA dilution	≥320	≤40	<20	≥320

Serum from OVA-immunized mice leads to a strong PCA reaction in untreated rat recipients. Dexamethasone or a combined treatment with promethazine and methysergide decreases the magnitude of the PCA in recipient rats. Ondansetron has no effects on the formation of edema as evaluated by PCA. The experiment was performed twice.

Discussion

Many studies have proved the existence of a direct relationship between food allergy and behavioral alterations. OVA-immunized mice given the opportunity to choose between water and a sweetened OVA solution (placed in two separate bottles positioned on opposite sides of their cages) prefer to ingest water, whereas control nonimmunized animals show a preference for the sweetened solution [3–5, 17, 24]. Employing this two-bottle preference test, we confirmed and extended our previous data, showing that the same phenomenon occurs in rats. We showed that nonimmunized rats choose to ingest the OVA-sweetened solution, whereas the OVA-immunized rats drink water instead, avoiding the supposedly preferable, allergen-containing solution (fig. 1b). Furthermore and importantly, no difference in total fluid intake was found between these two groups (fig. 1a).

To further test this immune-induced behavioral change, we developed a new food aversion test. In this model, rats were first conditioned to drink water for 10 min twice daily. The water was then replaced by a sweetened OVA-containing solution. As shown in figure 2b, whereas nonimmunized rats drank the sweetened OVA solution, those that were immunized to OVA, although deprived of water, avoided drinking the OVA solution. Again, no differences in fluid consumption were found between the animals of these two groups on the day before the test (fig. 2a). Thus, the present results, which were repeated here many times, reinforce the idea that OVA-immunized rats avoid the ingestion of solutions that contain the antigen. Therefore, and as reported for mice [3–5, 17], immunized rats undergo a shift in behavior that

makes them capable of avoiding the source of the allergen that would trigger an immediate allergic reaction.

Immunological tolerance is accomplished by offering a solution of OVA in tap water prior to, or along with, the primary OVA immunization. When animals receive oral OVA from days –7 to –2 (prior to OVA immunization), they do not produce either class (IgG1 or IgE) of OVA-specific antibodies [5]. We have now shown (fig. 3) that the induction of oral tolerance 5 days before OVA immunization was effective in preventing the development of food aversion in rats (measured in the new aversion test). This suggestion agrees with others we reported elsewhere. Indeed, we previously showed that the induction of oral tolerance in mice totally prevented the establishment of avoidance responses and the increase in Fos staining observed in the paraventricular nucleus of the hypothalamus and central nucleus of the amygdala following allergic reactions [4, 5]. We also showed that anti-IgE antibody given to mice 7 days before the challenge was able to block the development of OVA aversion in immunized animals [4, 5].

Accordingly, in the present experiment, oral tolerance inhibited the IgE-mediated PCA reaction. PCA is one of the most important *in vivo* models for anaphylaxis in a local allergic reaction [27, 28], and it is induced by several mediators, including histamine secreted by mast cells [29, 30]. Thus, it seems feasible to suggest that the process triggering the food-induced aversion relies, at least in part, on IgE-mediated mast cell degranulation. This suggestion is in line with our previous work on food-induced allergy and experimental allergic asthma in mice [5].

The new method we used to study immune-induced food aversion allowed some pharmacological manipulations. Indeed, since animals were conditioned to drink water for 10 min twice a day, we opened a ‘window in time’ to look at some possible effects of drugs interfering with mast cell degranulation and with inflammatory mediators involved in the immediate phase of the allergic response. This short period is also important to rule out pavlovian conditioning in our experimental conditions. Since aversion is observed in a single-session paradigm (i.e. without any previous learning or training sessions) and takes place shortly after the first time the antigen-containing solution is presented, behavioral conditioning could not be accountable for this response. In fact, we showed here that food aversion develops and can be detected 10 min after the antigen-containing solution is presented for the first time to OVA-immunized rats. Additionally, employing a paradigm in which the OVA solu-

tion was not sweetened, we found similar results (data not shown); i.e. the results are not influenced by association with sensory cues such as a sweet taste.

Glucocorticoids are among the most effective anti-inflammatory agents for the treatment of mast-cell-related allergic diseases [31, 32]. Our data showed that dexamethasone treatment significantly increased the consumption of sweetened OVA solution by OVA-immunized rats (fig. 4a). This observation agrees with data reported elsewhere showing that a similar treatment reversed the preference of OVA-sensitized mice for a sweetened egg white solution [24]. The anti-inflammatory actions of glucocorticoids in allergic processes are in part attributed to suppression of signaling events leading to mast cell degranulation and the release of lipid inflammatory mediators [35–37]. Thus, it could be thought that dexamethasone abrogated the food aversion due to its effects during the immediate early phase of the allergic response. Mast cell activation and degranulation are the first phenomena observed during the course of the immediate phase of the allergic response [8, 9]. Indeed, dexamethasone treatment decreased the IgE-mediated PCA reaction to about 22%. Altogether, our data point towards an involvement of IgE-mediated mast cell degranulation in the development of allergy-induced food aversion.

We then went on to investigate in more detail the participation of histamine and serotonin, which are both released by IgE-mediated mast cell degranulation [8, 9], in allergy-induced food aversion. In fact, there is substantial evidence that histamine and serotonin play important roles in the pathogenesis of allergic disorders [38, 39]. For instance, histamine and serotonin signaling through H_1 and $5-HT_2$ receptors, respectively, are known to be important mediators of edema formation during the immediate phase of allergic responses [40, 41]. Interestingly, our data showed that although effectively precluding the increase in vascular permeability seen in the PCA, treatment with a combination of promethazine and methysergide, which block H_1 and $5-HT_2$ receptors, respectively, was unable to modify the fluid consumption in OVA-immunized rats (fig. 4b). This finding confirms that although causing edema, signaling through H_1 and $5-HT_2$ receptors is not fundamental to the development of allergy-induced food aversion.

We then investigated a possible role played by $5-HT_3$ receptors in allergy signaling to the rat brain. $5-HT_3$ receptors have been shown to be expressed in vagal afferents present in the gut mucosa [42, 43], and evidence has been provided that they are activated following antigen-induced mast cell degranulation [44, 45]. Our results

showed that treatment with the $5-HT_3$ receptor antagonist ondansetron, although not totally abrogating the development of allergy-induced food aversion, increased the consumption of OVA-sweetened solution by OVA-immunized rats by about 100% (fig. 4c). In addition, as expected, $5-HT_3$ blockade did not modify edema formation following antibody-dependent mast cell degranulation in the skin. This might be an indication that, although not directly involved in allergic symptoms such as edema formation, $5-HT_3$ receptor signaling is responsible for conveying to the brain at least part of the information derived from the allergic response in the gut. In accordance, we have previously reported that sensory C fibers, which are reported to express $5-HT_3$ receptors [46], are partly responsible for signaling allergic responses to the brain. Indeed, in a similar way, the destruction of C fibers by neonatal capsaicin did not abrogate the development of allergy-induced food aversion but significantly reduced its magnitude [3].

Considering the findings described here, it is possible to conclude that immune-induced food aversion does occur in rats and can be assessed by a different experimental protocol to that used until now. We also showed that oral tolerance to OVA blocked allergy-induced food aversion and IgE-mediated mast cell degranulation (PCA reaction) in OVA-immunized rats. Altogether, these data support the notion that IgE-mediated mast cell degranulation and consequent $5-HT_3$ signaling are involved in the process that triggers avoidance to the source of the allergen in allergic rats.

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