

Hypersensitivity to ovalbumin induces chronic intestinal dysmotility and increases the number of intestinal mast cells

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Abstract Undiagnosed food allergies have been proposed as possible causes of promoting and perpetuating irritable bowel syndrome. Our aim was to find out if sensitization could induce chronic functional motor disturbances in the intestine and the mechanisms implicated. Rats were sensitized to ovalbumin (OVA) following three hypersensitivity induction protocols, two parenteral and one oral. Rat mast cell protease II (RMCP II) release in response to OVA challenge and immunoglobulin E (IgE) concentration were measured in serum. At least 1 week after challenge, small intestinal motility was evaluated using strain gauges. Intestinal tissue samples from orally sensitized rats were checked for *in vitro* stimulation with OVA. Mucosal mast cells were counted from duodenum sections. All sensitized rats showed intestinal hypermotility. Only rats sensitized by parenteral procedure showed an increase in RMCP II after OVA challenge in serum. IgEs increased only in the *Bordetella pertussis* sensitized group. Small intestine sections from orally sensitized rats released more RMCP II than sections from control rats. All sensitized rats showed an increase in the number of mucosal mast cells in duodenum. In conclusion, hypersensitivity to food proteins induces chronic motor alteration that persists long after antigen challenge and an excited/activated state of sensitized mucosal mast cells.

Keywords hypersensitivity, intestinal motility, irritable bowel syndrome, mucosal mast cells, rat mast cell protease II.

INTRODUCTION

The underlying cause of the physiopathological symptoms in irritable bowel syndrome (IBS) remains unclear. In recent years, a number of factors have been suggested as having a role in promoting and perpetuating IBS: previous episodes of infectious enteritis, genetic factors, changes in bacterial microflora and undiagnosed food allergies or food intolerance.^{1,2}

Allergic reactions are known to evoke inflammatory cell infiltration and activation at various mucosal sites, including the gastrointestinal tract.^{3,4} Mucosal mast cells lie in close proximity to enteric nerves⁵ and release a wide array of inflammatory mediators that are capable of affecting enteric nerve function^{6–8} and muscle contractility.²

Animal models of intestinal allergy, using food proteins or enteric parasites as sensitizing agents, demonstrate alterations of gut function, including changes in intestinal ion transport, permeability and motility.^{2,9}

However, because of the fact that allergy is considered a systemic response rather than a localized response, most animal models of food allergy research have been conducted using parenteral sensitization (with the use of adjuvants) and enteral challenges.^{10–12} Sensitization to food proteins administered through the diet or by gavage has only been used in high-immunoglobulin E (IgE) responders such as the Brown Norway rat.^{13,14}

Intestinal motility alterations have been associated with mucosal mast cell degranulation after antigen challenge in sensitized rats.^{9,15–17} However, there are no long-term studies evaluating chronic changes induced by food sensitization.

The aim of our study was to find out if sensitization could induce chronic functional motor changes and to assess the involvement of mucosal mast cells. Both parenteral and oral hypersensitivity induction protocols were used, and the motor activity of the intestine

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including intestinal mucosal mast cell number and activity were evaluated. Parts of this study were communicated previously in abstract form.¹⁸

MATERIALS AND METHODS

Animals

In this study, 59 male Sprague–Dawley rats (Charles River, Les Oucius, France) were used. Animals had free access to water and a standard pellet diet that did not contain any trace of ovalbumin (OVA) or any other egg derivative. Rats were pathogen free when purchased and they were kept in clean conventional conditions (between 1 and 10 weeks). They were maintained in an environmentally controlled room in groups of three to four animals. The night before the experiment, food was removed and each animal was caged individually. Control animals were handled the same way as treated animals. Rats were purchased at different ages according to the duration of sensitization protocol. In consequence, all animals had similar ages and weight at the time of the experiment.

Substances

Ovalbumin Grade V (Sigma, St Louis, MO, USA) was diluted in sterile saline to induce sensitization and in non-sterile saline for challenge. CCK-8, sulphated form (Peptide Institute, Osaka, Japan), was diluted in 1% sodium bicarbonate to 10^{-4} mol L⁻¹ and sterile saline to work concentration. Atropine (Merck, Darmstadt, Germany) and *N*_ω-nitro-L-arginine (L-NNA) (Sigma) were diluted in sterile saline. Compound 48/80 (Sigma) was diluted in Krebs buffer. Bovine serum albumin (BSA) Grade V (Sigma) was diluted in sterile saline.

Hypersensitivity to ovalbumin: induction protocols

Several protocols for inducing sensitivity to OVA were used. All of them have been previously reported by several authors.^{10,18}

Bordetella pertussis protocol Rats ($n = 9$) 8–10 weeks old were sensitized as described previously¹⁰ with 1 mg of OVA and 25 mg of Al(OH)₃ in 1 mL of sterile saline injected subcutaneously (s.c) and 0.5 mL of *B. pertussis* vaccine (2×10^{10} organisms; Berna Institute, Madrid, Spain) injected intraperitoneally (i.p).

11-day protocol Rats ($n = 9$) 8–10 weeks old were intraperitoneally (i.p) sensitized as described previously¹⁴

with 0.5 mL of 0.2 mg mL⁻¹ OVA solution in sterile saline injected on days 0, 2, 4, 7, 9 and 11. To potentiate the immune response, 0.2 mL of 25 mg mL⁻¹ Al(OH)₃ adjuvant suspension in sterile saline mixed with 0.5 mL of OVA was injected on day 0.

Oral protocol Following Knippels,¹⁹ 6-week-old rats ($n = 12$) were orally exposed to OVA by gavage (1 mg OVA mL⁻¹ saline solution, 1 mL/animal). Gavage dosing was performed daily during 6 weeks.

Controls

A group of rats ($n = 11$) not exposed to OVA was used as control (unsensitized control group). Three sham groups, *B. pertussis* sham ($n = 6$), 11-day sham ($n = 6$) and oral sham ($n = 6$), in which induction protocols were conducted as described before but replacing OVA by saline solution were used as control groups of the *B. pertussis*, 11-day and orally sensitized groups respectively.

Challenges and blood samples

After the sensitization period, animals were submitted to either saline (0.5 mL of saline solution 0.9% NaCl) or OVA (0.5 mL of 0.2 g mL⁻¹ OVA solution) challenge by gavage. Unsensitized control and sham animals were also challenged as described for sensitized animals. As a control of the specific response to OVA, two rats sensitized after the 11-day induction protocol were submitted to BSA challenge (0.5 mL of 0.2 g mL⁻¹ BSA solution). Following previous studies,^{7,20} challenge in the *B. pertussis* sensitized group was performed 14 days after hypersensitivity induction, 28 days after the first injection in the 11-day sensitized group and 7 days after the end of the induction period in the orally sensitized group. The night before the challenge, food was removed and the animal was caged individually. Blood samples from the saphenous vein were taken before (T_0) and 3 h after (T_f) challenge. Animals did not have access to food until the two blood samples had been taken. Serum obtained was kept at -30 °C until Rat mast cell protease II analysis was performed.

Motility experiments: animal preparation

A week after challenge each animal was fasted for 16–18 h before experiments. Anaesthesia was induced by inhalation of halothane to allow cannulation with polyethylene tubing of the right jugular vein. Stage III of anaesthesia was maintained with thiopental

sodium bolus infusion in the jugular vein as required. Body temperature was maintained at 37 °C by placing the rat on a heating pad. Rats were tracheotomized to facilitate spontaneous breathing. The abdomen was opened through a midline incision and the intestine was exposed. Three strain gauges (3 × 5 mm; Hugo Sachs Elektronik, March-Hugstetten, Germany) were placed to record circular muscle activity and sutured to the intestinal wall of duodenum (at 2 cm from the pylorus), proximal jejunum (at 2 cm from Treitz's ligament), and ileum (10 cm from cecum). Strain gauges were connected to high-gain amplifiers (Lectromed MTP8, Lectromed, New Jersey, UK) and amplified signals were sent to a recording unit (PowerLab/800; ADI Instruments, Sydney, Australia). Two electrode holders, with two platinum electrodes each were inserted into the intestinal lumen through a small incision of the intestinal wall to induce ascending excitation of the peristaltic reflex by stimulating of intestinal mucosa, as previously described.²¹ Electrode holders were placed 1 cm distally to the strain gauge of the duodenum and the ileum. Animals were killed at the end of the experiment with an overdose of anaesthesia. All experimental procedures were approved by the Ethical Committee of the Universitat Autònoma de Barcelona.

Experimental protocol for motor response evaluation

Before starting the protocol, a sample of blood from the jugular vein was taken for IgE analysis. After an equilibration period of 30 min, spontaneous motor activity was recorded during 60 min. Afterwards, CCK-8 (3×10^{-9} mol kg⁻¹ min⁻¹) was intravenously infused. After at least 1 h to allow a return to basal conditions, mucosal electrical stimulation of the duodenum and ileum to elicit ascending excitation was applied at 30 V, 0.6 ms and 2, 4 and 6 Hz. An intravenous bolus of L-NNA (10^{-5} mol kg⁻¹) was administered 10 min after electrical stimulation. After at least 30 min to allow motility normalization, the pattern of electrical stimulation was repeated. Afterwards, atropine (0.3 mg kg⁻¹) was also intravenously infused as a bolus and 10 min later the electrical stimulus was repeated. The area under the curve (AUC in mm²) was measured during the first 60 min-period of the protocol, during electrical stimulation and for the 10 min of CCK-8 infusion. Inhibitory tone was evaluated comparing the AUC taken for 10 min before L-NNA infusion with the AUC taken for 10 min starting 10 min after L-NNA infusion. Frequency expressed as contractions/minute was also measured

during spontaneous motility recording and after L-NNA infusion. This protocol has been previously validated.²²

Measurement of RMCP II

Rat mast cell protease II concentration in the serum was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Moredun Animal Health, Edinburgh, UK) as previously described.^{23,24}

Measurement of total IgE

Serum samples for IgE detection were frozen and stored at -30 °C until assayed. IgE concentration in the serum was measured by ELISA using a commercial kit (Crystal Chem Inc., Chicago, IL, USA).

In vitro stimulation of small intestine sections

Unsensitized control ($n = 5$) and orally sensitized ($n = 6$) rats were killed by decapitation and the abdominal cavity was opened to remove the small intestine. Three centimetre segments were taken from the duodenum, jejunum (beginning 10 cm distal to the Treitz's ligament) and ileum and placed in Krebs buffer bubbled with a mixture of 5% CO₂ and 95% O₂ (pH 7.35 and 37 °C) containing (in mmol L⁻¹): NaCl, 115; MgCl₂, 1.2; KH₂PO₄, 2; NaHCO₃, 25; glucose, 11.1; CaCl₂, 1.25. The tissue was then opened along the mesenteric border and sectioned into pieces of 1 cm² approximately. Each piece was placed in a tube with 2 mL of oxygenated Krebs buffer. After an equilibration period of 10 min, a 200 µL sample was taken from each tube (initial sample) and the volume was replaced by Krebs buffer (control) or OVA solution (1 mg mL⁻¹) or compound 48/80 (2 mg mL⁻¹) at each small bowel segment. Three hours later, 200 µL samples were taken from the tubes (poststimulation sample) and the tissue segments were weighed and placed in 2 mL of fresh Krebs buffer, ground and submitted to three cycles of freezing/thawing in order to break mucosal mast cell membranes and release cellular contents. After 5 min centrifugation at 3220 g, 200 µL samples from supernatants were taken (pellet sample). All the samples were frozen and stored at -30 °C until assayed for RMCP II analysis by ELISA as described. Rat mast cell protease II concentrations were standardized by the weight of the respective specimen. The sum of initial, poststimulation and pellet RMCP II was considered as the total RMCP II for each specimen.

RMCP II immunohistochemistry and mucosal mast cell count

Immunodetection of RMCP II was carried out on paraformaldehyde-fixed duodenum sections using a monoclonal antibody (Moredun). Detection was performed with avidin/oxidase (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA). Sections were counterstained with haematoxylin and counted at $\times 400$ magnification. Positively stained mast cells were counted in three to five sections per animal. Seven to 10 well-oriented villus-crypt units (VCU) were examined per section using an Olympus CH2 microscope (Olympus, Barcelona, Spain). Analysis of all morphological data was performed blinded to prevent observer bias.

Statistics

All data are expressed as mean $\pm$ SEM. Statistical analysis of the results was performed with ordinary ANOVA followed by a *post hoc* Bonferroni test for the motility studies and mast cell counts between control and sham groups. Unpaired student's *t*-test was used for comparison of each sensitized group with its respective sham group. Statistical analysis of results for the challenge was performed with two-way ANOVA followed by *post hoc* Bonferroni test. Statistical analysis of results for the *in vitro* stimulation was performed with unpaired student's *t*-test. Differences between groups were considered statistically significant when $P < 0.05$.

RESULTS

Spontaneous activity

Control unsensitized rats showed spontaneous activity consisting of isolated contractions with a frequency of 0.2–0.8 contractions min^{-1} in the duodenum (67% of the experiments), jejunum (34%) and ileum (50%). In sensitized rats, these contractions were substituted by intense bursts of clustered contractions followed by periods of inactivity. However, these changes varied according to the intestinal segment and the hypersensitivity induction protocol followed (Fig. 1). No differences were found in intestinal motility between control rats and sham groups.

The duodenum was the section of the small intestine most affected by sensitization. Frequency (contraction/min) and magnitude of contractions ($\text{mm}^2 \text{min}^{-1}$) significantly increased in all the three sensitized groups (Fig. 2). Motor alterations in the jejunum and ileum were not as evident as in the duodenum. Only the 11-day sensitized group showed a

significant increase in magnitude of contractions when compared with controls (Table 1).

CCK motor response

In control rats, intravenous infusion of CCK-8 [$3 \times 10^{-9} \text{ mol kg}^{-1} (10 \text{ min})^{-1}$] induced an increase of both tonic and phasic contractions in the duodenum and an inhibition of spontaneous mechanical activity in the jejunum. No difference was found between unsensitized controls [AUC (mm^2) $(10 \text{ min})^{-1}$, $(550.3 \pm 91.49, n = 6)$] and sham groups (*B. pertussis* $566.2 \pm 126.6, n = 6$; 11-day $458.1 \pm 82.32, n = 6$; oral $669.5 \pm 141.6, n = 6$; $P = 0.61$). The response to CCK was slightly greater in sensitized groups (*B. pertussis* $1017 \pm 267.9, n = 7, P = 0.21$; 11-day sensitized $635.7 \pm 116.9, n = 7, P = 0.26$; oral sensitized $960.6 \pm 172.7, n = 6, P = 0.22$) when compared with its respective sham group. However, the great variability in the responses prevented a statistical significance. Inhibitory response to CCK remained unchanged in all the sensitized groups.

Inhibitory tone

The magnitude of contractions in the jejunum increased after L-NNA both in control rats and in sham groups when compared with their respective spontaneous motility before L-NNA administration. This increase of magnitude was slightly higher at the animals receiving adjuvants. Similar results were found in the duodenum and ileum. In contrast, in the sensitized groups L-NNA induced no further increase in intestinal spontaneous motility (Fig. 3).

Effect of sensitization on the ascending contraction

Electrical stimulation on the intestinal mucosa always induced an ascending contraction recorded at the oral strain gauge. This response was increased by L-NNA and reduced by atropine. Table 2 shows responses obtained in both unsensitized and sensitized groups after electrical field stimulation at 2 and 6 Hz in duodenum and ileum (4 Hz data were omitted to simplify presentation of the results). In general, ascending contraction was not modified in any OVA-sensitized group.

Total IgE concentration

No difference was observed in IgE concentration between unsensitized rats ($13.41 \pm 4.32, n = 15$) and

sham groups (*B. pertussis* 8.84 ± 2.7 , $n = 5$; 11-day 15.32 ± 2.22 , $n = 5$; oral 15.05 ± 1.56 , $n = 6$). Serum total IgE concentration significantly increased only in the *B. pertussis* sensitized group (180.9 ± 58.43 ng mL⁻¹, $n = 9$; $P < 0.001$) when compared with *B. pertussis* sham group. Neither the 11-day sensitized (12.23 ± 5.6 , $n = 9$) nor orally sensitized (8.37 ± 4.45 , $n = 8$) groups showed any significant increase of IgE.

Systemic mucosal mast cell activation (RMCP II release)

Serum RMCP II concentrations, a marker of mucosal mast cell activation, were similar at prechallenge (T_0)

in all groups. No difference was observed in RMCP II concentration after sham challenge (T_t) with saline solution. In contrast, OVA challenge resulted in significantly high levels of serum RMCP II both in the *B. pertussis* sensitized group and in the 11-day sensitized group postchallenge (T_t) when compared with unsensitized groups (Fig. 4) (no difference was observed between pre- and postchallenge RMCP II concentration between unsensitized control and sham groups). No changes in RMCP II concentration were observed in the orally sensitized group after OVA challenge. Challenge with BSA in the 11-day sensitized rats did not modify RMCP II concentration.

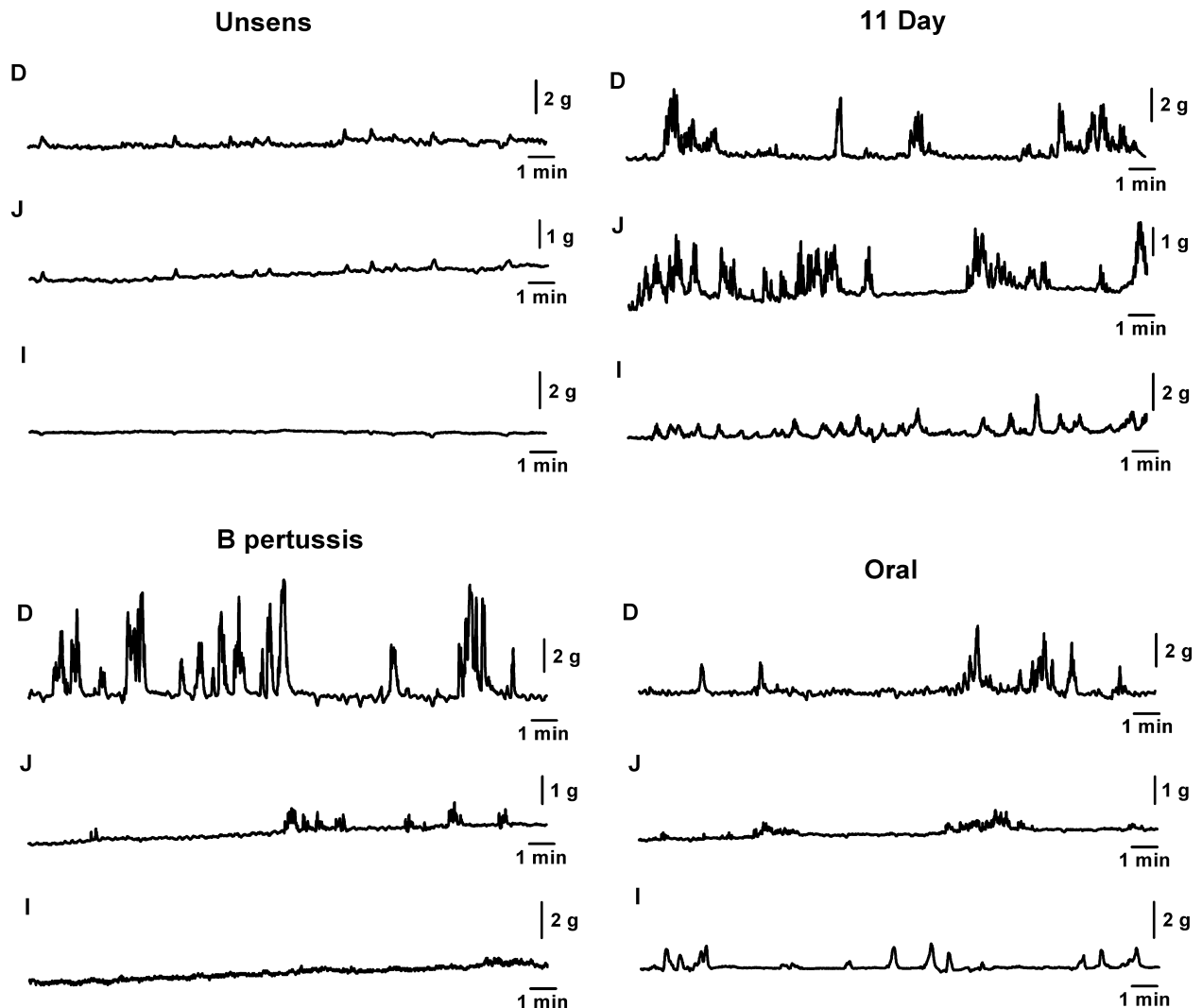


Figure 1 Representative recordings of mechanical activity showing the spontaneous motor events in duodenum (D), jejunum (J) and ileum (I) in unsensitized control (unsens) group and after OVA hypersensitivity induction by three different protocols (*Bordetella pertussis*, 11-day and oral). Similar recordings were obtained in all the rats of each group.

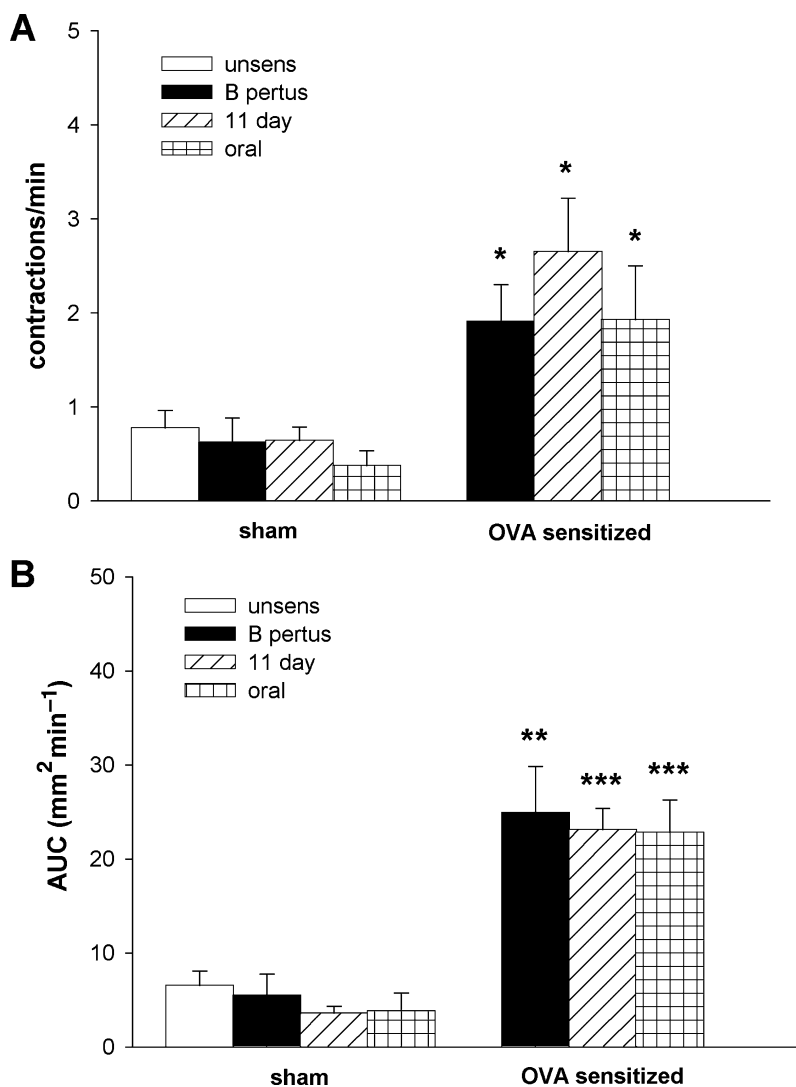


Figure 2 Quantification of the spontaneous motility (frequency and magnitude of the contraction) in the duodenum of unsensitized control ($n = 6$), sham sensitized [*Bordetella pertussis* ($n = 6$), 11 day ($n = 6$), and oral ($n = 6$)] and ovalbumin-sensitized rats [*B. pertussis* ($n = 9$), 11-day ($n = 9$) and oral ($n = 6$)]. * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$, respectively, vs respective sham sensitized group.

Table 1 Spontaneous motility in jejunum and ileum of unsensitized control, sham sensitized groups, *Bordetella pertussis* sensitized, 11-day sensitized and orally sensitized rats

	Unsens	<i>B. pertussis</i> sham	11-day sham	Oral sham	<i>B. pertussis</i> sensitized	11-day sensitized	Oral sensitized
Jejunum							
Freq	0.18 ± 0.096	0.13 ± 0.074	0.35 ± 0.19	0.18 ± 0.11	0.77 ± 0.41	0.89 ± 0.45	0.27 ± 0.14
Magn	2.15 ± 0.37	2.92 ± 0.43	1.73 ± 0.39	2.07 ± 0.62	11.46 ± 4.74	18.06 ± 5.02*	3.3 ± 0.77
Ileum							
Freq	0.21 ± 0.09	0.39 ± 0.11	0.34 ± 0.11	0.12 ± 0.07	0.28 ± 0.15	0.74 ± 0.21	0.4 ± 0.14
Magn	2.39 ± 0.71	4.14 ± 0.69	4.52 ± 0.79	2.69 ± 0.98	3.48 ± 0.83	17.4 ± 2.08***	4.26 ± 1.23

Freq, frequency is expressed as contractions min⁻¹; Magn, magnitude of contraction AUC (mm²) min⁻¹ (mean ± SEM). * $P < 0.05$ or *** $P < 0.001$ in relation to spontaneous motility of its respective sham group (*B. pertussis* sham, 11-day sham or oral sham).

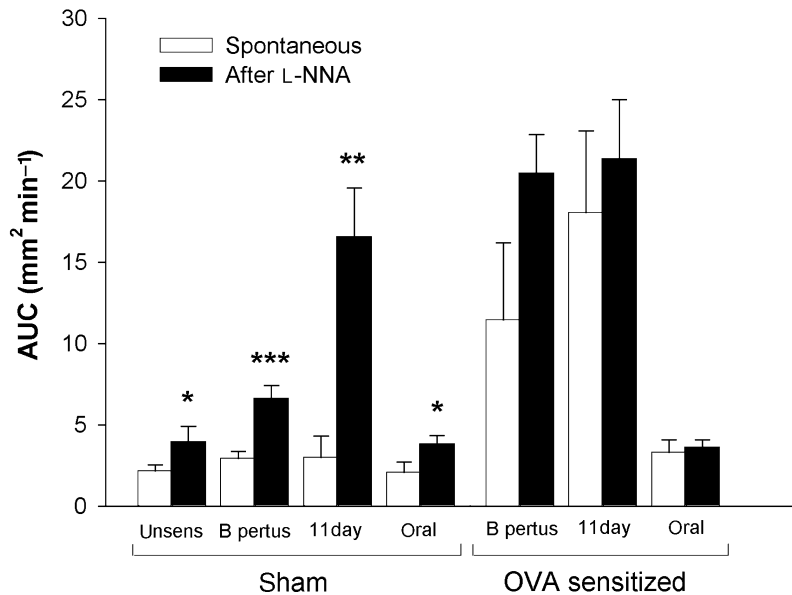


Figure 3 Effect of L-NNA infusion on magnitude of contractions in the jejunum of unsensitized control ($n = 6$), sham sensitized [*Bordetella pertussis* ($n = 6$), 11-day ($n = 6$) and oral ($n = 6$)], and ovalbumin sensitized rats [*B. pertussis* ($n = 9$), 11 day ($n = 9$), and oral ($n = 6$)]. * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$, respectively, vs spontaneous motility before L-NNA in the same group.

Local mucosal mast cell activation (RMCP II release from intestinal sections)

Spontaneous mucosal mast cell release of RMCP II in orally sensitized rats was higher than in unsensitized control rats in duodenum, jejunum and ileum (Fig. 5A). Rat mast cell protease II release was significantly higher in sensitized animals in relation to control unsensitized animals after OVA and 48/80 incubation (Fig. 5B).

RMCP II immunohistochemistry and mucosal mast cell count

Unsensitized control rats and sham groups showed similar number of mucosal mast cells (expressed per VCU). However, the number of mucosal mast cells increased significantly in all the three sensitized groups of rats compared with their respective sham groups (Fig. 6).

DISCUSSION

The results of this study demonstrate that food sensitized rats show chronic alterations in small intestine motility. These motor alterations are not due to an immediate OVA challenge, but are concurrent with increased number and activity of intestinal mast cells upon antigen challenge.

Allergy response to food is generally considered as a systemic response and, in consequence, most allergy studies are conducted using parenteral sensitization models. However, our results clearly show two

important facts: (i) that IgE increase due to OVA sensitization is not always present. Only the use of *B. pertussis*, a known IgE-selective adjuvant,²⁵ is able to increase IgE, and (ii) that it is possible to induce sensitization to food proteins, OVA in this case, without any detectable systemic increase of RMCP II. Our study corroborates that food sensitivity is very difficult to detect by conventional methods (IgE and mast cell mediators in serum), as previously proposed.²⁶

The results from the *in vitro* experiments clearly demonstrate that mucosal mast cells from orally sensitized animals release more RMCP II than those from unsensitized rats, even in the absence of any exogenous stimulus. Furthermore, the oral sensitized group showed an increase in the number of mast cells present in the intestinal mucosa. These results corroborate enteral sensitization to OVA and the implication of mast cells in the sensitization process. Moreover, the IgE and RMCP II increase observed in the serum after parenteral sensitization vs the absence of any detectable increase after enteral sensitization suggests that some allergic responses could be restricted to the intestine making them difficult to diagnose. Intestinal mucosal mast cells only represent 2–3% of the total lamina propria cells and RMCP II released by this relatively small number of cells could be non-detectable in the serum.

We previously demonstrated that intestinal mucosal mast cells from rats previously unexposed to OVA respond to OVA hydrolysate with an increase of RMCP II, modify nervous reflexes and modulate endocrine responses induced by intraluminal stimulus.^{23,24}

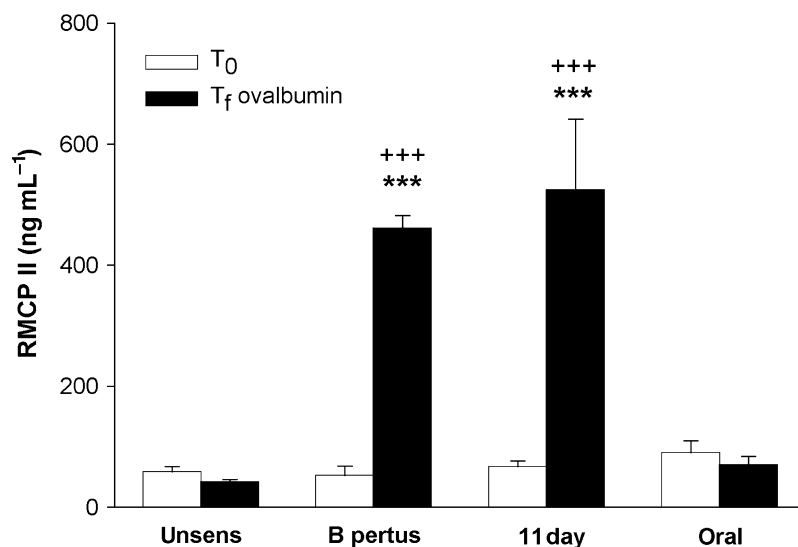
Table 2 Magnitude of ascending contraction expressed as AUC[mm² min⁻¹ (mean $\pm$ SEM)] after electrical stimulation at 2 and 6 Hz in duodenum and ileum before L-NNA infusion (control), after L-NNA infusion (L-NNA) and after atropine infusion (atropine)

	Control	L-NNA	Atropine
Duodenum			
2 Hz			
Unsensitized	18.27 $\pm$ 7.62	56.89 $\pm$ 8.8	3.83 $\pm$ 1.98
<i>B. pertussis</i> sham	18.31 $\pm$ 7.89	44.73 $\pm$ 13.3	1.66 $\pm$ 0.95
11-day sham	14.45 $\pm$ 7.43	28.54 $\pm$ 6.36	6.02 $\pm$ 1.77
Oral sham	21.5 $\pm$ 13.79	52.99 $\pm$ 6.47	8.15 $\pm$ 3.44
<i>B. pertussis</i> sensitized	46.8 $\pm$ 16.76	78.37 $\pm$ 27.07	0.39 $\pm$ 0.23
11-day sensitized	10.96 $\pm$ 3.21	57.42 $\pm$ 17.03	0.93 $\pm$ 0.54††
Oral sensitized	18.13 $\pm$ 8.26	37.55 $\pm$ 7.97	2.6 $\pm$ 1.16
6 Hz			
Unsensitized	32.76 $\pm$ 7.24	48.42 $\pm$ 15.81	3.41 $\pm$ 1.81
<i>B. pertussis</i> sham	21.49 $\pm$ 3.89	57.46 $\pm$ 14.46	1.86 $\pm$ 1.17
11-day sham	14.36 $\pm$ 3.63*	40.75 $\pm$ 9.72	2.2 $\pm$ 0.87
Oral sham	29.75 $\pm$ 5.63	38.96 $\pm$ 7.48	3.7 $\pm$ 2.16
<i>B. pertussis</i> sensitized	44.24 $\pm$ 16.39	94.43 $\pm$ 21.85	3.3 $\pm$ 0.5
11-day sensitized	18.33 $\pm$ 3.67	50.07 $\pm$ 9.73	1.81 $\pm$ 1.45
Oral sensitized	26.25 $\pm$ 7.63	66.02 $\pm$ 10.65†	1.53 $\pm$ 1.23
Ileum			
2 Hz			
Unsensitized	3.68 $\pm$ 1.1	5.06 $\pm$ 1.5	0.41 $\pm$ 0.23
<i>B. pertussis</i> sham	3.15 $\pm$ 2.025	30.01 $\pm$ 11.7	7.31 $\pm$ 4.94
11-day sham	0.37 $\pm$ 0.3	16.5 $\pm$ 14.03	8.91 $\pm$ 5.06
Oral sham	5.68 $\pm$ 5.6	9.89 $\pm$ 7.33	18.7 $\pm$ 8.37
<i>B. pertussis</i> sensitized	6.12 $\pm$ 3.36	15.24 $\pm$ 7.76	12.06 $\pm$ 5.14
11-day sensitized	8.16 $\pm$ 6.36	19.05 $\pm$ 6.96	19.92 $\pm$ 6.18
Oral sensitized	1.39 $\pm$ 0.48	2.31 $\pm$ 0.62	7.49 $\pm$ 3.9
6 Hz			
Unsensitized	39.85 $\pm$ 8.14	51.71 $\pm$ 14.72	13.27 $\pm$ 4.83
<i>B. pertussis</i> sham	47.49 $\pm$ 12.85	61.12 $\pm$ 16.12	24.28 $\pm$ 7.68
11-day sham	61.64 $\pm$ 13.59	93.42 $\pm$ 12.74	49.61 $\pm$ 7.87**
Oral sham	19.16 $\pm$ 6.25	28.44 $\pm$ 8.62	15.88 $\pm$ 5.74
<i>B. pertussis</i> sensitized	30.44 $\pm$ 6.83	107.1 $\pm$ 33.27	29.69 $\pm$ 12.32
11-day sensitized	56.52 $\pm$ 20.43	113 $\pm$ 24.55	34.16 $\pm$ 8.56
Oral sensitized	26 $\pm$ 7.68	39.9 $\pm$ 12.9	15.64 $\pm$ 7.51

* P < 0.05, ** P < 0.01 in relation to unsensitized control rats.

† P < 0.05, †† P < 0.01 in relation to respective sham sensitized group.

Figure 4 Rat mast cell protease II (RMCP II) concentration (ng mL⁻¹) in serum from unsensitized and ovalbumin (OVA) sensitized rats before (T_0) and after gavage challenge (T_f). All rats received OVA solution challenge (0.2 g mL⁻¹, 0.5 mL/rat). Bars represent mean $\pm$ SEM from unsensitized control (n = 4), *Bordetella pertussis* sensitized (n = 4), 11-day sensitized (n = 4), and orally sensitized (n = 6) rats. *** P < 0.001 compared with unsensitized controls; +++ P < 0.001 compared with T_0 values of the same group.



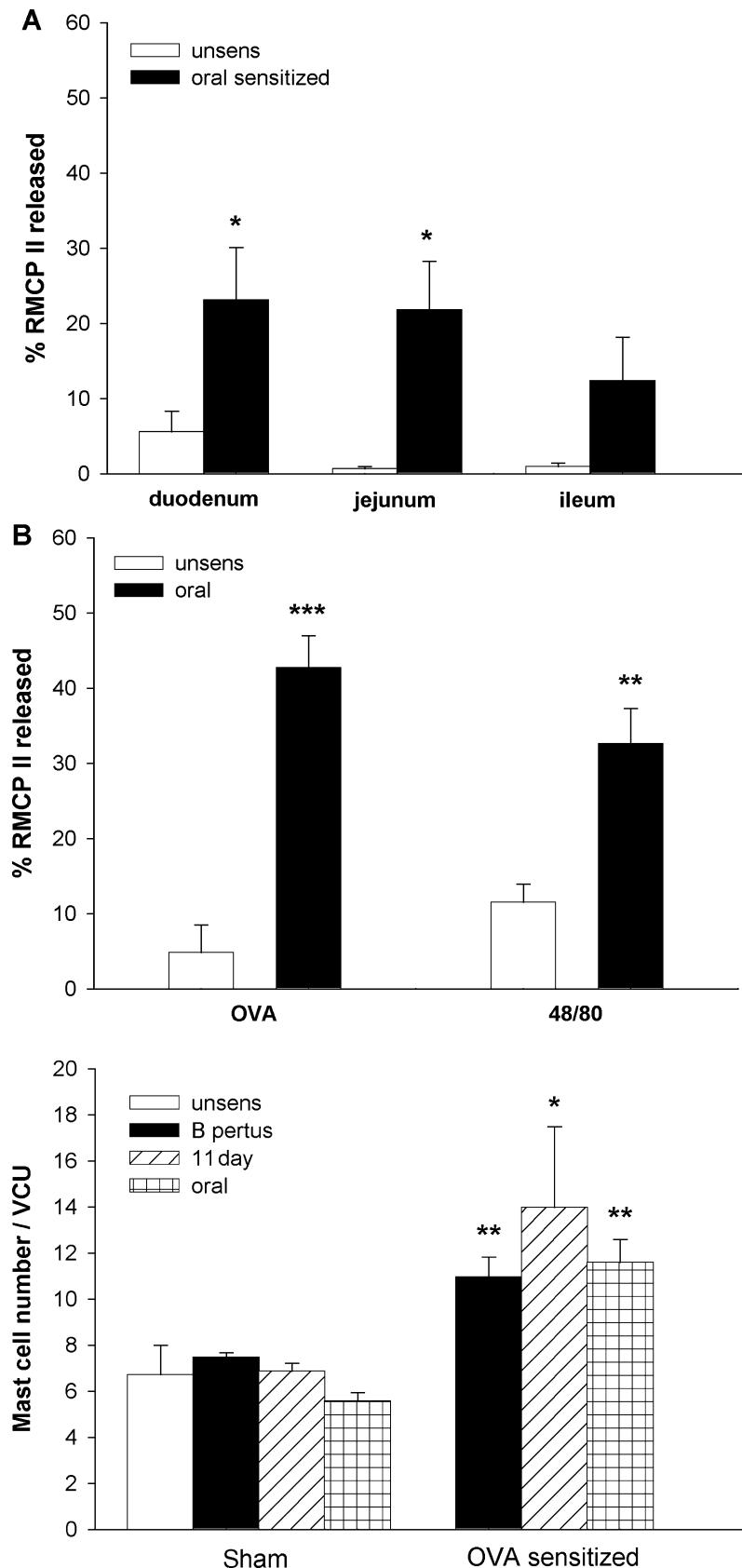


Figure 5 (A) Percentage of rat mast cell protease II (RMCP II) spontaneously released from duodenum, jejunum and ileum sections of unsensitized controls (unsens) and orally sensitized (oral sensitized) rats in basal conditions without stimulus. * $P < 0.05$ vs unsensitized control rats in the same intestinal section. (B) Percentage of RMCP II released from duodenum sections of unsensitized controls (unsens) and orally sensitized (oral) rats after stimulation with ovalbumin (OVA) and compound 48/80. ** $P < 0.01$ or *** $P < 0.001$, respectively, vs unsensitized control rats stimulated with the same secretagogue.

Figure 6 Duodenal mucosal mast cell number expressed per villus-crypt unit (VCU) counted in 7–10 units per section. Three to five sections were counted per rat. Bars represent mean $\pm$ SEM from four to six rats from each hypersensitivity induction protocol (sham and sensitized). * $P < 0.05$ or ** $P < 0.01$, respectively, compared with its respective sham group.

Therefore, it is possible that mast cell response to OVA in orally sensitized rats could be the consequence of the repeated exposure to OVA rather than a reaction of hypersensitivity. This hypothesis could also explain the absence of IgE or systemic RMCP II response to challenge. However, due either to allergic sensitization or continuous stimulation, the results of our study clearly show that animals with chronic intestinal motor disturbances have increased number of mast cells in the intestinal mucosa. There is a significant number of studies that demonstrate a correlation between mast cell activation and stimulation of the enteric nervous system located in close proximity to mast cells.^{27–29} The higher release of RMCP II even in basal conditions in orally sensitized animals also suggests that mucosal mast cells in these animals are in a continuous excited–activated state that maintains an increased excitability of the nervous structures of the intestine. Similarly, motility alterations observed in parenterally sensitized groups (*B. pertussis* and 11-day sensitized) could be related to an excited–activated state of their mast cells rather than due to the increase on mast cell numbers.

The proximity of mast cells to afferent nerve endings⁵ implies that mast cell mediator release could increase afferent sensitivity, explaining the alterations in visceral perception observed in IBS patients. Some substances released by mast cells are nociceptive molecules that not only decrease the threshold of sensitivity in mucosal sensitive afferent fibres, but also produce pain due to their action on nociceptive nerve pathways. Moreover, some substances released by mast cells can alter small intestine motility patterns^{30,31} and also contribute to IBS symptoms.

Our protocol was designed to evaluate the different structures controlling motility, therefore we briefly discussed each parameter. Concerning spontaneous motility, motor changes were observed in all sensitized animals but differ depending on the intestinal segment and the induction protocol considered. The three hypersensitivity induction protocols induced alterations in spontaneous motility in the duodenum. In contrast, alterations in jejunum and ileum motility were only evident in the 11-day protocol. Thus, hypersensitivity to OVA induces hypermotility mainly in the proximal areas of the intestine. The fact that 11-day protocol involved intraperitoneal injections could explain the wider reaction of the intestine in this model.

With respect to CCK response, an abnormal response to CCK has been suggested in patients with IBS.²⁰ We previously characterized the motor effects of CCK using the same experimental protocol as in the current

study.³² In addition, we also showed that inflammation induced by nematode infection increases the excitatory component of this response both at the duodenum and jejunum because of an increase of vagal sensitivity.^{22,33} In the present study, CCK response of sensitized animals, although of a larger magnitude, did not show a significant difference in relation to CCK response in unsensitized and sham animals. In consequence, while our results suggest a change in vagal afferent sensitivity induced by OVA sensitization, they do not actually confirm it.

L-NNA infusion blocks the tonic release of NO at the intestine and it is commonly used as a tool to evaluate functionality of the intestinal inhibitory tone. In a previous study, the jejunum showed the greatest response to L-NNA.³² However, in the present study, L-NNA did not increase motor activity in OVA sensitized rats, indicating that inhibitory tone in these animals is altered and that hypermotility observed after OVA sensitization could be, at least in part, a consequence of a decreased inhibitory tone.

Finally, regarding ascending contraction, distal electrical stimulation has been used extensively to induce ascending excitation, a well-characterized component of the peristaltic reflex.²⁴ The response to electrical stimulation observed in sensitized animals indicates that there is no significant effect of OVA sensitization at the nerve–muscle level.

In conclusion, both parenteral and enteral sensitizations to OVA induce chronic changes in mucosal mast cell sensitivity. Sensitized mucosal mast cells predispose the small intestine to overreact to intercurrent non-IgE-mediated stimulus. Chronic mast cell activation could be responsible for chronic changes in motor activity and could explain some of the clinical signs observed in IBS patients. This study demonstrates that enteral hypersensitivities to food could be a contributory factor of IBS symptoms and that such hypersensitivity is difficult to diagnose.

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