Rise of Prostanoids in Rat Small Intestinal Mucosa following Intestinal Protein Hypersensitivity

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ABSTRACT. The purpose of the present study was to establish whether there is an elevated prostaglandin concentration in the intestinal mucosa in rats suffering from an immediate type hypersensitivity reaction. Rats of the Hooded-Lister strain were sensitized and challenged with ovalbumin. Control rats were given adjuvant only. Prostanoid content of scraped mucosa was determined by radioimmunoassay. It was found that the prostaglandin E_2 content in the sensitized intestine was significantly elevated as compared to the controls. There was no significant rise of 6-keto prostaglandin $F_{1\alpha}$ or thromboxane E_2 in the sensitized rats. These results show that prostaglandin B_2 participates in intestinal immediate type responses and may explain some of the clinical manifestations of food protein allergy. (*Pediatr Res* 21: 414-416, 1987)

Abbreviations

PG, prostaglandin
TXB₂, thromboxane B₂
PCA, passive cutaneous anaphylaxis

The gastrointestinal manifestations of food hypersensitivity are similar to the effects elicited by administration of exogenous prostanoids (1). The possibility exists therefore, that prostanoids are involved in the symptomatology of immediate type hypersensitivity reactions in the intestine. In order to test this hypothesis, we used rats sensitized with ovalbumin, and subsequently challenged with this protein. We have previously shown that in these sensitized animals an immediate allergic type reaction in the small intestine can be developed upon challenge with ovalbumin (2). Herein we were able to show that the prostanoid content of such a challenged mucosa is raised.

MATERIALS AND METHODS

Sensitization and challenge. Rats of Hooded-Lister strain were used. Their weight ranged between 150 and 180 g. Nineteen rats were sensitized by an intraperitoneal injection of ovalbumin 250 μ g/0.5 ml with adjuvant consisting of killed *Bordetella pertussis* (1.6×10^3 /0.5 ml). On the 14th day a booster injection of ovalbumin (2.5 μ g/0.5 ml) was given intraperitoneally. In our experience this method of sensitization has always resulted in a

positive passive cutaneous anaphylactic reaction (2). The challenge procedure was based on that described by Byars and Ferraresi (3). On the 19th day the rats were challenged with 25 mg ovalbumin intragastrically (Sigma Chemical Co.) after an overnight fast. Forty-five min after the ovalbumin challenge, the rats were anesthetized with ether and then sacrificed. The first 5 cm of intestine immediately distal to the pylorus were removed, rinsed with ice-cold saline, and the mucosa was scraped and stored at 70° C for PGs determination. The control group consisted of 17 rats which were injected only with the adjuvant 19 days before challenge. They were challenged as the sensitized group.

Prostanoid determination. The tissue was sonicated in 1.0 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.0, containing 0.02 M EDTA. The homogenate was centrifuged and the supernatant was extracted twice with 2 vol of ether. The aqueous phases were assayed for their PG content.

PGE₂ was determined by radioimmunoassay as described by Bauminger *et al.* (4). TXB₂, the stable metabolite of TXA₂, and 6-keto PGF_{1 α} , the stable metabolite of prostacyclin, were determined by radioimmunoassay as previously described in detail (5). The protein concentration was determined by the method of Lowry *et al.* (6). Statistical evaluation of the data was based on the Student's *t* test for unpaired data.

RESULTS

PGE₂ content per wet weight in the small intestinal mucosa obtained from ovalbumin sensitized and challenged rats was significantly higher than its content in specimens obtained from the control group: 82 ± 29 and 51 ± 23 pg/mg (mean $\pm$ SD), respectively, $p < 0.005$ (Fig. 1). The data remained significant when calculated per mg protein. In the sensitized group consisting of 10 rats it was 691 ± 310 pg/mg protein *versus* 424 ± 155 (mean $\pm$ SD) in nine control rats ($p < 0.05$).

6-Keto PGF_{1 α} content in the small intestinal mucosa obtained from sensitized rats was 2.4 ± 1.7 pg/mg wet weight. This value was not significantly different from its content in specimens obtained in the control group, 2.9 ± 0.7 pg/mg wet weight (mean $\pm$ SD) (Fig. 1). TXB₂ in intestinal mucosa specimens obtained from sensitized rats did not differ significantly from the control group 0.8 ± 0.3 *versus* 1.3 ± 1.0 pg/mg wet weight (mean $\pm$ SD) (Fig. 1).

DISCUSSION

The gastrointestinal manifestations of food protein hypersensitivity include vomiting, diarrhea, malabsorption, and abdominal pain (7). It is likely that these symptoms have an immunological basis partly explained by an immediate type gastrointestinal allergic reaction to specific food protein. Exogenous PGs,

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Fig. 1. Prostanooid content of intestinal mucosa in ovalbumin-sensitized and control rats. Values in pg/mg wet weight, mean $\pm$ SD. C, control; T, sensitized group; NS, difference not significant.

in particular PGE₂, can affect the gastrointestinal tract by producing diarrhea and modifying intestinal motility. Diarrhea is a recognized adverse effect of PGs administered to pregnant women. Diarrhea has also been observed in volunteers who were treated with PGE₁ (8), PGE₂ (9), and PGF_{2α} (10). Experiments utilizing animal models have demonstrated enteropooling of fluid in response to treatment with PGE₂ (9).

The potential role of PG in food allergy has been suggested by Buisseret *et al.* (11, 12). They concluded that PGs (PGE₂ and PGF_{2α}) are involved in the mediation of increased intestinal motility and secretion. This hypothesis was based on their observation that in 11 of their 12 patients pretreatment with PG synthetase inhibitor, prior to the food challenge, prevented gastrointestinal as well as the other symptoms. In three of their patients blood and stool PG levels were determined and the changes in their concentrations correlated with the clinical symptoms. Subsequently two other reports on the role of prostanooid in food allergy have been published (13, 14). Similarly, Alun Jones *et al.* (15) have shown a rise in PGE₂ in rectal dialysate fluid following challenge by specific allergens in hypersensitive patients.

We have previously used a rat model to study an immediate type allergic response to ovalbumin (2) based on that described by Byars and Ferraresi (3). We believe that in this model the animal suffers from a local intestinal immediate type hypersensitivity for the following reasons. First, the PCA reaction was positive indicating that an immediate type allergy existed. Second, the rapid change in the permeability of the intestine which we measured in our previous experiments (2) was evidence that a local allergic response was taking place. In the present study we demonstrate a significant increase in PGE₂ content in the small intestinal mucosa after challenge with egg albumin as compared to unsensitized rats. On the other hand, the content of both 6-keto PGF_{1α} and TXB₂ in the control group was markedly low as compared to the content of PGE₂ and no significant change was observed in the contents of 6-keto PGF_{1α} and TXB₂ following challenge with ovalbumin. Other investigators (16) using an identical model have shown that the local intestinal anaphylactic reaction is also accompanied by degranulation of mast cells and release of histamine.

We have also shown an increased intestinal PG content in children suffering from active celiac disease (17). As celiac disease and food protein hypersensitivity share some similar clinical and

morphological characteristics it is possible that PG may play a role, at least in part, in the symptomatology of both disorders.

Although the precise cellular origin of PGs is unknown, its biosynthesis is presumed to occur in leukocytes, platelets, endothelial cells through the metabolic pathway of arachidonic acid (18). As the lamina propria of small intestinal mucosa obtained from patients suffering from food-protein hypersensitivity is infiltrated with mononuclear cells, it is conceivable that the increased PG content in the sensitized rats may be attributed to the increased infiltration of these cells in the lamina propria. It is suggested that PGs may contribute to diarrhea in food protein hypersensitivity by stimulating adenylate cyclase activity which increases intracellular cyclic adenosine monophosphate which in turn increases intestinal electrolyte and fluid secretion (19). Moreover PGE₂ was demonstrated to decrease jejunal Na⁺K⁺ATPase activity, an enzyme which has an important role in the modulation of sodium absorption (20).

Lake *et al.* (21), however, reported in the rat model that intraduodenal infusion of exogenous PGE₂ inhibited the histamine recovery after ovalbumin challenge. It has been shown that PGE₂ has cytoprotective properties to chemical injury in the intestinal tract (22). It is thus suggested that PGs may have a protective effect during acute intestinal allergy simultaneously with its known diarrheagenic mechanism. The effect of PGE₂ on the immune system may also be relevant. It has been shown that this prostanooid induces nonspecific T lymphocyte suppressor activity (23, 24). These studies relate to peripheral blood lymphocytes, however, and the effect of PGE₂ on gut-associated lymphoid tissue remains to be studied.

It is concluded that in the rat model of food hypersensitivity PG content of the intestinal mucosa is elevated. It is therefore suggested that the elevated PGs may contribute, at least in part, to the symptomatology of allergic enteropathy.

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