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In Vitro Activation of Adenylate Cyclase of Atrophic Celiac Intestinal Mucosa by Wheat Gliadin-Derived Peptides

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ABSTRACT. In order to demonstrate that gliadin peptides may interact with cell membranes of celiac small intestinal mucosa, the capacity of these peptides to activate the cell membrane enzyme adenylate cyclase was tested. The addition of peptides from *bread* wheat purified A-gliadin and whole gliadin (proteins that are toxic for celiac patients) enhanced the adenylate cyclase activity of crude cell membrane preparations obtained from atrophic small intestinal mucosa of celiac patients. No activation of adenylate cyclase of this tissue was observed with peptides from proteins nontoxic for celiac patients (*bread* wheat albumin and maize prolamin). Gliadin peptides did not activate adenylate cyclase of morphologically normal small intestinal mucosa from normal subjects or from celiac patients in remission. These results, therefore, suggest that peptides from *bread* wheat gliadin may interact with cell membrane of atrophic small intestinal mucosa of celiac patients. (*Pediatr Res* 20: 42–44, 1986)

Celiac disease is a gliadin-dependent enteropathy. Humoral- and cell-mediated immune hyperresponsiveness to wheat gliadin has been reported in celiac patients and it has been speculated that intestinal mucosa becomes a target of the immunological reaction after binding of gliadin-derived peptides (1). Gliadin peptides, obtained by simulating *in vivo* digestion, prevent *in vitro* morphological and biochemical recovery of atrophic small intestinal mucosa from celiac patients (2–7). They also cause reversible inhibition of *in vitro* differentiation and morphogenesis of rat fetal intestine (8), and reduce *in vitro* viability of human embryo and tumor cell lines (9). Interestingly, the same gliadin peptides do not exert any *in vitro* toxic effects on morphologically normal small intestinal mucosa from celiac patients in remission (2–8, 10) or from normal individuals (8), on the differentiated jejunum from 21-day-old rat fetuses (8) or on adult human fibroblast cells (9). All these data indicate that gliadin peptides only interact with immature and/or undifferentiated cells. Previously, binding of wheat gluten to enterocytes of celiac mucosa has been observed using impure preparations and large amounts of the protein (11). This evidence has been recently questioned since fluorescent techniques have failed to indicate binding of gliadin to the enterocytes (12).

Adenylate cyclase catalyses the formation of cAMP from ATP and increased levels of cAMP are associated with active secretion of electrolytes and secretion of water (13). This enzyme is situated at the lateral and basal membranes of the enterocyte and is activated by cholera and other bacterial toxins, prostaglandins and certain gut hormones. In the present paper we report that peptides obtained by sequential peptic and tryptic digestion of *bread* wheat purified A gliadin (14), as well as peptides obtained by peptic, tryptic and cotazym digestion of *bread* wheat whole gliadin (8), do interact with cell membranes from celiac patients, as shown by activation of the adenylate cyclase activity.

MATERIALS AND METHODS

Donors of intestinal mucosa specimens. Surgical biopsies with normal morphology were obtained from the first few centimeters of jejunum of 12 consenting adult patients undergoing surgery for peptic ulcer. Peroral biopsies with normal morphology were obtained from five celiac children in remission who had been on a gluten-free diet for at least 1 yr. Eighteen children with active celiac disease were new cases with typical clinical symptoms and subtotal mucosal atrophy of the small intestinal mucosa. Twelve biopsies revealing subtotal atrophy were obtained from children with celiac disease in relapse following 4 to 16 months of gluten challenge (mean 7.4 months). The ratio of villus height to crypt depth was less than 0.35 in all atrophic celiac mucosa. Three children affected by giardiasis and diarrhoea were also investigated; morphological appearance of these biopsied samples was that of a mild degree of partial mucosal atrophy.

Preparation of cell membrane pellets. Specimens obtained at biopsy were stored at −80° C for periods of up to 6 months. They were thawed, washed in 0.154 NaCl and homogenized in 0.5 ml of ice cold buffer (10 mM Tris HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA, 3 mM dithiothreitol, 0.25 M sucrose) with 10 strokes using a Teflon-glass homogenizer. The homogenate was then centrifuged at 7000 × *g* for 15 min, and the pellet used for enzymic assay after suspension; final protein concentration was less than 1.5 mg/ml.

Preparation of cereal protein digests. The peptic-tryptic-pancreatic digests of *bread* wheat gliadins or albumin and of maize prolamins were those described by Auricchio *et al.* (15, 16). The peptic-tryptic digest of A-gliadin was obtained as described by Frazer *et al.* (17); after the tryptic digestion, the sample was heated for 5 min in boiling water and clarified by centrifugation.

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Adenylate cyclase assay. Adenylate cyclase activity was assayed by measuring the conversion of α-[^{32}P]ATP to [^{32}P]cAMP (18). Total reaction volume was 70 μl and contained 0.1 mM ATP, 6 mM $MgCl_2$, 10 mM Tris-HCl at pH 7.4, 1 mg/ml bovine serum albumin, 10 mM theophylline, 5×10^5 α-[H^{32}P]ATP, an ATP-regenerating system consisting of 2.6 mM phosphoenol pyruvate and pyruvate kinase (70 μg/ml) and 0.5–0.8 mg/ml cell membrane proteins. Enzyme activity was assayed under basal condition and in the presence either of an optimal stimulating concentration of sodium fluoride (10 mM) or of 90 μg/ml of cereal protein digests.

After 10 min of incubation at 37° C the reaction was stopped by the addition of 0.1 ml of a solution containing 10 mM ATP, 1 mM cAMP, and 0.1 μCi of [^{3}H]cAMP. Aliquots of the reaction mixture were chromatographed on silica gel plates with unlabeled ATP, cAMP, ADP and 3'AMP as markers, using isopropyl alcohol:water:ammonium hydroxide (7:2:1) as solvent. The spots were located under ultraviolet light, scraped from the plates, and counted (19). The [^{3}H]cAMP served to determine the recovery of cAMP during the procedure. The recovery ranged from 70 to 80%.

Protein assay. Protein concentration was determined with the method of Lowry *et al.* (20) after solubilization of membrane proteins in 1 N NaOH and heating at 95° C.

Reagents. ATP, cAMP, and ADP were obtained from Boeringher (Mannheim, West Germany), silicagel plates from Merck (Bracco, Milano, Italy), Hα-[^{32}P]ATP, and [^{3}H]cAMP from Amersham (Bucks, England); all the other materials were of reagent grade.

RESULTS

Basal adenylate cyclase activity was significantly higher in atrophic small intestinal mucosa of celiac patients than in morphologically normal small intestinal mucosa of celiac patients in remission or of control subjects. The enzyme activity was increased also in patients with giardiasis who were characterized by a mild degree of mucosal atrophy (Table 1).

The addition of the peptic-tryptic digest of purified A-gliadin from *bread* wheat or of the peptic-tryptic-cotazym digest of whole gliadin from *bread* wheat further increased basal adenylate cyclase activity of cell membrane preparations from atrophic celiac mucosa. On the contrary, these digests had no effect on adenylate cyclase activity from mucosa of patients with giardiasis and from morphologically normal mucosa from celiac patients in remission or control subjects (Tables 1 and 2). Furthermore, no activation of adenylate cyclase from atrophic celiac intestinal mucosa was observed with either the peptic-tryptic-cotazym digest of *bread* wheat albumin or maize prolamin (Table 2).

DISCUSSION

In agreement with the earlier findings by Tripp *et al.* (21), basal adenylate cyclase activity was increased in atrophic small intestinal mucosa of celiac patients; patients in remission had activities similar to the control group. As intestinal crypt cells are known to have a higher adenylate cyclase activity, these findings have been interpreted as an indication of a more immature character of surface enterocytes in active celiac disease (21). The similar increase of the adenylate cyclase activity found in the mucosa of patients with giardiasis and partial villous atrophy might be due to a similar mechanism (22) or to the stimulation of the enzyme activity by the protozoal infection.

The addition of peptides from *bread* wheat A-gliadin and whole gliadin, proteins that are toxic for celiac patients (14), further enhanced the adenylate cyclase activity of crude cell membrane preparations obtained from atrophic small intestinal mucosa of celiac patients. No activation of the adenylate cyclase of this tissue was observed with peptides from *bread* wheat albumin and maize prolamin, known to be nontoxic in celiac disease (23). Gliadin peptides did not activate the adenylate cyclase of mucosa from normal subjects or from celiac patients in remission.

These findings suggest that the enzyme activated by gliadin peptides in the atrophic celiac mucosa is either that of the immature surface enterocytes or, less probably, that of immunocytes, which are present in a high number in the mucosa of celiac patients with active disease. Studies on isolated enterocytes, if feasible, could clarify this question. It should also be clarified whether gliadin-induced activation of adenylate cyclase is specific for atrophic celiac mucosa, or whether it is also observed in other pathological conditions resulting in intestinal mucosa atrophy.

Our results suggest that *bread* wheat gliadin peptides interact with cell membranes of atrophic small intestinal mucosa of celiac patients, whereas peptides from proteins nontoxic in celiac disease do not. This effect could be related to the capacity of gliadin

Table 2. *Adenylate cyclase in patients with celiac disease: in vitro* effects of wheat gliadin and albumin peptides and of maize prolamins peptides

Celiac patients with active disease (patient no.)	Adenylate cyclase activity (pmol cAMP/mg protein/10 min)			
	Basal	Peptic-tryptic digest of A-gliadin	Peptic-tryptic-pancreatic digest of:	
1*	80	140	78	
2†	100	165	140	Total wheat gliadins
3†	130	200	180	
4†	55	186	53	
5*	152	269	151	Wheat albumins
6†	224	393	245	
7*	80	160	78	
8†	150	250	145	Maize prolamins

* New cases.

† In relapse after gluten challenge.

Table 1. *Adenylate cyclase activity in patients with celiac disease or giardiasis: in vitro effects of sodium fluoride and of wheat gliadin peptides (mean ± 1 SD)*

Subjects	*n*	Adenylate cyclase activity (pmol cAMP/mg protein/10 min)		
		Basal	Sodium Fluoride (10 mM)	Peptic-tryptic digest of A-gliadin (90 μg/liter)
Control subjects	12	68.0 ± 24.1	573.3 ± 199.2	72.8 ± 35.9
Celiac patients in remission	5	65.8 ± 14.6	381.4 ± 93.4	85.0 ± 34.7
Celiac patients with active disease:				
New cases	18	148.6 ± 85.7*	614.4 ± 202.3	248.6 ± 110.6‡
On relapse after gluten challenge	12	185.7 ± 94.0*	783.2 ± 172.5	291.4 ± 173.3‡
All cases	30	163.5 ± 89.5*	663.7 ± 205.8	265.7 ± 137.9‡
Giardiasis patients	3	314.2 ± 60.6†	743.1 ± 72.8	282.6 ± 43.8

Statistically different from controls: * $p < 0.01$, † $p < 0.001$ (Student's t test).

Statistically different from basal value: ‡ $p < 0.001$ (paired t tests).

peptides to exert *in vitro* toxic effects on immature and/or undifferentiated cells (15). The disappearance during enterocyte maturation of cell receptor sites for gliadin peptides could well explain the transient susceptibility of some tissues and cells to the *in vitro* toxic action of these peptides (15).

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