EFFECT OF PROTEIN INHIBITORS OF PANCREATIC PROTEINASES OF DEGRADATION AND ABSORPTION OF IMMUNOREACTIVE CHICK OVALBUMIN IN THE ADULT RAT INTESTINE

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Protin inhibitors of proteinases can inhibit proteolysis of food protein in the intestine and thereby reduce its biological value and assimilability [3]. Absorption of undergraded protein from the intestine may arise under these circumstances in both newborn [11] and adult animals [5]. The writers showed previously [5] that proteolysis of chick ovalbumin (OVA) is considerably inhibited in the adult rat intestine if administered simultaneously with soybean trypsin inhibitor (SBTI). The aim of this investigation was to study the effect of another protein proteinase inhibitor — chick ovomucoid (OM) — on proteolysis in the gastrointestinal tract and also the effect of SBTI and OM on absorption of undegraded OVA in the adult rat intestine.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 230-280 g. The animals were deprived of food for 24 h before the experiment but allowed water ad lib. Protein mixtures containing OVA, bovine serum albumin (BSA), SBTI, and OM were administered to the animals by gastric tube. The compositions of the mixtures used for feeding are shown in Table 1. Protein preparations manufactured by the Olaine "Biokhimreaktiv" Research-Production Combine were used. The solvent for the mixture was 0.01 M Na-phosphate buffer, pH 7.4, with 0.15 M NaCl (PSB). In the study of protein digestion, laparotomy was performed under hexobarbital anesthesia on the animals 20 and 60 min after feeding and the stomach and the proximal and distal parts of the small intestine were perfused with PSB. Immunoreactive (binding specific antibodies) OVA was determined in the perfusates quantitatively by a modified immunofluorescence method. The technique was described in detail previously [4]. To study absorption of OVA, the animals were exsanguinated under hexobarbital anesthesia 3 h after administration of the mixtures, by bleeding from the posterior vena cava, and the concentration of immunoreactive OVA in the blood serum was determined by competitive radioimmunoassay [7]. Antibodies against OVA obtained by immunization of rabbits by the scheme described previously

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TABLE 1. Composition of Mixtures (in mg protein) Used for Intragastric Injection into Rats in Experiments to Study Degradation and Absorption of OVA

Stage of proteolysis	Group of animals	Composition of mixtures			
		OVA	BSA	OM	SBTI
Degradation of OVA in gastrointestinal tract	1	20	80	—	—
	2	20	60	20	—
Absorption of OVA into blood stream	3	500	—	—	—
	4	500	—	—	5
	5	500	—	—	10
	6	500	—	—	25
	7	500	—	10	—
	8	500	—	25	—
	9	500	—	50	—

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TABLE 2. Quantity of OVA Preserving Its Antigenic Properties in Contents of Rat Gastrointestinal Tract (in % of dose fed, M ± m)

Time after feeding, min	Stomach		Small intestine					
			proximal portion		distal portion		total	
	control	experim.	control	experim.	control	experim.	control	experim.
20	2,72±0,08	2,89±0,05	0,98±0,26	1,15±0,58	0,85±0,16	0,89±0,28	1,83±0,31	2,04±0,31
60	0,26±0,06	0,36±0,1	0,19±0,08	0,5±0,12*	0,72±0,18	1,42±0,22*	0,91±0,29	1,92±0,37*

Legend. *P < 0.05 compared with group 1.

[10], and purified by affinity chromatography on OVA-sepharose 4B [6], were used in the determination. OVA was labeled with ^{125}I by means of chloramine T [8], and the protein was purified on microcolumns with Sephadex G-25 [12], and later on columns with immobilized rabbit antibodies against OVA [6]. The preparation of [^{125}I]OVA used for radioimmunoassay had specific radioactivity of 17 MBq/mg and was precipitated by more than 90% by an excess of specific rabbit antibodies. The results were subjected to statistical analysis by Student's test and also by Fisher's angular transformation test [1].

EXPERIMENTAL RESULTS

As Table 2 shows, when OVA was administered to rats together with OM and an excess of heterologous protein (BSA) some inhibition of degradation of the antigenic structures of OVA in the small intestine was observed. However, this effect became appreciable only after digestion for 60 min, and after 20 min the difference between the experimental and control groups was not statistically significant. Significant differences in the degree of inactivation of antigen in the stomach for the experimental and control groups were not found whether after 20 or 60 min of digestion. The writers showed previously [4] that SBTI inhibits proteolysis of antigenic determinants of OVA in the rat small intestime after digestion for both 20 and 60 min.

It will be clear from Fig. 1 that the concentration of immunoreactive OVA in the rats' blood serum was increased if this protein was given together with any of the doses of SBTI used (from 5 to 25 mg per animal) or together with OM in a dose of not less than 25 mg. These results confirm the view that depression of the stage of intestinal intraluminal proteolysis of protein by administration of inhibitors of pancreatic proteinases increases the quantity of food protein preserving its original antigenicity into the internal medium of the body. It will be recalled that absolute quantities of undegraded antigen penetrating into the blood stream are extremely small and, in these experiments, did not exceed 0.003% of the dose fed, in agreement with observations made by other workers using radioimmunoassay [2, 7].

The results are in agreement with previous results [5] showing an increase in absorption of undegraded insulin from the adult dog intestine after injection into the intestine together with pancreatic protein proteinase inhibitor. However, it was not clear from the data given in [5] whether the proteinase inhibitor can affect degradation and absorption of protein when injected into the stomach, because we know that in experiments *in vitro*, many inhibitors (SBTI in particular) undergo considerable inactivation under the influence of gastric juice [9]. The present results show that in a system *in vivo* inactivation of protein

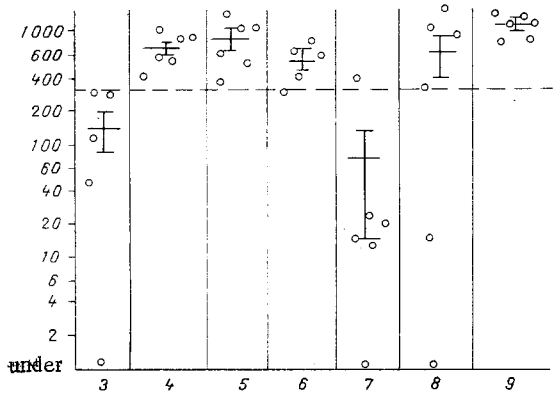

Fig. 1. Absorption of immunoreactive OVA into blood stream in rats of groups 3-9 (Table 1). Data for individual animals and mean values (with standard deviations). Abscissa, groups of animals; ordinate, OVA concentration in blood serum (in ng/100 µl, logarithmic scale). Broken line shows level of comparison of data by Fisher's angular transformation method.

inhibitors in the stomach does not proceed to sufficient depth to rule out their subsequent influence on intestinal intraluminal proteolysis and absorption of protein.

The question whether protein proteinase inhibitors, in the quantities in which they are present in food products, can have an unfavorable action on the body, by increasing the secretion of pancreatic juice and thereby causing hypertrophy of the pancreas and delaying the growth of young animals on account of reduced assimilation of proteins, has been widely discussed in the literature [3]. It follows from our own data (Fig. 1) that even a relatively low content of SBTI (unlike OM) in food products can cause increased entry of undegraded protein into the internal medium of the body. The presence of other more powerful proteinase inhibitors and which, at the same time, have increased resistance to gastric digestion (for example, the Bowman–Birk inhibitor [9]), in the composition of food products increases the potential risk of entry of undegraded antigenic material into the internal medium of the body.

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