DIP-α-thrombin (Fig. 3b), in the range of concentrations studied (10^{-12}-10^{-8} M) did not stimulate the mast cells and the level of heparin secreted did not exceed the control.

Thus analysis of interaction of α-thrombin and its analogs with rat peritoneal mast cells showed that α-thrombin stimulates heparin secretion provided that the enzyme molecule contains both its catalytic center and its recognition center for high-molecular-weight compounds. The low effective concentration of the ligand suggests that specific receptors for thrombin, controlling the mechanism of secretion, exist on the surface of the mast cells.

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CHARACTERISTICS OF PROTEOLYSIS IN THE GASTROINTESTINAL TRACT IN THE EARLY POSTNATAL PERIOD

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KEY WORDS: artificial feeding; casein; glycomacropeptide

An important problem at this time is the study of proteolysis of experimental samples of casein in the neonatal gastrointestinal tract with a view to creating substitutes for human milk that will satisfy the physiological demands of the neonatal period. Experimental results have shown the functional immaturity of the proteolytic system for protein digestion in the stomach and the considerable maturity of the proteolytic system for protein digestion in the small intestine [1, 2]. The combination of these effects leads to a shift of the peak of protein digestion in the period of milk feeding toward the distal portion of the small intestine.

The aim of this investigation was to study the specific nature of hydrolysis and assimilation of an "experimental" sample of casein, purified from glycomacropeptide.

EXPERIMENTAL METHOD

Experiments were carried out on 90 Wistar rats transferred from the 15th to the 21st day to artificial feeding on a milk substitute, with a control or experimental sample of casein as the protein component. The fractional composition of the chyme was studied by gel-chromatography with Sephadex G-75 [2]. Protein was determined by Lowry's method [6]. Concentrations of amino acids in the experimental and control samples of casein were determined jointly

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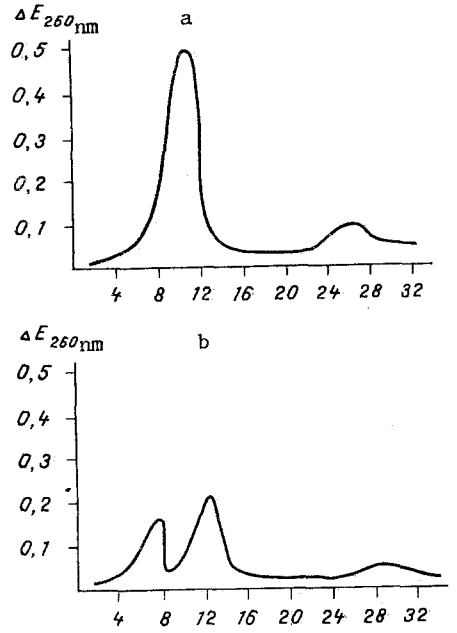

Fig. 1

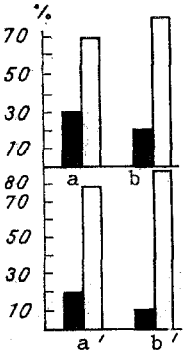

Fig. 2

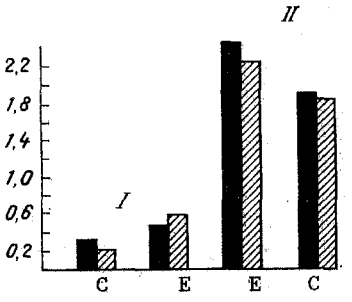

Fig. 3

Fig. 1. Fractional composition of gastric contents of rats during artificial feeding with the use of control (a) and experimental (b) samples of casein as the protein component of a milk substitute. Abscissa, Nos. of fractions; ordinate, E_{280}, nm.

Fig. 2. Fractional composition of chyme from jejunum (a) and ileum (b) during artificial feeding, using control (a, b) and experimental (a', b') specimens of casein. Black column – protein fraction; white column – low-molecular-weight fraction. Ordinate, concentration of fractions (in %).

Fig. 3. Activity of pancreatic proteinases (I) and of acid phosphatase (II) in chyme of small intestine during artificial feeding with milk substitute using control (C) and experimental (E) specimens of casein. Black columns – proximal, obliquely shaded columns – distal portions of small intestine. Ordinate, activity of enzymes (in optical density units at a wavelength of 280 nm per milligram protein per hour).

with M. L. Ioffe by ion-exchange chromatography on an automatic amino-acid analyzer [4]. Activity of gastric proteinases was studied by Helander's method [3] and activity of pancreatic proteinases by Robberecht's method [7]. Acid phosphatase activity was determined in the chyme from the small intestine [5].

EXPERIMENTAL RESULTS

The study of the pepsinogen concentration in the gastric mucosa of rats fed artificially on a milk substitute containing experimental or control samples of casein, purified from glycomacropeptide, revealed a marked increase (by 1.5 times) in the pepsinogen concentration in the gastric mucosa when the control sample of casein was replaced by the experimental (Table 1). Replacement of the control sample of casein by the experimental during artificial feeding was accompanied by acidification of the gastric contents and accelerated evacuation of protein (Table 1). The use of the experimental sample of casein was accompanied by a marked increase in the activity of the lysosomal marker enzyme, acid phosphatase, in the gastric contents.

Pepsin activity, determined at the same pH as that observed in the gastric contents in the case of artificial feeding using the experimental specimen of casein, was increased, further evidence of activation of proteolysis in the stomach.

The results of investigation of changes in fractions of the gastric chyme in different parts of the small intestine, obtained by gel-chromatography with Sephadex, showed that the concentration of the protein fraction in the gastric contents of rats artificially fed with milk substitute containing the experimental sample of casein was much lower than when the

TABLE 1. Proteolysis in the Stomach during Artificial Feeding of Rats with Control and Experimental Samples of Casein as Protein Component of Milk Substitute (M ± m; n = 6)

Protein component of milk substitute	pH of chyme	Pepsinogen of gastric mucosa, U/mg/h	Pepsin of gastric chyme, U/mg/h	Acid phosphatase, U/mg/h	Protein, mg/ml
Control sample of casein	5,4	0,75±0,06	0,36±0,09	0,11±0,09	1,3±0,1
Experimental sample of casein	5,0	1,12±0,09	0,5±0,09	0,77±0,05	0,7±0,05

TABLE 2. Concentration of Amino Acids (in %) in Experimental and Control Samples of Casein

Amino acid	Control sample	Experimental sample
Lysine	7,1	8,0
Histidine	3,6	4,2
Arginine	3,9	4,0
Aspartic acid	7,8	7,6
Threonine	3,2	3,2
Serine	4,6	4,2
Glutamic acid	19,7	18,2
Proline	9,0	7,9
Glycine	2,1	2,1
Alanine	3,2	2,6
Valine	6,3	6,0
Methionine	2,5	2,3
Isoleucine	6,0	4,4
Leucine	8,3	7,8
Tyrosine	4,7	4,4
Phenylalanine	4,7	4,4

TABLE 3. Increase in Body Weight and Efficiency of Utilization of Casein Samples during Artificial Feeding of Rats from the 15th through the 21st Days (M ± m, n = 6)

Protein component of milk substitute	Increase in body weight, g	APPU, %
Control sample of casein	6,7±0,3	89±0,3
Experimental sample of casein	3,75±0,2	75±0,2

control sample was used (Fig. 1). A fall in the concentration of the protein fraction also was observed in chyme from the jejunum and ileum, evidence of optimization of protein evacuation in the case of artificial feeding with an experimental sample of casein as the protein component of the milk substitute (Fig. 2).

Activity of pancreatic proteinases in the chyme of the small intestine was increased during artificial feeding using an experimental sample of casein compared with the control (Fig. 3).

Acid phosphatase activity was uniformly distributed in the mucosa of the proximal and distal portions of the small intestine, being rather higher when the control sample of casein was used for artificial feeding. In the course of the increase in activity of both gastric and pancreatic proteinases, responsible for protein digestion, the decrease which we observed in activity of the lysosomal marker enzyme was compensatory.

The results of a study of amino-acid concentrations in the experimental and control samples of casein are given in Table 2.

The potential biological value of the control casein sample was 52.4%, and that of the experimental 57.2%.

The use of the control casein sample in the course of the experiment led to a greater increase in the animals' body weight. On the basis of data on the quantity of protein consumed with the diet and the increase in the protein concentration in the animal's cadaver, the value of the apparent pure protein utilization (APPU) was determined. The increase in body weight and protein utilization were reduced when the experimental casein sample was used (Table 3).

The experimental data are evidence that replacement of the control casein sample by an experimental sample leads to considerable changes in hydrolysis and the initial stages of protein assimilation, promoting its utilization. The results suggest an anobolic effect of the glycomacropeptide. The experimental casein sample is readily assimilated and may be used for dietetic products.

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NEUROPEPTIDES IN MECHANISMS OF ACTIVATION OF VENTROMEDIAL HYPOTHALAMIC STIMULATION-INDUCED AVOIDANCE REACTIONS DURING SATIATION

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KEY WORDS: ventromedial hypothalamus; food motivation; avoidance reactions

A dominant focus of avoidance as a rule inhibits food motivation. Meanwhile the demand for food has a modulating effect on the character of the avoidance behavior of animals. It has been shown, for example, that avoidance reactions of hungry mice [6, 7] and of the marine mollusk Aplysia [10] are considerably depressed compared with those of satiated animals. Shevelkin [4] showed that food sensory satiation activates avoidance reactions in snails. It can be postulated that neurochemical factors participating in the organization of animals' food behavior may also exert a modulating action on avoidance motivation. For instance, endogenous oligopeptides such as opioids and gastrointestinal peptides, involved in the formation of food behavior [1, 8], in particular inhibit the organization of avoidance behavior [3, 5, 12].

The aims of the present-investigation were accordingly as follows: 1) to determine the effect of satiation, starting from the first contact of hungry rabbits with food until complete refusal to eat any more, on the character of avoidance reactions induced by ventromedial hypothalamic stimulation; 2) to assess the role of β-endorphin, cholecystokinin, and gastrin in mechanisms of modulation of avoidance reactions during changes in the level of food motivation in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 25 male rabbits weighing 2.5 kg, allowed free access to food before the experiment, and on 25 rabbits deprived of food for 48 h before the experiment. Bipolar nichrome electrodes were implanted in all animals into the right ventromedial hypothalamus and a cannula was introduced into the left lateral cerebral ventricle. Stimulation of the ventromedial hypothalamus led to passive avoidance behavior of the animals. The parameters of the stimulating currents were: amplitude 20-80 μA, duration of square pulses 1 msec, frequency 20-100 Hz. After testing for avoidance reactions so that, at a frequency of stimulation of 100 Hz, the latent period of the reaction was 1 sec, 40 μliters of Ringer's solution for warm-blooded animals, 1 nmole pentagastrin in 30 μliters of Ringer's solution, 0.3 nmole of the octapeptide cholecystokinin in 30 μliters of Ringer's solution, and 1 mmole of β-endomorphin in 30 μliters of Ringer's solution were injected into the lateral cerebral ventricle of animals of 4 groups, respectively, each consisting of 5 hungry and 5 satiated rabbits; 1 μmole naloxone in 0.5 ml of Ringer's solution also was injected intravenously into 5 hungry and 5 satiated rabbits. Next, with an interval of 1-10 min, a series of stimulations was applied to the ventromedial hypothalamus in the form of square pulses of different frequency: from 20 to 100 Hz consecutively. The latent period of the avoidance reaction to stimulation of each

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