FORMATION OF A PEPTIDE INHIBITOR OF GASTRIC SECRETION FROM RAT MILK PROTEINS *IN VIVO*

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When milk enters the gastrointestinal tract of a newborn animal, it has a many-sided regulatory effect on its function [15]. Milk proteins not only supply the newborn organism with amino acids in optimal proportions and amounts, but can also be the source of physiologically active peptides, formed during their restricted proteolysis [6, 11-14]. It is in the caseins of milk that their alimentary specificity is most clearly reflected. Maximal hydrolysis of caseins by gastrointestinal proteinases [7] and their ability to undergo coagulation enable caseins to exert a regulating influence on the rate of gastric evacuation [2] and amino acid absorption [8]. A product of restricted proteolysis of β-casein by milk proteinase — a component of the 5-proteosopeptone fraction — has a controlling action on the cerebral circulation during functional loading of the CNS [3]. Caseins are a source of peptides with opioid [13] and bifidogenic [11] activity and of phosphopeptides which take part in Ca^{++} absorption [14]. For the formation and preservation of these peptides and other biologically active milk proteins (γ-globulins, lactoferrin, lysozyme) *in vivo* in the neonatal gastrointestinal tract, they must evidently be protected against digestion by gastrointestinal proteinases and, above all, from excessive digestion in the stomach. Digestion of rat milk proteins in the stomach of newborn rats is characterized by a slow and small-scale process of proteolysis, on account of the low secretory activity of the stomach during natural milk feeding [4]. The low level of acid and pepsin secretion in the rat stomach during the period of milk feeding is associated with weakness of the gastric secretory apparatus. However, this explanation is incomplete. There is evidence that on the change to artificial feeding, the secretory activity of the stomach rises sharply [4], evidence of a definite degree of maturity of the secretory apparatus. It can be tentatively suggested that during natural milk feeding the secretory function of the stomach is inhibited by a certain inhibitor, for intensive secretion can create unfavorable conditions for the formation and preservation of physiologically active milk peptides.

Previous investigations suggest that peptides formed during partial proteolysis of milk caseins in the neonatal stomach may be such inhibitors of gastric secretion during the period of milk feeding. It has been shown, for instance, that a glycomacropeptide formed from cows' κ-casein by partial proteolysis with pepsin *in vitro* is a powerful inhibitor of gastric secretion [1]. Its inhibitory activity is resistant to the action of pepsin and pancreatic proteinases [9], and it is due not to the whole glycomacropeptide molecule, but only to its low-molecular-weight fragment [5].

In connection with the facts described above, it was decided to study the possibility of formation of a similar peptide inhibitor of gastric secretion *in vivo* in the neonatal rat stomach as a result of digestion of rat milk proteins.

EXPERIMENTAL METHOD

The water-soluble protein fraction was isolated from the gastric contents of fed unweaned 10-day-old rats by mixing 50 g of the crude contents with 200 ml distilled water and removing the insoluble protein fraction for centrifugation at 10,000 rpm for 15 min. Protein and large polypeptides in the supernatant were precipitated with 12% (final concentration) TCA solution and separated by centrifugation. The protein-free supernatant, containing proteolysis products of milk proteins, was freed from TCA by repeated extraction with ether and lyophilized. The

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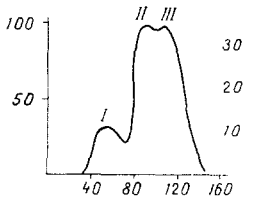

Fig. 1

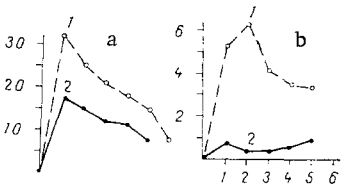

Fig. 2

Fig. 1. Chromatogram of proteolysis products of rat milk proteins from gastric contents of 10-day-old rats. Sephadex G-25 column (1.5 × 72 cm) was equilibrated with 55 mM ammonium acetate buffer, pH 8.0, in 5 M urea, and 150 mg of material in 2.5 ml of eluate was applied to it. Rate of filtration 20 ml/h. Abscissa, elution volume of fractions I, II, and III (in ml); ordinate, absorbance at 260 nm (in %).

Fig. 2. Inhibitory action of peptide fraction Ia on gastric secretion of dogs with high (a) and low (b) secretory activity. Abscissa, time of experiment (in h); ordinate, volume of gastric juice (in ml). 1) Control; 2) secretion after intravenous injection of peptide fraction Ia.

material thus obtained was fractionated on a Sephadex G-25 column. The peptide fraction coming out into the elution volume of the column was collected and freed from urea on a Sephadex G-10 column (1.5 × 50 cm), equilibrated with distilled water. The volume of the fraction applied to the column was 20 ml, the rate of elution was 15 ml/h, and the resulting eluate was lyophilized.

The inhibitory action of the peptide material thus obtained on gastric secretion was studied in two dogs with Pavlov gastric pouches. The dogs were deprived of food for 18 h and, their gastric reaction being neutral or alkaline, 15 mg peptide was injected intravenously into each animal and a food stimulus (raw meat) given 1 h later. The quantity of gastric juice secreted, its pH, and the total, free and bound acid were determined every 15 min by a titration method and the proteinase content was determined [10].

EXPERIMENTAL RESULTS

TCA-soluble proteolysis products of milk proteins from the gastric contents of 10-day-old unweaned rats were separated by chromatography on a Sephadex G-25 column into three fractions (Fig. 1): fraction I (peptide) came out in the elution volume of the column and was more clearly separated from the rest of the material, whereas fractions II and III came out in the hold-up volume of the column and were poorly separated. On filtration through a Sephadex G-10 column fraction I separated into two fractions: fraction Ia came out in the elution volume of the column and fraction Ib in the hold-up volume. Fraction Ia was found to have a powerful inhibitory action on gastric secretion induced by the food stimulus. Fraction Ia, in an amount of 15 mg, when injected intravenously into a dog with a Pavlov gastric pouch, caused a decrease of 50% in the volume of gastric secretion (Fig. 2a). Its inhibitory action was manifested on both the neurohumoral and the humoral phase and it lasted 5 h. The total acidity and free hydrochloric acid concentration in the gastric juice fell a little under these circumstances, but the proteinase concentration was increased on average by 10%. In another dog with a low level of gastric secretion, fraction Ia in the same dose caused almost complete inhibition of gastric secretion during the 5 h of the experiment (Fig. 2b).

The investigation thus showed that under natural conditions of digestion of maternal (rat) milk proteins a peptide capable of inhibiting gastric secretion to a food stimulus in dogs by intravenous injection is formed in the stomach of 10-day-old rats. In its physiological action this peptide-inhibitor is similar to the peptide isolated previously from restricted proteolysis products of κ-casein by pepsin *in vitro* [5]. It can be tentatively suggested that this protein has an inhibitory action on gastric secretion in the newborn animal during natural breast feeding, by reducing the intensity of proteolysis of maternal milk proteins in the stomach.

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EFFECT OF BIVALENT CATIONS ON PROPERTIES OF NaCl-STIMULATED ATPase ACTIVITY IN RABBIT SMALL INTESTINAL MUCOSA

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The writers' previous investigation showed that the mucosa of the rabbit's small intestine contains a highly labile enzyme with ATPase activity, the properties of which suggest that the function of this ATPase may be linked with secretion [1]. A particularly interesting fact is that Ca^{++} or Mg^{++} ions, if added to the incubation medium in a concentration of 0.2-0.5 mM, cause sharp changes in the properties of the ATPase reaction, expressed as loss of the stimulating effect of NaCl and $NaHCO_3$ and disappearance of the inhibitory effect of ethacrynic acid on ATPase activity. The results suggest that this ATPase functions in the cell under conditions which exclude the possibility of access of bivalent cations to it.

The aim of this investigation was to continue the study of the effect of bivalent cations on ATPase activity of the mucosa of the small intestine.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 1.2-1.8 kg. The secretogenic agent was histamine, which was injected subcutaneously into the animals in a dose of 2.0-2.5 mg/kg body weight. The animals were decapitated 2-2.5 h after injection of histamine. The mucosa of the small intestine was washed with cold physiological saline and wiped with filter paper, after which the epithelium was carefully scraped off and a 10% homogenate prepared in isolation medium containing 0.25 M sucrose, 5 mM EDTA, and 5 mM HEPES-Tris, pH 7.0. After centrifugation at 700g for 10 min a residue of the membranes was obtained. The residue was resuspended in isolation medium in which EDTA was reduced to a concentration of 0.5 mM or was completely absent. ATPase activity in these membrane preparations was determined from the accumulation

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