ANTIGASTRIN ACTION OF GLYCOMACROPEPTIDE

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Experiments on dogs with isolated Pavlov and Heidenhain gastric pouches showed that intravenous injection of glycomacropeptide leads to the almost complete inhibition of the second phase of gastric secretion evoked by feeding with meat. Glycomacropeptide strongly inhibits secretion evoked by the analog of gastrin, which is reduced under these circumstances by two-thirds or more. By contrast, the first phase of gastric secretion and also the secretion induced by insulin hypoglycemia were reduced, although their intensity still remained quite high. After administration of the glycomacropeptide certain changes took place in the composition of the juice. It is concluded from the results that glycomacropeptide can delay the action of gastrin on the fundal glands of the stomach.

Recent investigations have shown that casein is the source of substances with an active influence on digestive functions [5, 7]. Experiments were accordingly carried out to determine the possible effect of certain components of casein. The results of these experiments, showing that glycomacropeptide (GMP), a cleavage product of kappacasein, can delay the action of gastrin on the fundal glands of the stomach, are described below.

EXPERIMENTAL METHOD

Pavlov and Heidenhain isolated gastric pouches were formed in dogs. To reproduce the action of gastrin, its synthetic analog (the amide of the C-terminal tetrapeptide of gastrin), which has qualitatively the same action as gastrin itself [10, 12], was used. The gastrin analog was injected subcutaneously into the fasting dogs in a dose of 200 μg in 1 ml physiological saline. In other experiments food stimuli and injections of insulin were given. The total acidity and the free hydrochloric acid were determined in the gastric juice by the usual methods, and pepsin content was estimated by a modified spectrophotometric method [1], using dried bovine serum proteins as the substrate [2].

GMP was first isolated from the casein of cows' milk by the action of renin [8]; investigations have shown that it is liberated as the result of the partial hydrolysis of kappacasein [16]. In the present investigation, the GMP was obtained by the method of Chernikov and Nikol'skaya, based on the hydrolysis of kappacasein by pepsin. The kappacasein was briefly (2.5 min) hydrolyzed by pepsin at pH 5.5 and at 37°C, with the enzyme and substrate in the ratio of 1 : 200. To reduce the absorption of GMP, calcium chloride was added to the residue of para-kappacasein in the concentration of 0.8%. The para-kappacasein was precipitated by TCA in a final concentration of 12%. Before lyophilization the GMP solution was dialyzed against distilled water.

According to the literature GMP is heterogeneous and has a molecular weight of 6,000-8,000; it contains about 30% of carbohydrate, including 15.2% galactose, 4.3% galactosamine, and 8% N-actylneur-

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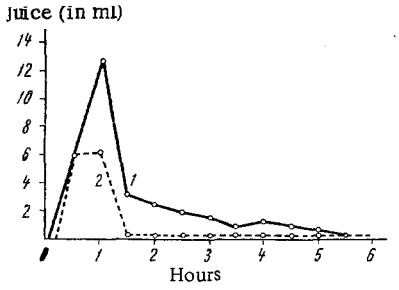

Fig. 1

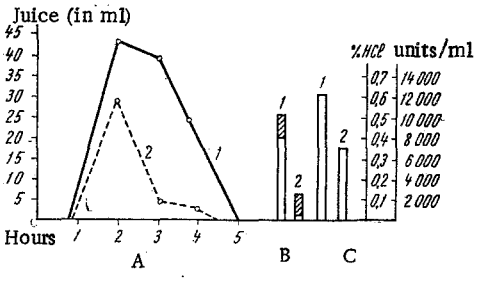

Fig. 2

Fig. 1. Secretion of gastric juice in dog with isolated Pavlov gastric pouch: 1) control experiment (administration of 100 g meat); 2) preliminary parenteral injection of 50 mg GMP and feeding with 100 g meat; almost complete inhibition of the second phase of gastric secretion is observed.

Fig. 2. Effect of GMP on secretion of gastric juice and its composition after injection of insulin into a dog with an isolated Pavlov gastric pouch: A) secretion of juice; B) acidity: shaded part of column represents total, unshaded part, free; C) pepsin content in juice in control experiment without GMP (1) and after injection of GMP (2).

aminic acid, as well as 0.4% phosphorus [14]. GMP contains no arginine, histidine, cysteine, or aromatic amino-acid residues [11]. GMP has been shown to participate in the coagulation of casein, by increasing the degree of dispersion of the curds of this protein [4].

The lyophilically dried specimen of GMP was injected intravenously into the dogs in a dose of 50 mg in 10 ml physiological saline.

EXPERIMENTAL RESULTS

Injection of GMP into the experimental dogs 15 min before administration of the food stimulus evoked sharp changes in the secretion of gastric juice. In control experiments on the same animals, the food stimuli were given without GMP.

After injection of GMP into the dogs with an isolated Pavlov gastric pouch, the secretion of juice was reduced during the first 1-1.5 h after administration of 100 g meat, but it still remained at a very high level. The volume of juice was reduced by not more than half the control value. Later the secretion fell almost to zero ($P < 0.001$). The acidity of the juice during the first hour of secretion showed a tendency to diminish, but the pepsin content remained high. Good reproducibility of the results was obtained. The results of one of the experiments are illustrated in Fig. 1.

The experiments showed that under the influence of GMP the first (reflex) phase of secretion remained largely intact but the second phase was completely or almost completely inhibited. Experiments on dogs with a Heidenhain gastric pouch gave similar results. Absence of the first phase of secretion is a characteristic feature of such a pouch; its secretory response is due entirely to the mechanisms of the second phase. Injection of GMP into such dogs before feeding them with meat led to the total cessation of juice secretion (disregarding the secretion of gastric mucus) throughout the secretory period.

For example, in the control experiment when the dog was given 100 g meat the volume of juice secreted in 5 h was 10.6 ml, while in a similar experiment but with the preliminary injection of GMP the volume of juice was zero.

Preliminary experiments showed that GMP must be injected 15-20 min before application of the stimulus. If injected at the same time as the stimulus was given the effect was reduced, while if given after it, no effect whatever could be detected. These experiments also showed that GMP can be injected only twice into the same animals. After subsequent injections its action becomes weaker and disappears completely, evidently because of fixation of the GMP by antibodies as a result of the development of an immunological response of the animal to this substance.

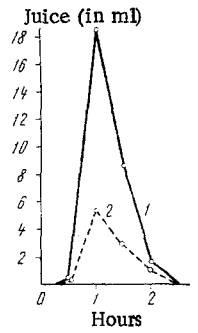

Fig. 3. Effect of GMP on secretion of gastric juice evoked by subcutaneous injection of gastrin analog into dog with isolated Heidenhain gastric pouch: 1) secretion without GMP; 2) secretion after injection of GMP.

The second phase of gastric secretion is due mainly to gastrin. It could therefore be postulated that GMP acts by preventing the action of gastrin.

To test this hypothesis, in the next experiments the "nervous" secretion and the "gastrin" secretion were reproduced separately. Nervous secretion was reproduced by injecting insulin subcutaneously into the dogs in a dose of 0.6-0.7 unit/kg. The hypoglycemia induced by insulin leads to excitation of the centers in the lateral hypothalamus, and these evoke gastric secretion via the vagus nerves. Division of the vagus nerves abolishes this effect on the gastric glands.

The results given in Fig. 2 show that in the control experiment a large volume of juice was secreted by the dogs with the isolated Pavlov pouch under the influence of insulin hypoglycemia. The juice exhibited high acidity and a very high pepsin content, a feature of nervous secretion. Injection of GMP 15 min before the beginning of secretion (30 min after injection of insulin) reduced the volume of secretion, just as during the first phase of secretion in response to meat; the volume of juice was reduced to approximately 50% of the control value. However, the intensity of secretion still remained fairly considerable and the pepsin content of the juice was high.

In the experiments with insulin, the acidity of the juice showed characteristic changes. Under the influence of GMP the total acidity was greatly reduced, while the free hydrochloric acid fell even more (by 2-4 times). The mechanism of these changes in acidity is not yet clear.

To reproduce the "gastrin" secretion, the synthetic analog of gastrin, the amide of the C-terminal tetrapeptide with the composition butylhydroxycarbonyl-Try-Met-Asp-Phe-NH_2, was used. Injection of this analog causes a marked secretory response in dogs with both Pavlov and Heidenhain gastric pouches, which continues for 1.5-2 h [3, 6]. GMP was injected 15 min before administration of the gastrin analog.

The experiments showed that GMP sharply inhibits the action of the gastrin analog. The volume of juice secreted after its administration to dogs with Pavlov and Heidenhain gastric pouches was reduced by two-thirds or more under the influence of GMP ($P < 0.01$). The acidity of the juice was slightly reduced. The results of experiments on different dogs were reasonably consistent. The results of one of the experiments are illustrated in Fig. 3.

Although GMP strongly inhibited the action of the gastrin analog, it nevertheless did not suppress it completely as the second phase of gastric secretion in response to feeding the animal with meat. This may be because GMP more effectively inhibits the action of gastrin (which has a much larger molecule) than that of its analog. However, it may be that the experimental conditions under which the effect of GMP on the action of the gastrin analog would be maximal have not yet been discovered.

It must also be remembered that in some dogs the repeated administration of GMP was followed by an aftereffect in the form of slight contamination of the gastric juice with blood and a reduced food intake by the animals. This was particularly noticeable when GMP was combined with insulin hypoglycemia. The results described above were obtained only when two injections of GMP were given to each experimental dog.

The experiments thus showed that GMP completely or almost completely inhibits the second phase of gastric secretion to a food stimulus (meat) and, in addition, that it sharply inhibits the secretory response of the gastric glands to injection of the analog of gastrin. This suggests that GMP has an antigastrin action, possibly by blocking the effect of gastrin on the fundal glands of the stomach.

The marked decrease produced by GMP in the secretion in response to stimulation of the vagus nerves may be due to weakening of the potentiating effect of gastrin. Not only do the vagus nerves potentiate the effect of gastrin [9, 14, 15], but conversely, gastrin strongly potentiates the vagal effect. Blocking of gastrin by glycomacropeptide thus perhaps weakens this effect, and thereby reduces the secretory response to the influence of the vagus nerves.

A precise explanation of the mechanism of action of GMP is a matter for future investigation. All that can be said at present is that it can inhibit the effect of gastrin; other aspects of the action of GMP are possibly connected with this ability.

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