The results are evidence of inhibition of the early insulin response, although the A cells remain capable of inhibiting glucagon secretion in response to exogenous glucose. The reduction of glucagon secretion combined with reduction of insulin release in chronic pancreatitis leads to correction of their molar ratio to a level similar to that observed in intact dogs, so that the normal glucose concentration in the body is maintained in the first stages of the disease.

LITERATURE CITED

1. L. I. Geller, Chronic Recurrent Pancreatitis [in Russian], Khabarovsk (1976).
2. L. N. Dagaeva, V. G. Vladimirov, O. I. Podotykina, et al., Patol. Fiziol. Éksp. Ter., No. 2, 48 (1977).
3. L. N. Dagaeva, O. I. Podotykina, V. G. Vladimirov, et al., Byull. Éksp. Biol. Med., No. 8, 152 (1980).
4. A. A. Shelagurov, Diseases of the Pancreas [in Russian], Moscow (1970).
5. L. Bottgar, E. M. Schlein, S. Witt, et al., J. Clin. Endocrinol., 89, 329 (1978).
6. F. M. Matschinsky, A. A. Pagliara, W. S. Zawalich, et al., Endocrinology (New York), 2, 935 (1979).
7. L. Orci, Acta Histochem. (Jena), 55, 147 (1976).
8. E. F. Pfeiffer, C. Thum, and A. H. Clemens, Horm. Metab. Res., 6, 339 (1974).
9. B. D. Ross, R. Hems, and H. A. Krebs, Biochem. J., 102, 942 (1967).
10. B. Schulz, M. Ziegler, S. Witt, et al., Acta Endocrinol. (Copenhagen), 89, 329 (1978).
11. E. Struck, J. Ashmore, and O. Wieland, Adv. Enzyme Regul., 4, 219 (1966).
12. F. Tasso, N. Stemmelin, H. Sarles, et al., Biomedicine, 18, 134 (1973).
13. R. H. Unger, E. Aguilar-Parada, W. A. Muller, et al., J. Clin. Invest., 48, 837 (1970).
14. R. H. Unger, Diabetes, 12, 834 (1971).
15. B. W. Volk and K. F. Wellmann, The Diabetic Pancreas, New York (1977), pp. 294-309.

INHIBITION OF GASTROINTESTINAL MOTILITY BY LOW-MOLECULAR-WEIGHT PEPTIDES FROM KAPPA CASEIN

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UDC 612.327-064:/612.332.4:547.963.2

KEY WORDS: motility; inhibition; peptides of K (kappa) casein.

Since gastroduodenal pathology associated with hypersecretion is one of the most widespread diseases of the digestive system, the search for new and effective remedies of a peptide nature for the treatment of this disease is a very important problem. The search for such preparations is mainly proceeding on the lines of isolation of endogenous regulatory peptides and the synthesis of their longer-acting analogs. A new and very promising trend may be the search for analogous regulatory peptides among proteolysis products of food proteins. For instance, α_S- and β-caseins from cows' milk contain in their structure amino acid sequences which, in the course of proteolysis, are liberated in the form of physiologically active peptides [1, 7, 14], capable of influencing CNS activity [1, 10] and the hormonal status of the body [8, 9].

The writers found previously that a glycomacropeptide (GMP) released from Kappa (K) casein during curdling of milk is an inhibitor of gastric secretion [6] and motility [3]. Since the inhibitory effect of GMP on gastric and duodenal motility was accompanied by side effects,

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 423-426, October, 1987. Original article submitted May 14, 1986.

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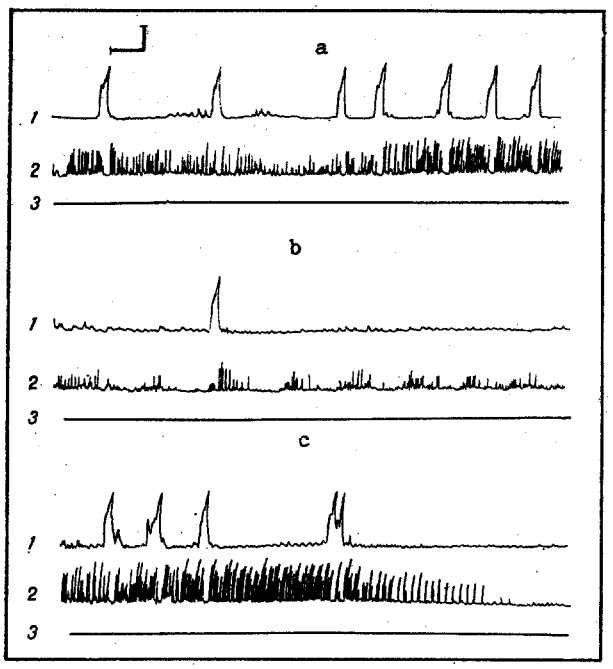

Fig. 1. Effect of peptide IV-1 on fasting gastric and duodenal motility. a) Time of intravenous injection of peptide in a dose of 6 μg/mg; b) chaotic gastric and duodenal contractions during first period of rest after injection of peptide; c) end of a regular period of work and beginning of rest period. Here and in Figs. 2 and 3: 1) motility of gastric fundus, 2) motility of duodenum, 3) line is marker of stimulation. Calibration: 20 cm water, 1 min.

in the form of nausea and vomiting [3], the aim of this investigation was to study the inhibitory action of low-molecular-weight peptide fragments formed during short-term digestion of K casein by pepsin, which might be free from such side effects.

EXPERIMENTAL METHOD

K casein was obtained from fresh defatted milk by Zittle's method and purified twice to remove α_s- and β-caseins, present as impurities, by precipitation with ammonium acetate from 50% ethanol [15]. The methods of enzymic hydrolysis of K casein by pepsin and of obtaining the initial peptide preparation were as described previously [4]. To isolate low-molecular-weight peptides 100 mg of the initial preparation in 2 ml of 0.01 M ammonia was passed through a column of Sephadex G-50 (1.5 × 100 cm) at the rate of 25 ml/h. Fractions II and IV were collected from the beginning of the chromatogram and freeze-dried. Fraction IV, in a quantity of 100 mg in 2 ml of 0.1 M acetic acid, was refractionated on a BioGel P-2 100 × 200 mesh (1.5 × 100 cm) column, equilibrated with 0.1 M acetic acid, at the rate of 35 ml/h, and fraction IV-1, eluted as the first peak in the external volume of the column, was collected and freeze-dried. Peptide fractions III and IV-1 were tested for their ability to inhibit motility of the gastric fundus and duodenum.

Experiments were carried out on six dogs with gastric and duodenal fistulas. The innervation of the gastrointestinal tract was intact in two dogs, two dogs underwent the operation of selective proximal vagotomy, and another two dogs underwent bilateral trunk vagotomy in the thorax.

Gastric and duodenal motility was recorded by a balloon-graphic method, using highly sensitive electric manometers and an RPCh-2 ink-writing recorder. The gastric balloon was inflated with 12 ml of air and the duodenal with 1.5 ml. Special experiments on fasting animals showed that these volumes of air did not themselves induce motor responses in the stomach or duodenum.

Experiments were carried out on fasting animals, in which periodic movements of the stomach and duodenum were recorded (during two or three periods of work and rest), and on fed dogs, which were fed with 75 g bread + 75 g of stewed lean meat in lumps, 15-30 min after the

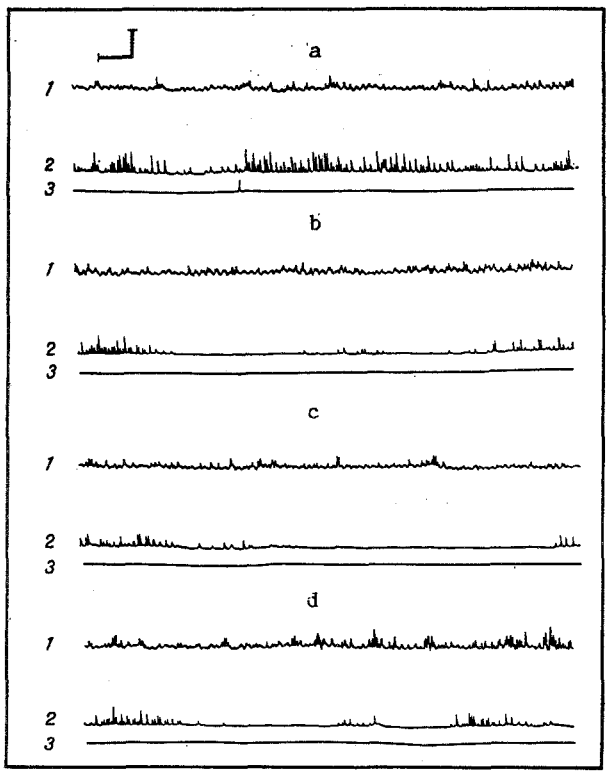

Fig. 2. Effect of intravenous injection of peptide IV-1 on food-induced gastric and duodenal motility. a) Time of injection of peptide; b) first phase of inhibition of motility 30 min after injection of peptide; c) second phase of inhibition a further 35 min after end of first phase; d) third phase of inhibition 40 min after end of second phase of inhibition. Method of injection and dose the same as in Fig. 1.

beginning of recording of motility; 20 min after feeding the peptides were injected intravenously or subcutaneously into these animals in doses of 3 and 6 μg/kg body weight. After injection of the peptides, recording of motility continued for a further 3-5 h.

EXPERIMENTAL RESULTS

Two fractions of low-molecular-weight peptides (III and IV), numbered in accordance with the order of their elution from the column, were isolated from products of short-term hydrolysis of K casein by hog pepsin, by gel-chromatography on a Sephadex G-50 column. After additional purification of peptide IV on BioGel P-2, a peptide IV-1, eluted in the external volume of the column, was isolated. Experiments on dogs showed that peptide III, in a dose of 6 μg/kg, immediately after intravenous injection, caused inhibition of gastric and duodenal digestive movements which lasted for 10 min. Motility was then restored, and 60-75 min after the injection, the dogs began to vomit while normal contractility of their gastrointestinal tract was restored. The same dose of peptide III, when injected subcutaneously, induced a distinct inhibitory response after a latent period of 7-8 min, which continued for 6-8 min, but vomiting did not arise in this case.

Intravenous injection of peptide IV-1 in a dose of 6 μg/kg, against a background of periodic movements of the gastrointestinal tract, did not change the cyclic character of the onset of periods of work and rest, but during a regular rest period after injection of the peptide, small chaotic movements developed, although they did not disturb differentiation between the regular periods of work and rest (Fig. 1).

Peptide IV-1 had more marked effects on gastric and duodenal motility induced by food. As a rule, intravenous injection of the peptides in a dose of 6 μg/kg, after a latent period of 15-20 min, induced definite inhibition of gastric and duodenal motility for 6-12 min. In most experiments this inhibitory effect after a single injection of the peptide was repeated in a fresh cycle every 20-40 min another two or three times (Fig. 2). If a smaller dose

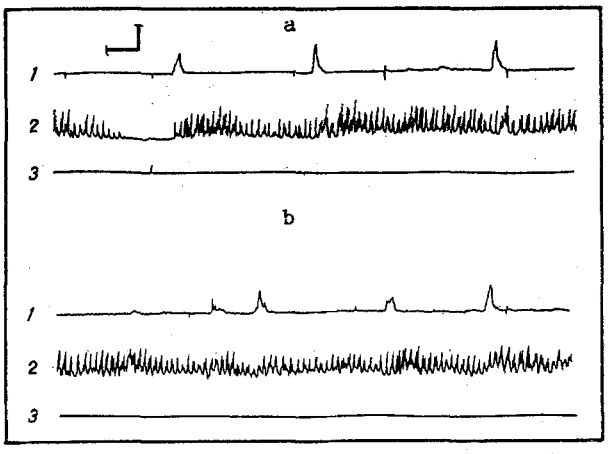

Fig. 3. Absence of inhibitory effect of peptide IV-1 on gastric and duodenal motility in dogs undergoing trunk vagotomy: a) time of injection of peptide during food-induced motility; b) gastric and duodenal motility 20 min later. Mode of injection and dose the same as in Fig. 1.

of the peptide (3 μg/kg) was given, gastric and duodenal motility was more frequently inhibited only once. Subcutaneous injection of the same dose of peptide IV-1 had no effect. In dogs undergoing the operation of selective proximal vagotomy, the inhibitory action of peptide IV-1 was short and weak. It was completely absent in a dog on which bilateral trunk vagotomy was performed (Fig. 3).

In most experiments intravenous injection of peptide IV-1 was followed by changes in the animals' emotional state: they became more lively or, conversely, more inhibited.

It follows from the time course of the inhibitory action of peptide III that it contains two different inhibitors of motility. Inhibitor 1 exerts its action immediately after intravenous injection, whereas the effect of inhibitor 2 appears after a latent period of 60-75 min, and it is accompanied by vomiting. Vomiting indicates that inhibitor 2 evidently can pass through the blood–brain barrier and interact with corresponding brain structures.

In the latent period and character of its physiological action, inhibitor 2 resembles GMP [3]. Similar data on the biphasic character of its action also have been obtained for inhibition of gastric secretion [4]. In response to subcutaneous injection of peptide III, vomiting by the animals were not observed, evidently due to partial degradation of inhibitor 2 under the influence of tissue proteases.

Peptide IV-1 preserves its inhibitory influence on gastric and duodenal motility induced by food, but its action is no longer biphasic in character, probably as a result of enzymic degradation of inhibitor 2 under the influence of pepsin to fragments with lower molecular weight. The duration of the inhibitory action shortens with shortening of the length of the peptide fragment, evidently due to its more rapid degradation and inactivation in the body.

For the peptides to exhibit their inhibitory action, the vagus innervation must be intact. Partial or total destruction of it led to reduction or absence of the inhibitory effect, respectively (Fig. 3). It is a very interesting fact that the inhibitory action of the inhibitors was manifested only against food-induced motility (Fig. 2), and in the experiments conducted in the fasting state, some stimulation of motility was observed during a regular rest period after injection of the peptides (Fig. 1). This opposite character of action depending on the food status of the animal is also characteristic of another regulatory peptide, namely β-casomorphine, which is a fragment of the β-casein molecule. The potentiating action of β-casomorphine on secretion of insulin [12], somatostatin [13], and pancreatic polypeptide [11] is manifested only during preliminary infusion of glucose and amino acids into the animal, whereas β-casomorphine either has no action on the basal level of these hormones or depresses it. Peptide-inhibitors of gastric motility and secretion, which are released from K casein during digestion of milk proteins in vivo [2], are evidently natural inhibitors, formed in the process of evolution [5], whose action is realized only during the response to food. Being proteolysis products of a widely distributed food protein, namely milk casein, these isolated peptide inhibitors may become promising therapeutic agents for the treatment of gastroduodenal pathology associated with a hyperacid state.

LITERATURE CITED

1. E. Ya. Stan, A. V. Popova, and M. P. Chernikov, Vopr. Pitan., No. 1, 18 (1979).
2. E. Ya. Stan and M. P. Chernikov, Byull. Éksp. Biol. Med., No. 8, 64 (1982).
3. E. Ya. Stan, S. D. Groisman, K. B. Vasil'shchikov, and M. P. Chernikov, Byull. Éksp. Biol. Med., No. 7, 10 (1983).
4. E. Ya. Stan, A. P. Ekimovskii, S. I. Aleinik, et al., Vopr. Med. Khim., (1987) (in press).
5. M. P. Chernikov, G. V. Nikol'skaya, E. Ya. Stan, et al., Vopr. Pitan., No. 2, 21 (1974).
6. G. K. Shlygin, L. S. Vasilevskaya, M. P. Chernikov, and G. V. Nikol'skaya, Byull. Éksp. Biol. Med., No. 2, 9 (1971).
7. V. Brantl, H. Teschemacher, A. Henschen, and F. Lottspieck, Z. Physiol. Chem., 360, 1211 (1979).
8. T. Mitsuma, T. Nogimori, and M. Chays, Exp. Clin. Endocrinol., 84, 324 (1984).
9. J. Nedvidkova, E. Kasafirek, A. Dlabac, and V. Felt, Exp. Clin. Endocrinol., 85, 249 (1985).
10. K. G. Reymann, A. N. Chepkova, K. Sculzeck, and T. Ott, Biomed. Biochim. Acta, 44, 749 (1985).
11. R. Schick, A. Holland, A. Fuente, et al., Acta Endocrinol. (Copenhagen), Suppl. 253, 80 (1983).
12. V. Schusdziarra, R. Schick, A. Fuente, et al., Endocrinology, 112, 885 (1983).
13. V. Schusdziarra, R. Schick, A. Fuente, et al., Endocrinology, 112, 1948 (1983).
14. C. Zioudrou, R. A. Streaty, and W. A. Klee, J. Biol. Chem., 254, 2446 (1979).
15. C. A. Zittle and J. H. Custer, J. Dairy Sci., 46, 1183 (1963).

FREE SERUM AMINO ACIDS IN RATS WITH HEPATIC FAILURE DUE TO SMALL INTESTINAL FISTULAS

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UDC 616.36-008.64-092.9-02:616.341-007.253/-07:616.153.466-074

KEY WORDS: free amino acids; small intestinal fistulas; hepatic failure.

Clinical observations on patients with hepatic failure (HF) of varied etiology [12, 13] and experimental investigations [7, 11] have revealed uniform changes in the free amino acid (AA) spectrum of the blood (Fig. 1). An increase in the total AA content with a considerable rise in the level of aromatic AA and a fall in the content of branched-chain AA has now begun to be used as a diagnostic test for HF [14, 15]. The metabolic basis for the development of changes in the ratio between AA and their participation in the development of hepatic coma have been partly elucidated [10, 12]. It is noteworthy that all pathological processes studied were the result of primary damage to hepatocytes (hepatotoxic poisons, viral and nonviral hepatitis) or of a slowly developing dystrophic process (cirrhosis of the liver, etc.). It will be noted that the catabolic reactions in these processes are of moderate intensity [1]. Previously, however, the writers showed that HF against a background of high catabolic activity appears after exhaustion of the compensatory–adaptive reactions of metabolism [4, 5]. The biochemical and morphological symptoms of HF of this pathogenesis have the characteristic features of developing HF and of active reorganization of metabolism in order to maintain gluconeogenesis. About 50-60% of free AA and of AA obtained in the course of proteolysis is utilized for gluconeogenesis and ketogenesis by the body when there are functioning small-intestinal fistulas [2]. This degree of AA utilization after total exhaustion of the energy reserves of the body, as takes place in the presence of small intestinal fistulas, must have some effect on the AA spectrum in HF, developing as a result of functioning of small intestinal fistulas. Despite the acute necessity of determining the AA spectrum in the presence of

Department of General Surgery of the Pediatric Faculty, and Department of Biochemistry of the Interlaboratory Complex, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 426-428, October, 1987. Original article submitted October 17, 1986.

