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EFFECT OF χ-CASEIN GLYCOMACROPEPTIDE ON GASTROINTESTINAL MOTILITY IN DOGS

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Casein, the principal dietary protein of newborn mammals, has features of alimentary specificity, elaborated as a result of prolonged evolution and expressed in a surprising adaptibility of the caseins to differences in the character of function of the gastrointestinal tract in the neonatal period [8]. Maximal vulnerability of native casein to attack by gastrointestinal proteinases [3, 9] and its ability to undergo curdling enable the newborn animals to digest this protein with minimal expenditure of energy and ensure the optimal rate of entry of protein into the intestine and absorption of amino acids [4, 10].

The writers have shown that physiologically active peptides, capable of affecting the circulation [5] and of inhibiting gastric secretion [11] can be formed from the caseins of cows' milk as a result of their limited proteolysis. It has also been shown that a peptide inhibiting gastric secretion in the newborn rat stomach can be formed in vivo by digestion of rat milk proteins [6].

The object of this investigation was to study the action of casein glycomacropeptide (an inhibitor of acid secretion by the stomach [11]) on motor activity of the fundal portion of the stomach and of the duodenum.

EXPERIMENTAL METHOD

χ-Casein was obtained by Zittle's method and subjected to brief (2 min) action of pepsin with an enzyme–substrate ratio of 1:100 (37°C, pH 5.8). Proteolysis was stopped and protein and large peptides precipitated with a 12% solution of TCA (final concentration). The precipitate was discarded and the filtrate dialyzed and lyophilized. The resulting peptide material, in a quantity of 400 mg in 10 ml, was applied to a Sephadex G-25 column (2.5 × 80 cm), equilibrated with 0.5 M NaCl solution. The filtration rate was 80 ml/h. Elution of the fractions from the column was recorded by means of an RÉPPS-1M recorder on the basis of the change in percentage absorption at 260 nm. The high-molecular-weight fraction, namely the glycomacropeptide, eluted in the void volume of the column, was desalted and lyophilized on a Sephadex G-10 column (2.5 × 80 cm).

Experiments were carried out on two dogs weighing 15 kg with fistulas to the gastric fundus and duodenum. Motor activity was recorded graphically using a balloon and mechanotron electromanometer, with RPCh-2 electromechanical recorder. An ink-writing x-y recorder also was used and gastric and duodenal motor activity was expressed in conventional units as a motor index by means of an electronic integrator. The concept of "motor index" was based on analysis of the work done by the stomach and duodenum in the course of 15-min time intervals. In control experiments on dogs the background feeding motor activity was recorded. For this purpose hungry dogs were fed with a test meal (75 g bread and 75 g meat), after which their gastric movements were recorded and the motor index calculated for a period of 3-5 h. Glycomacropeptide was injected intra-

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TABLE 1. Effect of Glycomacropeptide on Gastric and Duodenal Movements in Dogs (expressed as 15-min motor index: 1 impulse equivalent to 6.6 g/min)

Time of experiment	Dog No. 1				Dog No. 2			
	stomach		duodenum		stomach		duodenum	
	background	peptide	background	peptide	background	peptide	background	peptide
15 min	84±13,9	94±4,0	175±48	223±35	124±30		116±17,4	
	112±6,8	114±11,0	231±11,3	261±35	127±15	120±20	157±19,6	148±33
	133±9,7	117±9,5	267±29	206±47	128±13,5	118±6,1	175±26	175±31,5
1 h	125±4,04	113±2,5*	235±20	193±24*	110±6,5	119±4,0*	150±12	176±32*
	119±10,3	101±1,5	218±14,3	203±29	113±7	109±6,06	178±16	179±24
	115±7,5	100±3,8	225±13	175±18	107±18	100±9,4	147±13	152±20
	113±3,6	92±6,2	221±31	173±15	98±7	135±16,8	135±16,8	169±28,9
2 h	102±6,6	93±4,8	169±39	172±15	112±14	68±17,4	135±21	141±30
	93±5,6	93±2,8	115±34	164±26	93±11	74±7,3	122±17	125±29,3
	97±1,4	99±5,3	176±18	144±32	98±7	97±19	141±21	140±19
	97±6,8	95±3,6	174±5,7	164±21	96±10	94±19	139±15	145±32,7
3 h	97±4,7	87±3,5	209±27	166±9	87±10	82±10	133±14	144±35
	91±8,5	85±4,8	182±6,3	164±8	85±9,5	75±6	129±22	133±13
	88±0	97,5±6,0	192±26	181±16	74±5,5	86±1	154±12,5	150±3,5
	88±0	88±3,8	170±15	181±14	70±5	103±7	142±3	244±55
					70±10	108±23	—	191±55

*Injection of peptide.

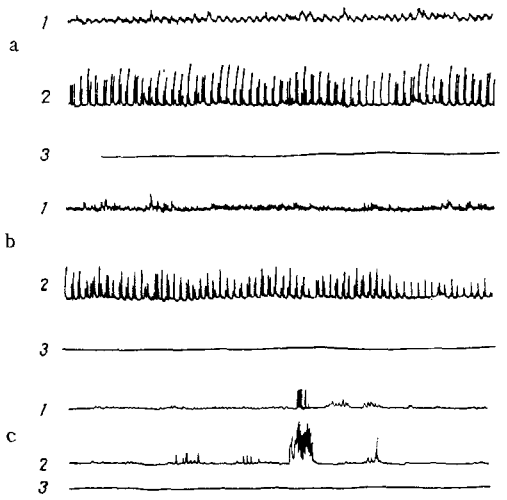

Fig. 1. Effect of glycomacropeptide on movements of stomach and duodenum in dogs: a) without injection of peptide, b) 1.5 h after intravenous injection of 10 mg glycomacropeptide, c) cyclic vomiting after injection of glycomacropeptide into hungry dogs. 1) Gastric fundus, 2) duodenum, 3) marker of integrator.

venously in a dose of 10 mg 20-40 min after feeding. Five experiments were carried out on each dog. Since feeding motor activity of dogs has a tendency to diminish spontaneously 60-90 min after feeding (the so-called second phase of feeding motor activity), to assess the action of glycomacropeptide more objectively the whole series of values of the motor index in control and background investigations was compared. To avoid the action of the natural decline of feeding movements with time, experiments were carried out in which the peptide was injected into fasting animals.

EXPERIMENTAL RESULTS

The glycomacropeptide was isolated from short-term pepsin hydrolysis products of χ-casein by gel-chromatography on a Sephadex G-25 column and desalting on a Sephadex G-10 column. After intravenous injection into dogs in a dose of 10 mg per animal the glycomacropeptide caused statistically significant inhibition of feeding movements of the gastric fundus in both dogs (Table 1); in dog No. 2, moreover, this inhibition clearly began 60 min after injection of the peptide and continued for 30-40 min. It will be clear from Fig. 1 that the

glycomacropeptide caused a decrease in amplitude of the peristaltic contractions of the gastric fundus. Inhibition of duodenal motor activity was observed only in dog No. 1 (Fig. 1b, 2).

The glycomacropeptide, when injected into fasting dogs, induced persistent cyclic vomiting, repeated every 30-50 min, in the animals after 60-90 min (Fig. 1c). The animals lost their appetite. At this time the periodic contractions of the stomach and duodenum were found to be inhibited. When the peptide was injected into fed dogs vomiting developed in 3 of 10 experiments.

An important role in the mechanism of protein hydrolysis in the stomach is played by motor activity of the gastric fundus, which is responsible for mixing the chyme with the secretion, for changing the juxtamural layer of chyme and moving it from the central zone to the juxtamural zone and, consequently, for accelerating protein hydrolysis. The physiological action of glycomacropeptide from cows' χ-casein consists of inhibiting the secretion of acid by the stomach and its motor activity, and it is thus aimed at depressing proteolysis of protein in the stomach. This phenomenon may be exceptionally important during the period of milk feeding, when the chief source of protein is the milk proteins, during the digestion of which in vivo in the neonatal stomach a peptide with similar physiological action may be formed [6].

Because of the resistance of the glycomacropeptide to the action of gastrointestinal proteinases (pepsin, trypsin, and chymotrypsin [12]), it is perhaps preserved in the small intestine and, because of the low molecular weight of the active fragment of the peptide [7] and the high permeability of the intestinal mucosa to biopolymers, it may penetrate into the blood stream in the period of milk feeding, where it exerts its physiological action, inhibiting proteolysis of milk proteins in the stomach and thereby facilitating preservation of biologically active proteins (γ-globulins, lactoferrin, lysozyme) in vivo in the neonatal gastrointestinal tract. This peptide, which is constantly being formed in the stomach during digestion of the proteins of the mother's milk, can evidently prevent premature stimulation of gastric secretion during stress of nonalimentary origin. Inhibition of motor activity of the gastric fundus and vomiting are typical effects of central excitation of opiate receptors [1]. Endogenous opiates also are very powerful inhibitors of gastric secretion [2, 14]. Meanwhile there are data in the literature on opiate-like peptides of alimentary origin, namely exorphins formed as a result of proteolysis of β-casein [13] and α-casein [15]. On the basis of these results and data in the literature it can be postulated that the glycomacropeptide has an opiate-like action.

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