EFFECT OF AN INHIBITOR OF GASTRIC SECRETION FROM KAPPA-CASEIN ON MESENTERIC LYMPHATIC MICROCIRCULATION AND INTESTINAL MOVEMENTS IN RATS

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Bioregulators of peptide nature are currently at the focus of attention of research workers as promising therapeutic agents for the correction of various pathological states. A peptide preparation has been isolated [9] from a peptic digest of the food protein kappa-casein from cows' milk which can inhibit gastric secretion and food-induced movements of the stomach and intestine [3, 6]. This substance combines an inhibitory action with neurotropic activity, similar in its electroencephalographic picture to food satiation or to the action of cholecystokinin [7]. Individual fractions of this preparation exhibit analgesic activity when injected intraperitoneally [4]. The stimulating action of some opioid peptides on the lymph flow in the microvessels, discovered previously [5, 10, 11], and also stimulation of the lymph flow after food intake suggest that this peptide preparation, that inhibits gastric secretion, may have a similar action.

The aim of this investigation was to study the effect of the isolated peptide preparation from milk kappa-casein on contractile activity (CA) of the mesenteric lymphatic microvessels (LM) and on intestinal movements, involved in the mechanism of the lymphatic drainage.

EXPERIMENTAL METHOD

The investigation was carried out on a fraction of a preparation of casein inhibitor of gastric secretion (CIGS) which, on a Sephadex G-50 column, is eluted at the second peak on the chromatogram (fraction II [6]) and contains peptide material with mol. wt. of 1000-5000 daltons. To obtain an amino acid mixture equivalent to CIGS we used 1-forms of amino acids (Serva, West Germany). The experiments were conducted on 36 noninbred male albino rats weighing 250 g, anesthetized with pentobarbital (0.1 g/kg body weight, intramuscularly). Intravital microscopy of the small intestinal mesentery was carried out by the method in [13]. CA of the wall and valves of the mesenteric LM and the wall of the intestine was studied by the method in [2]. CIGS in a dose of 0.001-200.0 μg in 0.1 ml of isotonic sodium chloride solution and the equivalent mixture of amino acids in the same concentration were applied to the surface of LM and to the intestinal wall. In the experiments of series II CIGS or the equivalent mixture of amino acids was introduced into the lumen of the intestine in a dose of 5 mg in 0.5 ml of isotonic sodium chloride solution. The results were subjected to statistical analysis by Student's test.

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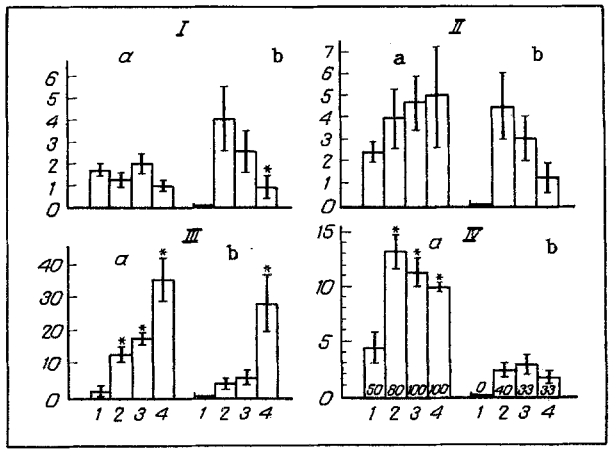

Fig. 1. Effect of application of CIGS on CA of wall (a) and valve (b) of mesenteric LM of rat intestine. I) Latent period before beginning of contraction (in min), II) time of onset of maximal response (in min); III) duration of contraction (in min); IV) maximal frequency of contraction during 1 min. Abscissa, doses of CIGS: 1) 0.01 μg, 2) 0.1 μg, 3) 1.0 μg, 4) 10.0 μg; ordinate: for I, II, and III — time (in min); for IV — frequency of contraction per minute. Numbers inside columns show number of activated LM (in %). Asterisk indicates significance of difference ($p < 0.05$) compared with threshold dose of CIGS.

EXPERIMENTAL RESULTS

In the control, LM contracted with a frequency of 3-5 contractions per minute for 5-10 min. The investigation was carried out on LM not contracting initially, for the different initial background of contraction affected the intensity of the subsequent response. Application of CIGS in different doses (0.001-200.0 μg/0.1 ml) to the surface of the mesenteric LM of the rats led to maximal activation of CA of the vessels when used in a dose of 0.1-10.0 μg. A greater increase in the dose of CIGS was accompanied by weakening or absence of CA of LM. The threshold dose of CIGS, causing contraction of the wall of LM was 0.01 μg, and of the valve 0.1 μg. With an increase in the dose of CIGS from 0.01 to 10.0 μg the latent period before the beginning of contraction of the wall of LM was unchanged, but that of the valve was reduced (Fig 1, I). A similar dependence was observed in the case of the time of the maximal response of LM (Fig. 1, II). The duration of contraction of the wall and valve, and also the number of functioning LM (contraction of the wall) increased with an increase in the dose (Fig. 1, III-IV).

Consequently, CIGS, when applied, activates CA of LM (the wall to a greater degree than the valve), which is characterized by dose-dependence of parameters such as the number of functioning LM, the duration of their contraction, and the latent period to the beginning of contraction.

Since individual amino acids may affect CA of LM [12], for comparison we studied the action of a mixture of amino acids equivalent to CIGS Application of the mixture of amino acids in low concentrations (up to 50.0 μg) had no appreciable effect on CA of LM, whereas application of high doses (50-200 μg) caused disturbances of the microcirculation of the blood in the mesenteric microvessels, in which stasis developed. This action of the mixture of amino acids was evidently due to the presence of arginine [12].

To determine the possible action of CIGS under conditions as close as possible to natural, it was injected into the lumen of the intestine (5 mg in 0.5 ml). Under these circumstances its stimulating action on CA of LM was preserved (Fig. 2). The maximal frequency and duration of contraction of the wall of LM were the same as with application to the mesentery in a dose of 0.1-10.0 μg (compare Figs. 1 and 2). The latent period and the time of onset of maximal CA of the wall and valve were increased by more than 15 times, which can be attributed to the time taken in overcoming the intestinal barrier. The response of the valve of LM to intraintestinally injected CIGS was weaker than during application, and the maximal frequency and duration of contraction of the valve were 3-4 times less.

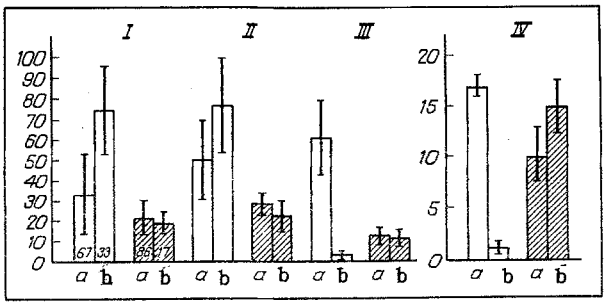

Fig 2. Effect of intraintestinal injection of CIGS (5 mg/0.5 ml) and of equivalent mixture of amino acids (5 mg/0.5 ml) on CA of wall (a) and valve (b) of mesenteric LM of rat intestine. Unshaded columns — CIGS; shaded columns — mixture of amino acids. Remainder of legend as to Fig. 1.

A mixture of amino acids, equivalent to CIGS, injected into the intestine caused CA of the wall of LM which was weaker in strength and duration; the latent period to the beginning of contraction was shorter than for the action of CIGS (Fig. 2).

Thus intraintestinal injection of CIGS is accompanied by activation of CA of the wall and a very weak effect on CA of the valve of LM. The equivalent mixture of amino acids, absorbed more rapidly by the intestinal wall, had a more rapid but shorter action on CA of LM; the effect on the wall and valve, moreover, was the same These results are evidence that both individual fragments of CIGS and the amino acids of which it consists, and which possess lymphotropic activity, can penetrate through the intestinal wall.

Intensification of movements of the intestinal wall led to an increase in CA and lymphatic drainage along the mesenteric LM. To study the role of the intestine in this process, the effect of CIGS was studied on motor activity of the muscular membrane of the intestinal wall. CIGS, if applied to the intestine from the side of the serosa, potentiated its CA on average by 33%; its action, moreover, was directed mainly at increasing the minimal frequency of contraction, which rose by 51% (Fig. 3). With intraintestinal injection of CIGS (5 mg in 0.5 ml) an increase in intestinal CA on average by 31% also was observed, but it was weaker than after intraintestinal injection of the same dose of the equivalent amino acid mixture, which increased intestinal CA on average by 84%.

It can accordingly be postulated that amino acids, removed from CIGS within the intestinal lumen under the influence of proteinases, may participate in the realization of the stimulating action of CIGS on intestinal CA when injected into the intestinal lumen, and that activation of the lymph flow under the influence of the amino acid mixture is largely connected with activation of CA of the muscles of the intestinal wall.

Thus CIGS stimulated CA of the wall and valve of LM in a dose-dependent manner. Its action when applied to the mesentery was exhibited within a narrow dose range, namely from 0.01 to 10.0 μg, and with a further increase in the dose to 50-200 μg it disappeared. This sliding of the physiological action with an increase in the dose is found with many known peptide regulators [10-12].

An important advantage of CIGS is that its activating action on CA of LM is preserved on intraintestinal injection, just as was observed previously with CIGS in acute experiments on rats [1]; in this case the latent period of realization of the effect increased compared with the latent period observed on application. Since on replacement of CIGS by the equivalent mixture of amino acids, the stimulating effect on CA of LM weakened and was actually replaced by lymph stasis, it can be postulated that the action of the inhibitor on CA of LM was due mainly to its peptide fragment.

CIGS potentiated CA of the intestinal wall on application, in agreement with stimulation of the periodic fasting movements of the duodenum noted previously in dogs in response to intravenous injection of one fragment of CIGS [3]; this potentiation of CA may make a contribution of its own to the increased lymphatic drainage from the mesentery.

A characteristic difference of CIGS from endogenous regulatory peptides is its alimentary origin from a widely distributed food protein, namely casein from cows' milk. In the primary structure of casein there are several amino acid sequences which, in the course of proteolysis, are released in the form of physiologically active peptides [8]. One such peptide is contained in the preparation tested in the present study.

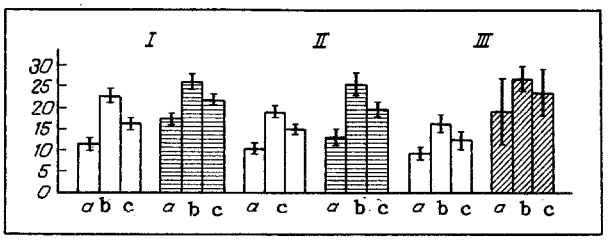

Fig. 3. Effect of application (I) and of intraintestinal injection (II, III) of CIGS (I, II) and of equivalent mixture of amino acids (III) on CA of musculature of rat small intestine. a) Minimal frequency of contraction of intestinal wall, b) maximal, c) average Ordinate, frequency of contraction, per minute. Unshaded columns — initial CA of intestinal wall; horizontal shading — CIGS; oblique shading — mixture of amino acids. Asterisk indicates significant differences ($p < 0.05$).

Although the lymphotropic action of CIGS is weaker than that of Leu-enkephalin and its synthetic analog dalargin [11, 12], and is realized in doses two orders of magnitude higher, nevertheless the ability of the inhibitor to preserve its activity when injected into the intestinal lumen makes its use as an additive, in the composition of a therapeutic diet, highly promising. A combination of inhibitory action on gastric secretion and a stimulating effect on the lymphatic microcirculation suggests that this preparation may be used for the treatment of diseases of the gastrointestinal tract associated with a state of hyperacidity, and in inflammatory conditions.

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