A PHYSIOLOGICAL INHIBITOR OF GASTRIC SECRETION, THE ACTIVATION PEPTIDE OF TRYPSINOGEN

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Received 12 June 1973

1. Introduction

Numerous peptides produced by proteolytic cleavage of proteins such as kinins or angiotensins, have been shown to have powerful pharmacological actions [1]. The thrombin-catalyzed conversion of fibrinogen to fibrin results in the release of several peptides. The β-peptide possesses the ability to potentiate the bradykinin-induced contraction of rat-uterus muscle [2].

The duodenal activation of pancreatic zymogens, trypsinogen, chymotrypsinogens, prophospholipases, proelastase, for example, releases a number of peptides [3–5] which may have physiological activities. The peptide with the most peculiar sequence and which is liberated in highest amounts is that formed during the conversion of trypsinogen to trypsin. In this paper we study the effect of Val–$(Asp)_4$–Lys, the hexa-peptide [3] that is liberated during the conversion of bovine trypsinogen to trypsin, on pancreatic and gastric secretions of the dog.

2. Methods

2.1. *Preparation of the activation peptide of bovine trypsinogen*

5 g of bovine trypsinogen (Worthington, USA) in 500 ml of a 10 mM Tris-Cl buffer at pH 8.0, containing 100 mM NaCl and 50 mM $CaCl_2$, were converted to trypsin, at 0°C (3 hr), by 200 mg of crystalline trypsin (Worthington, USA). The peptide was isolated from the activation mixture by filtration through Sephadex G-25 and ion-exchange chromatography at 50°C on a column of Bio-Rad. Elution was carried out with an ionic strength and pH gradient as follows. Starting buffer: 1 ℓ of a 0.2 M pyridine–formic acid buffer at pH 3; final buffer: 1 ℓ of a 1 M pyridine–formic acid buffer at pH 5. Migration of the peptide was followed spectrophotometrically at 570 nm after alkaline hydrolysis and reaction with ninhydrin [6]. The purity of the peptide was checked by a determination of the amino acid composition after acid hydrolysis.

2.2. *Evaluation of the physiological effect on the pancreatic and gastric secretions of the dog of the activation peptide of trypsinogen*

Seven dogs, weighing between 12 and 15 kg, were prepared with chronic gastric and pancreatic Thomas cannulae [7]. Experiments were started no less than 4 weeks after surgery. Each experiment was carried out with the animal in the conscious state, standing

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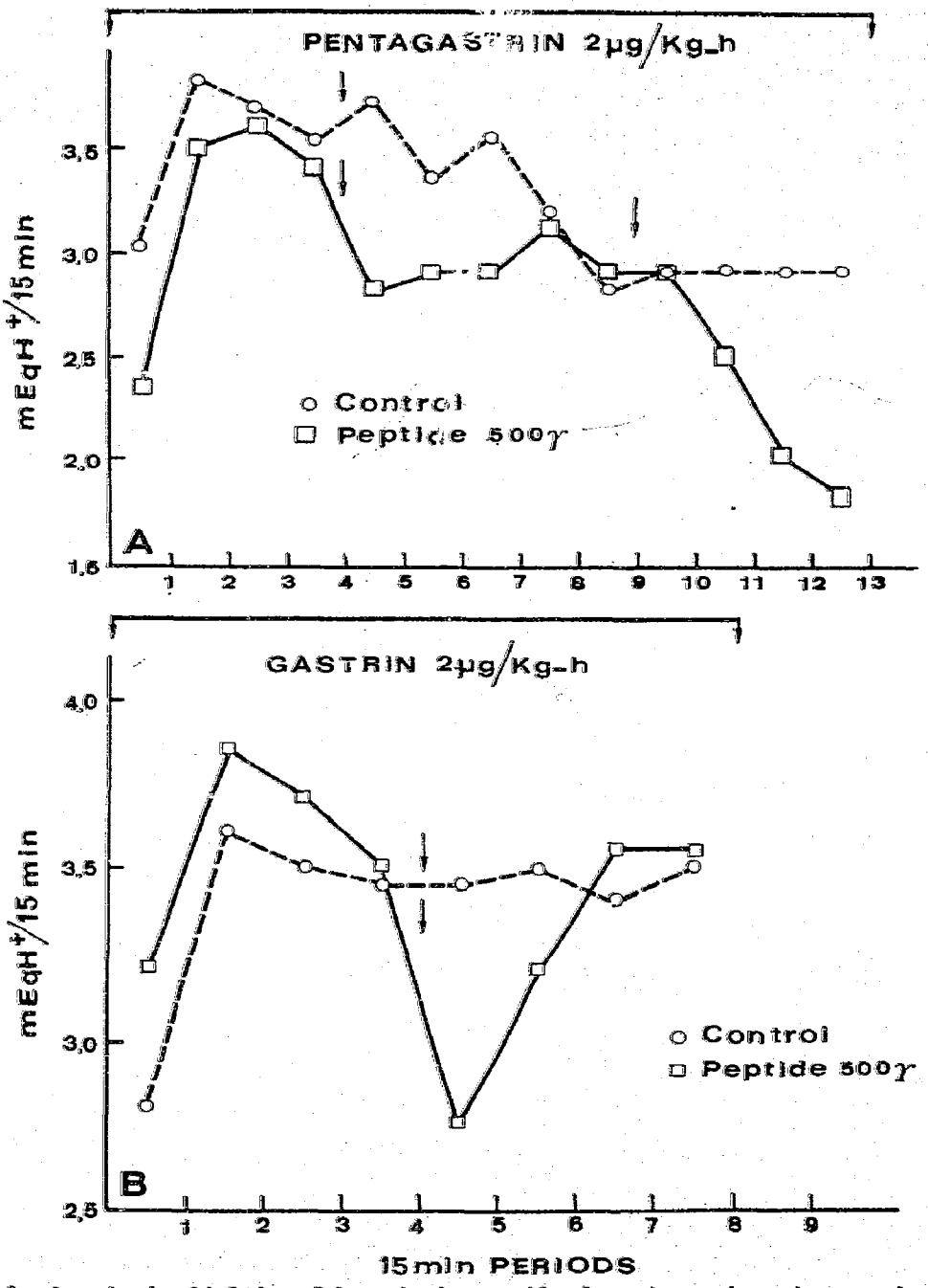

Fig. 1. A) The effect of an intraduodenal infusion of the activation peptide of trypsinogen (arrow) on gastric secretion stimulated by intravenous infusion of pentagastrin. The control received an intraduodenal infusion of isotonic saline (5.0 ml) instead of peptide. Before it was administrated, peptide I (700 µg) was diluted in 5.0 ml of isotonic saline, pH 7. The time of infusion was of 10 min. B) The effect of an intraduodenal infusion of the activation peptide of trypsinogen (arrow) on gastric secretion stimulated by intravenous infusion of gastrin. Control received an intraduodenal infusion of isotonic saline (5.0 ml) instead of peptide. In test, peptide I (500 µg) was diluted in 5.0 ml of isotonic saline, pH 7. The time of infusion was of 10 min.

quietly in a harness. The dogs were deprived of food, but not water, for at least 18 hr before the experiment.

Gastric secretion was stimulated by a continuous constant-rate (100 ml/hr) intravenous infusion of either penta-gastrin (Imperial Chemical Industries, Ltd., England), 2 μg/Kg-hr, human gastrin I (Imperial Chemical Industries Ltd., England), 2 μg/Kg-hr, or hog gastrin (Eurorga Laboratories, France), 2 μg/Kg-hr. During each experiment gastric secretion was collected continuously. Its volume was measured every 15 min and the acid concentration was estimated with a glass electrode. Acid outputs were derived by multiplication of the volume and concentration values. The plateau of acid secretion from the cannula was achieved 60 min after the start of the hormone I.V. injection. Once a plateau acid response had been established, the activation peptide of trypsinogen (peptide I), or those used as control (peptides II and III)*, were given intravenously or intraduodenally in doses ranging between 300 and 2500 μg. Differences between test and control mean values for each 15 min period were tested for statistical significance by Student's *t*-test for paired values. $P < 0.05$ was taken as the level of significance.

3. Results and discussion

Fig. 1 A shows the effect of 700 μg of peptide I, infused intraduodenally by means of a catheter introduced through the duodenal Thomas cannula, on the acid response to pentagastrin 2 μg/Kg-hr. The HCl concentration of the gastric juice remained unchanged. However, as can be seen in fig. 1, an inhibition of acid secretion occurred rapidly after the infusion of peptide I, being statistically significant for 1 hr, after which there was a return to the control level. A second infusion of peptide I produced a new inhibition of gastric secretion.

Fig. 1 B shows the effect of an intraduodenal infusion of 500 μg of peptide I on the acid response to a 2 μg/Kg-hr dose of hog or human gastrin I; as with pentagastrin, a temporary inhibition of gastric secretion was also observed.

Table 1

Effect of an intravenous infusion of the activation peptide of trypsinogen (700 μg) on gastric secretion stimulated by a continuous infusion of pentagastrin (2 μg/Kg-hr).

	Volume (ml/15 min)	HCl outputs (mEq/15 min)
Control	35.2 ± 1.2	3.62 ± 0.16
Expti.: 1st period	29.6 ± 2.9	3.03 ± 0.25
2nd period	27.6 ± 2.6	2.89 ± 0.25
3rd period	27.3 ± 2.3	2.87 ± 0.46
4th period	28.6 ± 1.4	2.63 ± 0.41

Values are means of 7 tests ± S.E. Control = average values of the last two periods of 15 min preceding the infusion of the peptide. Expti. = the four periods of 15 min following the infusion of the peptide. $P < 0.05$ was taken as the level of significance.

Table 1 shows that, as in the intraduodenal experiment, the intravenous administration of peptide I also produced an inhibitory effect. The average depression of volume and HCl output was about 20%.

The inhibitory effect is specific to peptide I. Intraduodenal infusion of isotonic saline or of peptides II (2500 μg) and III (650 μg) did not modify the plateau levels of gastric secretion stimulated by a continuous infusion of pentagastrin (2 μg/Kg-hr).

The range of peptide I doses used in these experiments is between 300 μg and 700 μg. It corresponds to activations of 10.6 to 24.8 mg of trypsinogen. These amounts of the zymogen coincide with those liberated in 15 min in the pancreatic juice under the present experimental conditions. The average trypsinogen, chymotrypsinogen and lipase outputs of the pancreatic secretion stimulated by a continuous infusion of either pentagastrin or gastrin (2 μg/Kg-hr) were 13.3 mg/15 min, 6.4 mg/15 min, and 1.4 mg/15 min respectively. (The total protein output was 63.6 mg/15 min).

The N-terminal peptide liberated from bovine trypsinogen, during the course of the activation step, has a very peculiar sequence, Val–$(Asp)_4$–Lys, with four adjacent aspartyl residues. This cluster of aspartyl residues in the activation peptide has been found in all trypsinogens studied till now, including dogfish trypsinogen, with only one exception, the lungfish

* Peptide II: Val–Ala–Ala–Lys–Ile–Val–Gly was synthetized as previously described [8]; peptide III: pyroGlu–Glu–Gly–Ile–Ser–Ser–Arg, the activation peptide of hog prophospholipase A [4] was a gift of Dr. G.H. de Haas, Utrecht, Holland.

Table 2
Sequence of activation peptides of trypsinogen.

Species	Activation peptide	References
Ox	Val–Asp–Asp–Asp–Asp–Lys	[3]
Pig	Phe–Pro–Thr–Asp–Asp–Asp–Asp–Lys	[9]
Wild boar	Phe–Pro–Thr–Asp–Asp–Asp–Asp–Lys	[10]
Sheep	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys Val–Asp–Asp–Asp–Asp–Lys	[10]
Goat	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys Val–Asp–Asp–Asp–Asp–Lys	[10]
Red deer	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys Val–Asp–Asp–Asp–Asp–Lys	[10]
Roe deer	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys Val–Asp–Asp–Asp–Asp–Lys	[10]
Horse	Ser–Ser–Thr–Asp–Asp–Asp–Asp–Lys	[11]
Dromedary	Val–Pro–Ile–Asp–Asp–Asp–Asp–Lys	[10]
Dogfish	Ala–Pro–Asp–Asp–Asp–Asp–Lys	[12]
Lungfish	Phe–Pro–Ile–Glu–Glu–Asp–Lys	[13]

trypsinogen, (table 2) where the $(Asp)_4$ sequence is replaced by a Glu–Glu–Asp sequence [3, 9, 13].

The N-terminal peptide which is liberated from trypsinogen during the activation has already been found to contain considerable "information".

i) The cluster of 4 aspartyl residues is an essential element for the "recognition" of trypsinogen by enterokinase, the enzyme which initiates the duodenal activation of the zymogen [14].

ii) As it is well known, the initiation of the activation is followed by an autoactivation process involving trypsin itself. The 4 aspartyl residues serve the role of "floats" at the N-terminal sequence of trypsinogen and make the activation bond Lys_7–Ile_8 readily available to trypsin, while the other lysyl or arginyl bonds of the trypsinogen sequence are protected within the three-dimensional structure of the protein (at least in the presence of Ca^{2+}) and therefore cannot be cleaved by the enzyme [15, 16].

iii) Because of the 4 aspartyl residues near the Lys_7–Ile_8 activating bond of the trypsinogen sequence, this bond is hydrolysed very slowly by trypsin ($k_{cat} = 2.5 \times 10^{-3}$ sec^{-1}). This ensures that pancreatic trypsin inhibitors (Kunitz and Kazal inhibitors) have time to intervene in case of an accidental activation of the zymogen [16, 17]. This also permits a "suicidal" activation (formation of inert proteins) of trypsinogen in the absence of high enough calcium concentrations.

Trypsinogen is the key zymogen of the pancreatic secretion. It gives rise to trypsin which is the essential initiator enzyme of other activations e.g. pro-carboxypeptidases A and B, chymotrypsinogens A, B and C, prophospholipase, proelastase. On the other hand, this zymogen is present in high amounts (ca. 20%) compared to other zymogens in the pancreatic juice and will give rise during activation to high quantities of peptide. For all these reasons the activation peptide of trypsinogen appeared to be the most probable candidate among other activation peptides for a physiological effect. It appeared to us that it could be an excellent chemical signal for linking zymogen activations and pancreatic or gastric secretions.

The results presented in this paper show that this peptide produces an appreciable decrease of the gastric secretion output stimulated by gastrin or pentagastrin (a 20–30% decrease in HCl outputs will have drastic effects on the pH control in the duodenum). The gastric effect of the trypsinogen activation peptide is very specific. It was not observed with the synthetic peptide or with the activation peptide pyroGlu–Glu–Gly–Ile–Ser–Ser–Arg from prophospholipase. That the trypsinogen activation peptide interferes with the gastrin effect does not come as a surprise. It has been found that gastrins from man, pig, cat, cow and sheep have in their sequences a succession of 4 or 5 contiguous glutamic acid residues [18]. Until now no physiological role has been given to this cluster of glutamic residues; the essential physiological activity

of the hormone was found to be localized in the C-terminal tetrapeptide. The C-terminal tetrapeptide alone however displays an activity which is one fifth that of the intact hormone molecule [19]. This result strongly suggests that the N-terminal tridecapeptide sequence of gastrin, which included the $(Glu)_4$ or $(Glu)_5$ cluster, comprises recognition sites (or subsites) of the hormone for its receptor. The structural analogy between gastrin and the activation peptide suggests that both molecules recognize the same receptor site, the activation peptide working as an antagonist of gastrin.

The finding that the activation peptide of trypsinogen inhibits gastric juice secretion emphasizes that both components of the pancreatic secretion, the alkaline and the proteic, play a role in the regulation of pH at the duodenal level, the former acting directly by neutralizing the HCl that enters the duodenum and the latter acting indirectly via the activation peptide of trypsinogen (and maybe other components of the pancreatic secretion yet to be discovered) to counteract the gastrin stimulation of the stomach. HCl in the duodenum controls the pancreatic secretion via pancreozymin and secretin, and the pancreatic secretion of proteins controls partly gastric secretion of HCl via the activation peptide of trypsinogen. It seems likely, therefore, that this activation peptide is one of the links which connects duodenal activation of the zymogens and their secretion by the pancreas.

Acknowledgements

We are very grateful to professor H. Sarles, Director of the Unité de Recherches de Pathologie digestive INSERM, for offering us the facilities available in his institute, for his interest in the work and for information which has been essential in the course of the experiments. We also thank Dr. Maroux for advice in the course of the purification of the N-terminal peptide. This work benefited from the help of the Fondation pour la Recherche Médicale.

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