TRYPSINOGEN ACTIVATION PEPTIDE AND RELATED PEPTIDES AS INHIBITORS OF GASTRIC SECRETION

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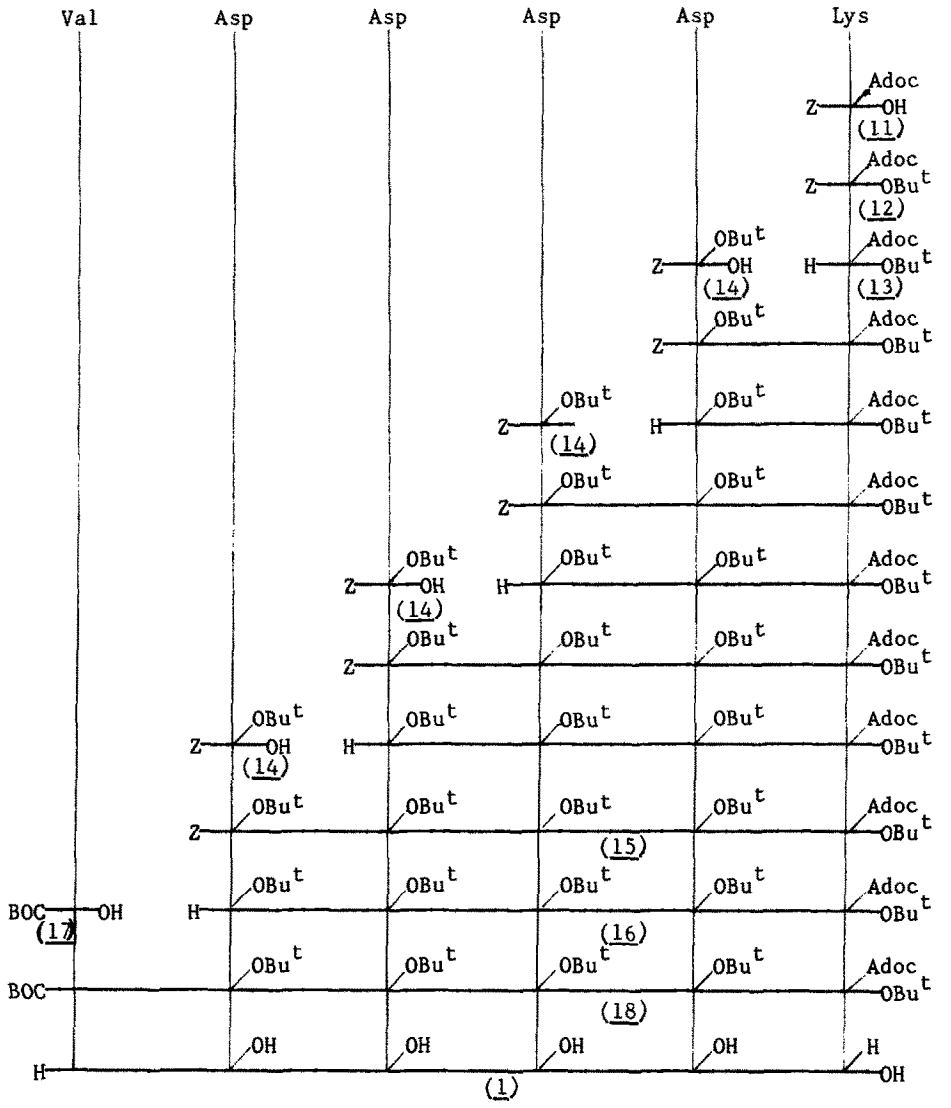
Summary. The inhibitory effects on gastric acid secretion of bovine trypsinogen activation peptide [Val-$(Asp)_4$-Lys] have been confirmed with the pure synthetic peptide. However the effects are considered to be too weak to be of physiological significance. Four related peptides and a gastrin octapeptide sequence [$(Glu)_5$-Ala-Tyr-Gly] have similar effects.

Abita et al (1) report that the peptide Val-$(Asp)_4$-Lys (trypsinogen activation peptide), liberated during the conversion of bovine trypsinogen to trypsin, inhibits gastrin- or pentagastrin- stimulated gastric acid secretion in dogs when dosed intravenously or intraduodenally, and suggest a physiological role for the peptide in the control of gastric secretion. Activation peptide from natural bovine trypsinogen was used in these studies. We now report results with synthetic bovine trypsinogen activation peptide, the corresponding C-terminal methyl ester and amide, and analogues in which the N-terminal valine residue is replaced by Thr, Pro, Ile, Ala, D-Val or deleted (the first three replacements are found in trypsinogen activation peptides of other species). Since Abita et al (1) relate the activity of the activation peptide to structural features present in gastrin, the gastrin octapeptide sequence, $(Glu)_5$-Ala-Tyr-Gly, was also examined.

Syntheses. Bovine trypsinogen activation peptide was synthesised by the route shown in the Fig. N^{α}-Benzyloxycarbonyl-N^{ϵ}-adamantyloxycarbonyl-L-lysine (11) was converted into the t-butyl ester (12) by treatment with t-butanol-phosphorus oxychloride, and hydrogenolysis of the ester afforded N^{ϵ}-adamantyloxycarbonyl-L-lysine t-butyl ester (13) in 76% overall yield. The pentapeptide derivative (15) was built up by successive coupling reactions (N,N'-dicyclohexylcarbodi-

Synthesis of bovine trypsinogen activation peptide.



imide and 1-hydroxybenztriazole) (DCCI-HOBt) and hydrogenolyses, using β-t-butyl N-benzyloxycarbonyl-L-aspartate (14) as the carboxyl component at each coupling stage. Hydrogenolysis of the pentapeptide derivative (15), followed by coupling (DCCI-HOBt) of the product (16) with N-t-butoxycarbonyl-L-valine (17), gave the protected hexapeptide (18). Treatment of this with cold trifluoroacetic acid gave bovine trypsinogen activation peptide (compound 1) in

high overall yield. The C-terminal methyl ester (compound 8) was prepared by a similar series of reactions starting from N^{ϵ}-t-butoxycarbonyl-L-lysine methyl ester. The amide (compound 9) was prepared by ammonolysis (methanolic ammonia at room temperature) of the methyl ester. Other hexapeptides (compounds 2-6) were prepared by coupling Z-Thr, Z-Pro, Z-Ile, Z-Ala or Z-D-Val with the pentapeptide derivative (16) by means of DCCI-HOBt, following by deprotection of the resulting products by (a) 90% aqueous trifluoroacetic acid, and (b) hydrogenolysis in aqueous acetic acid. The gastrin octapeptide (compound 10) was prepared by successive cleavages of the penta-t-butyl methyl ester (ref. 2) with trifluoroacetic acid and aqueous sodium hydroxide. Purification of products was readily achieved by means of chromatography on Bio-Rad AG1X resin (acetate form) or aminoethyl cellulose. All compounds (1-10) examined biologically were judged homogeneous by the following three criteria: amino-acid analysis, thin-layer chromatography, and high voltage paper electrophoresis at pH 6.5.

Methods. Beagle dogs, each weighing 20 kg and provided with a Heidenhain pouch and gastric fistula, were used in our studies. Gastric secretion was stimulated by intravenous infusion of pentagastrin [1 μg/kg/hr] or synthetic human gastrin [0.2 μg/kg/hr], and secretion was collected from both the pouch and fistula every 15 min. When steady state conditions were reached, a saline solution of the peptide under examination was given by i.v. infusion for 1 hr at a dose of 50 μg/kg. Collections were continued throughout the infusion of the peptide and for at least 1 hr afterwards, and the pentagastrin or gastrin infusion was continued throughout the experiment. Acid in each 15 min collection was measured by potentiometric titration. For study of the effect of oral administration, a beagle dog with a Pavlov pouch was used, and the peptide was given orally in a gelatine capsule known to disintegrate in the pyloric antrum 20-25 min after ingestion.

Results and Discussion. In the experiments where the peptides were given intravenously (Table), synthetic bovine trypsinogen activation peptide (compound 1) and analogues in which the N-terminal valine residue is replaced by Thr,

Table. Effect of trypsinogen activation peptide (compound 1) and related peptides on Heidenhain pouch (HP) and gastric fistula (GF) acid secretion in dogs [dogs 20 kg; stimulant pentagastrin, 1 μg/kg/hr; all peptides 50 μg/kg/1 hr].

Compd. No.	Structure	% Inhibition of acid	
		HP	GF
1	Val-$(Asp)_4$-Lys	-30	-42
2	Thr-$(Asp)_4$-Lys	-37	-15
3	Pro-$(Asp)_4$-Lys	-28	-16
4	Ile-$(Asp)_4$-Lys	0	0
5	Ala-$(Asp)_4$-Lys	-21	-48
6	D-Val-$(Asp)_4$-Lys	-8	-5
7	$(Asp)_4$-Lys	0	0
8	Val-$(Asp)_4$-Lys-OMe	-48	-32
9	Val-$(Asp)_4$-Lys-NH_2	0	0
10	$(Glu)_5$-Ala-Tyr-Gly	-36	-29

Pro, or Ala (compounds 2, 3, and 5), but not Ile (compound 4), caused 15-48% inhibition of pouch and fistula acid secretion. The methyl ester (compound 8) and the gastrin octapeptide (compound 10) were equally effective. Other peptides had no significant effect. No effect was observed when synthetic bovine trypsinogen activation peptide (compound 1) (10 mg) was given orally, or at a lower dose [10 μg/kg/1 hr] intravenously.

The weak inhibitory effect of bovine trypsinogen activation peptide on gastric acid secretion after i.v. administration is thus confirmed with pure synthetic material. It would appear that the N-terminal valine residue may be replaced by L-amino acid residues of comparable or smaller size without significant effect on the activity, but that for activity there are steric requirements at this position not met or fully met when the residue is deleted (compound 7) or replaced by D-valine (compound 6) or the slightly more bulky isoleucine residue (compound 4). The activity of the ester (compound 8) may possibly be accounted for by assuming *in vivo* hydrolysis to the acid (compound 1).

The finding of comparable activity in the gastrin octapeptide (compound 10) might appear to support the speculation of Abita et al (1) that the inhibitory effects arise through competition for a recognition site for part or all of the cluster of five glutamic acid residues in gastrin, which may be saturated, thus preventing binding of the whole gastrin molecule, by molecules (eg. bovine trypsinogen activation peptide or the gastrin octapeptide used in the present study) containing a similar cluster of acidic residues. However, we reject the speculation for two reasons. First, the argument advanced for the existence of the recognition site (decreased potency of gastrin C-terminal tetrapeptide amide in in vivo assays, as compared with the whole molecule) is not justified [see, for example, Morley (2)], and is not supported by structure-function studies (3). Second, the observed inhibitory effects are no different when pentagastrin, lacking any sequence corresponding to the supposed recognition site, or gastrin is used as the stimulant.

The observed inhibitory effects of trypsinogen activation peptide, even at the substantial dosages used, seem much too weak to support a possible physiological role for the peptide in the control of gastric secretion. N-Terminal fragments of gastrin, structurally related to the gastrin octapeptide used in the present study, have recently been demonstrated at least in the plasma of Zollinger-Ellison patients (4); it is attractive to postulate a physiological role for these fragments but here too the inhibitory effects of the gastrin octapeptide seem much too weak to support the argument. The effect claimed by Abita et al (1) after intraduodenal administration of trypsinogen activation peptide is nevertheless of considerable general interest. Bearing in mind that the effect is also seen after intravenous injection, this suggests that the peptide may be capable of absorption from the duodenum. Although we have not been able to demonstrate an effect after oral administration, further study is indicated.

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