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ISOLATION OF A PORCINE INTESTINAL PEPTIDE WITH C-TERMINAL SOMATOSTATIN

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SUMMARY. A peptide showing somatostatin-like immunoreactivity has been isolated from acid extracts of porcine upper small intestine. Purification was followed by radioimmunoassay, high pressure liquid chromatography, thin layer chromatography and isotachophoresis. Preliminary chemical characterization shows that it has N-terminal serine and that it is composed of somatostatin extended from its N-terminus by an additional peptide.

INTRODUCTION. Somatostatin[1] (G.H.-R.I.F.) is a tetradecapeptide which has been isolated from ovine and porcine hypothalamic extracts (1, 2, 3). Somatostatin-like immunoreactivity (S.L.I.) and bioactivity have been shown to be widely distributed in the central nervous system and digestive tract tissues (4, 5, 6, 7). Immunohistochemical localisation of S.L.I. in the digestive tract was first reported in islet-pancreatic cells (8) and subsequently in the gastrointestinal mucosa. The S.L.I. material from human urine has been shown to be similar to G.H.-R.I.F. (3) but size heterogeneity has been suggested (3, 10, 11). No final purification of these molecules has however been described. We report here the isolation of a peptide extracted from porcine upper small intestinal tissue which, according to a preliminary charac-

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terization corresponds to a N-terminus extended somatostatin.

MATERIALS AND METHODS

Materials. The peptide was obtained from a porcine intestinal peptide concentrate previously described. The immediate starting material for its purification is denoted "post-secretin", which is a side fraction used for the isolation of the vasoactive intestinal peptide (V.I.P.) (13).
Synthetic G.H.-R.I.F., used as standard, was a gift from Dr. Harrant, Serono Lab. (Freiburg, Germany).

Methods. Purification was followed by S.L.I. determinations (14). The partial purification performed using column chromatographies on Sephadex G25 and carboxymethylcellulose has already been described in a preliminary note (15). Final purification of the peptide was obtained on H.P.L.C. followed by T.L.C.
The H.P.L.C. was performed on Waters Associates model 204 apparatus equipped with model U6K injector, and 6000 A pump. The column (300 x 3.9 mm I.D.) was packed with 10 µm octadecyl silane coated particles (µ Bondapak C_{18} Waters). The solvent consisted of ammonium acetate 0.01 M, pH = 4.0 63% - Ethanol 37%. The flow-rate and pressure were respectively 1 ml/min and 1500 psi. Eluated components were detected at 280 nm (Waters Ass. M 440 detector).
T.L.C. were run on Riedel de Haehn (Seelze, Hannover, Germay) silica gel plates in a butanol-pyridine-acetic acid-water (15-10-3-12) solvent system (16). After a 6 hours migration spots were revealed by spraying with ninhydrin. Isotachophoretic analyses were carried out on a LKB, 2127 Tachophor instrument (LKB, Bromma, Sweden) equipped with a 23 cm capillary tube. Runs were performed at a constant temperature of 13°C and at a constant current of 100 µA. Leading and terminating electrolytes (L.E., T.E.) were as follows: L.E.: 10mM potassium acetate pH = 5.3, 0.20% H.P.M.C., 4 M urea. T.E.: 10mM α alanine.
N-terminal amino-acid analysis was performed using the "dansyl" method of Hartley (17). Identification of D.N.S. amino-acids was by chromatography on 5x5 cm polyamide thin layer plates (Schleicher & Schüll, Dassel, Germany) using the three solvent systems described by Woods (18). Performic acid oxidation was performed as described by Moore (19). For amino-acid analysis, peptides, c-a 50 µg, were hydrolyzed for 24 hours at 110°C in 50 µl of 6N hydrochloric acid Aristar B.D.H. grade containing 0.5% 2-mercaptoethanol or 50 µl of 3N M.E.S. Analyses were performed with a Beckman model 121 M amino-acid analyzer.
Tryptic digestion was performed for 6 hours at room temperature using 1 µg trypsin (Worthington T.P.C.K. treated) for 100 µg of peptide with further 1 µg addition of trypsin after 2 and 4 hours incubation.

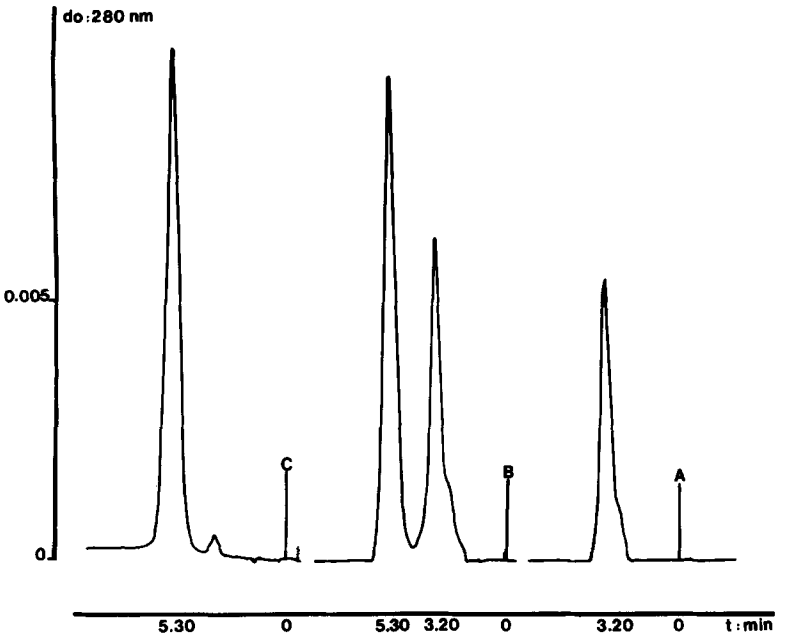

Fig. 1. High pressure liquid chromatography of the isolated porcine intestinal peptide and synthetic G. H. -R. I. F.

A- 10 μg of the intestinal peptide

B- Mixture in equal amounts (10 μg each) of the isolated intestinal peptide and synthetic G. H. -R. I. F.

C- 10 μg of synthetic G. H. -R. I. F.

RESULTS.

COMPARATIVE STUDIES BETWEEN THE INTESTINAL PEPTIDE AND G. H. -R. I. F.

High pressure liquid chromatography: Fig. 1 illustrates the different retention times (3 min. 20 s. for the intestinal peptide, 5 min. 30 s. for G. H. -R. I. F.) during H. P. L. C. as described in methods. The intestinal peptide, first eluted from the mixture is well resolved from G. H. -R. I. F.

Thin layer chromatography: Rf values for G. H. -R. I. F. and the intestinal peptide were respectively 0. 65 and 0. 43.

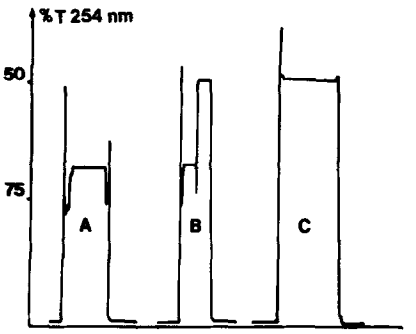

Fig. 2. Isotachophoregrams of the isolated intestinal porcine peptide (A), synthetic G. H. -R. I. F. (C) and mixture in equal amounts of the two peptides (B). Each run was performed as described in Methods on a total peptide amount of 10 μg/dry weight.

Isotachophoresis: The relative behaviours of the intestinal peptide and G. H. -R. I. F. are illustrated in Fig. 2. The intestinal peptide gives a main plateau (diagram A) at 70% T. 254 nm while, the one corresponding to G. H. -R. I. F. (diagram C) is at ≃ 50%. The mixture in equal amounts of the two peptides (diagram B) illustrates their different mobilities; the intestinal peptide moving slower than G. H. -R. I. F.

CHARACTERIZATION OF THE INTESTINAL PEPTIDE

N-terminal Amino-acid: Thin layer chromatography of the 6N HCl hydrolyzate from the dansylated material after the H. P. L. C. purification step exhibited two main spots corresponding to D. N. S. -serine and ε-D.N.S. -lysine. Three other faint spots, D. N. S. -Ala, -Gly and-o-tyrosine, could be visualized on this preparation. The same experiment carried out on the peptide eluated from the silica gel T. L. C. gave only serine as N-terminal amino-acid.

Tryptic degradation: Thin layer chromatography of the intestinal peptide tryptic digests resolved four distinct peptides (RfIP1 = 0.71, RfIP2 = 0.35, RfIP3 = 0.29, RfIP4 = 0.25). Treated in the same way synthetic G.H.-R.I.F. gave two tryptic peptides (RfSP1 = 0.72, RfSP2 = 0.34). The corresponding areas of the plate were scraped off, tryptic peptides eluted with 0.2 M acetic acid and recovered by freeze-drying. SP1 and IP1, when subjected to N-terminal amino-acid analysis showed an Asx residue while both SP2 and IP2 had two N-terminal residues Ala and Thr. The N-terminal amino-acids of IP3 and IP4 were Ser and Glx respectively.

Amino-acid analysis: Results of the amino-acid analysis carried out on the intestinal peptide and G.H.-R.I.F. under various hydrolysis conditions are given in table 1. These results suggest that the peptide might have the composition: Ala 3, Arg 2, Asx 2, Cys 2, Glx 1, Gly 1, Lys 2, Phe 3, Ser 2, Thr 2, Trp 1.

DISCUSSION

This report deals with the first biochemical characterization of a possible larger molecular form of G.H.-R.I.F. In course of partial purification of S.L.I. material from porcine intestine (14) we had noticed a different behaviour between this molecule and synthetic G.H.-R.I.F. during column chromatographies and counter-current distribution. The purified intestinal peptide is well differentiated from synthetic G.H.-R.I.F. by H.P.L.C. and isotachophoresis. Furthermore the finding of a N-terminal serine confirmed that this molecule was different from G.H.-R.I.F. Evidence for the identity of two of the four tryptic digest

Table 1: Amino acid analysis of the intestinal peptide and synthetic G.H.-R.I.F. run in parallel.

A.A. in sample GH-RIF	Lys 2	Cys 2	Asn 1	Thr 2	Ser 1	Gly 1	Ala 1	Phe 3	Trp 1	Total 14
1	1.9	1.14	0.9	1.8	0.9	1.0	1.0	2.9	- -	
3	1.85	0.8	0.96	1.85	0.8	1.1	1.1	2.86	0.3	
5	2.0	[x]1.99	1.03	1.91	0.97	0.99	1.0	3.18	- -	

A.A. in intestinal peptide	Lys 2	Cys 2	Asx 2	Thr 2	Ser 2	Gly 1	Ala 3	Phe 3	Trp 1	Arg 2	Glu 1	21
2	1.7	0.64	1.7	1.5	1.8	0.9	2.0	2.8	0.2	2.4	1.1	
4	1.96	0.13	1.85	1.3	1.8	0.98	2.07	1.7	0.3	1.4	1.0	
6	2.7	[x]2.02	1.94	1.67	2.2	1.0	3.2	2.93	- -	1.87	1.18	

1. Synthetic G.H.-R.I.F. after 6N HCl, 0.5 % mercaptoethanol hydrolysis
2. Intestinal peptide in the same conditions as 1
3. Synthetic G.H.-R.I.F. after 3N MES hydrolysis
4. Intestinal peptide in the same conditions as 3
5. Synthetic G.H.-R.I.F. oxydized in performic acid and hydrolyzed like under 1
6. Intestinal peptide oxydized in performic acid and hydrolyzed like under 1

[x] in 5 and 6: Cysteine was found as cysteic acid

peptides from S. L. I. peptide with those obtained from G. H. - R. I. F. is given by the identity of their Rf and N-terminal residues. The presence of two Arg residues is in good agreement with the two extra tryptic peptides encountered and it can be assumed that one of these residues is linked to the N-terminal alanine of G. H. -R. I. F. These observations suggest that the isolated intestinal peptide is a larger molecule than the tetradecapeptide of G. H. -R. I. F. and is the latter extended in its N-terminal part. The amino-acid composition of the intestinal peptide combined with the findings from the tryptic degradation experiments and from N-terminal amino-acid determination suggest that the extension might be by the heptapeptide Ser (Ala 2, Arg 1, Asx 1, Glx) Arg. The possible conversion of the intestinal peptide to G. H. -R. I. F. in body fluids might be carried out by a trypsin-like enzyme. The complete aminoacid sequence of the peptide must, however, be determined before giving to it the possible status of a G. H. -R. I. F. precursor.

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