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# CHROMATOGRAPHIC CHARACTERIZATION OF NEUROTENSIN FRAGMENTS, NEUROTENSIN, AND OTHER INTESTINAL PEPTIDE INHIBITORS OF GASTRIC ACID SECRETION

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## ABSTRACT

Neurotensin and other small intestinal peptides that may inhibit gastric acid secretion have not previously been separated chromatographically from each other. We report two HPLC gradient systems that effectively resolve 11 different peptides of small intestinal origin, and that may be useful in the characterization of new peptides isolated from the small intestine or from portal plasma.

## INTRODUCTION

Neurotensin is a biologically active peptide isolated from bovine hypothalamus (1) and bovine (2) and human small intestine (3). Among its activities is the inhibition of gastrin-stimulated gastric acid secretion in humans (4), dogs (5) and

rats (6). Although neurotensin is present in plasma (7) and is released from the small intestine in response to lipid (8), it is rapidly metabolized into biologically inactive fragments (9). In attempts to extract and purify neurotensin-related biologically active peptides from the small intestine, it is necessary to demonstrate that such new peptides are distinct from other known inhibitors of gastric acid secretion which may also be extracted from the intestine. These include cholecystokinin (10), secretin (11), gastric inhibitory polypeptide (12), vaso-active intestinal peptide (13), neuropeptide Y (14), peptide YY (15), and perhaps motilin (16). The complete HPLC separation of these peptides has not been reported in the literature.

We have developed two HPLC gradient systems that provide resolution of the above-listed (synthetic) peptides, neurotensin and the inactive metabolites of neurotensin that have been found in plasma.

#### MATERIALS

Reagents: Motilin, cholecystokinin (26-33) (CCK-8), secretin, peptide YY (PYY), neuropeptide Y (NPY), vaso-active intestinal peptide (VIP), and gastric inhibitory polypeptide (GIP) were from Peninsula Labs or Sigma. Neurotensin (NT) was from Peninsula. The amino-terminal NT fragments (1-8), (1-10), (1-11), and (1-12) were prepared by proteolytic digestion of NT as previously described (17) and purified on high pressure liquid chromatography (HPLC). HPLC grade acetonitrile, methanol,

$\text{NaH}_2\text{PO}_4$ , and trifluoroacetic acid (TFA) were from Fisher Scientific, and water was purified on a Milli-Q System. A Waters HPLC system, with two M-510 pumps, a Model 680 automated gradient controller and a U6K injector was used, and column eluates were monitored at 210 nm with a Waters Model 450 variable wavelength detector. Absorbance was recorded on a Houston recorder.

#### METHODS

Details of the solvent composition and gradient timing are provided in Table 1. Individual peptides (2-100  $\mu\text{g}$ ) were injected in 2-10  $\mu\text{l}$  of HPLC grade water and each full gradient was run. Peptides whose retention times were similar on the final gradients (NT(1-8) and NT(1-10), 20 and 21 min; and GIP, NPY, and secretin, 34, 35, and 36 min) were injected together to demonstrate their near-baseline resolution, and all peptides were injected together as detailed in the Figure legends.

#### RESULTS

Near-baseline resolution was achieved in both buffer systems for NT(1-8), NT(1-10), NT(1-11), NT, motilin, CCK-8, secretin, and neuropeptide Y (Figs. 1 and 2). Retention times of the peptides are indicated in Table 2. VIP was not injected in sodium phosphate because it did not elute sharply in that system, but it resolved well in 0.1% TFA. PYY and GIP did not resolve in 0.1% TFA, but separated well in sodium phosphate. NT(1-12) was not injected on the TFA gradient because degradation of the

Table 1

Buffers and Gradient Systems Utilized in Figure 1 (Left) and Figure 2 (Right).

Buffer A: 0.05 M  $\text{NaH}_2\text{PO}_4$

Buffer A: 0.1% TFA, pH 2.05

Buffer B: 60%  $\text{CH}_3\text{CN}$  in A

Buffer B: 60%  $\text{CH}_3\text{CN}$  in A

<u>Time</u>	<u>Flow rate</u>	<u>% B</u>	<u>Time</u>	<u>Flow rate</u>	<u>% B</u>
initial	1 ml/min	0	initial	2 ml/min	0
5 min	1	0	5 min	2	0
10	1	20	10	2	29
13	1	20	12	2	29
27	1	50	14	2	40
32	2	50	20	2	40
34	1	50	23.33	2	50
46	1	100	27.33	2	50
51	1	100	34	2	70
55	1	0	37	2	100
			40	2	100
			45	2	0

Table 2

Retention Times of Peptides on the Two HPLC Systems\*

Peptide (Number of residues )	Retention Time (min)	
	<u><math>\text{NaH}_2\text{PO}_4</math> System</u>	<u>0.1% TFA System</u>
NT (1-8) (8)	23.0	12.4
CCK (26-33) (8)	33.6	18.4
NT (1-10) (10)	23.6	18.4
NT (1-11) (11)	25.8	13.4
NT (1-12) (12)	27.8	not tested
NT (13)	30.0	16.3
Motilin (22)	32.0	19.3
Secretin (27)	44.0	29.9
VIP (28)	broad peak	22.4
Peptide YY (36)	32.8	27.8
Neuropeptide Y (36)	43.4	33.4
GIP (42)	42.8	27.5

\*peptides are listed in order of increasing molecular weight

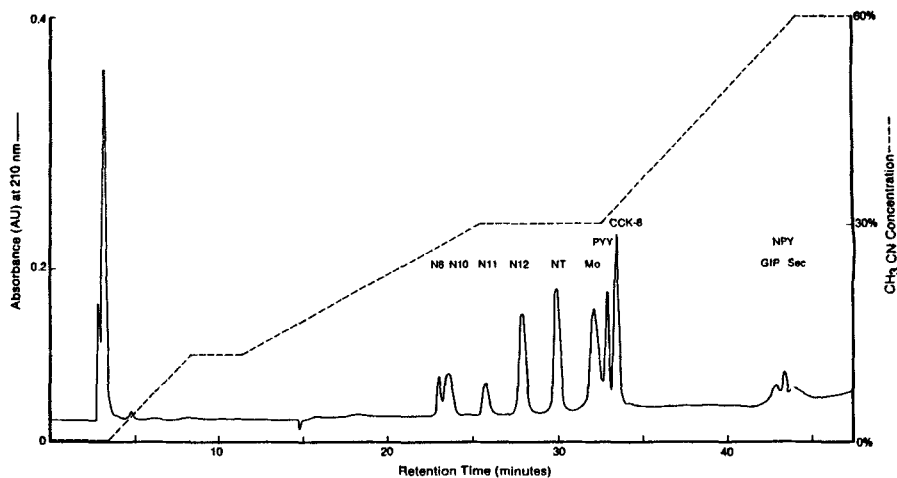


Figure 1. Separation of peptides on the  $\text{CH}_3\text{CN}:\text{0.05 M NaH}_2\text{PO}_4$  gradient system. Details of the gradient are in the left half of Table 1. 100 to 200 pmoles of the individual peptides, in a volume of 2 to 100  $\mu\text{l}$  each, were combined in a 1 ml Hamilton syringe and injected onto the column to start the gradient program. Retention times of the peptides are indicated on the left in Table 2. Abbreviations: N8, NT(1-8); N10, NT(1-10); N11, NT(1-11); N12, NT(1-12); NT, neurotensin; Mo, motilin; PYY, peptide YY; CCK-8, cholecystokinin (26-33); GIP, gastric inhibitory polypeptide; NPY, neuropeptide Y; Sec, secretin.

standard into 3 components during storage prevented our assigning a definite retention time to that peptide.

#### DISCUSSION

The order of retention times for these intestinal peptides and fragments is generally in keeping with their molecular weights, except for the relatively prolonged retention time of CCK-8. Commercial CCK (Pancreozymin, Adria, 95% pure) contained 6 to 8 peaks of absorbant material eluting in all regions of the

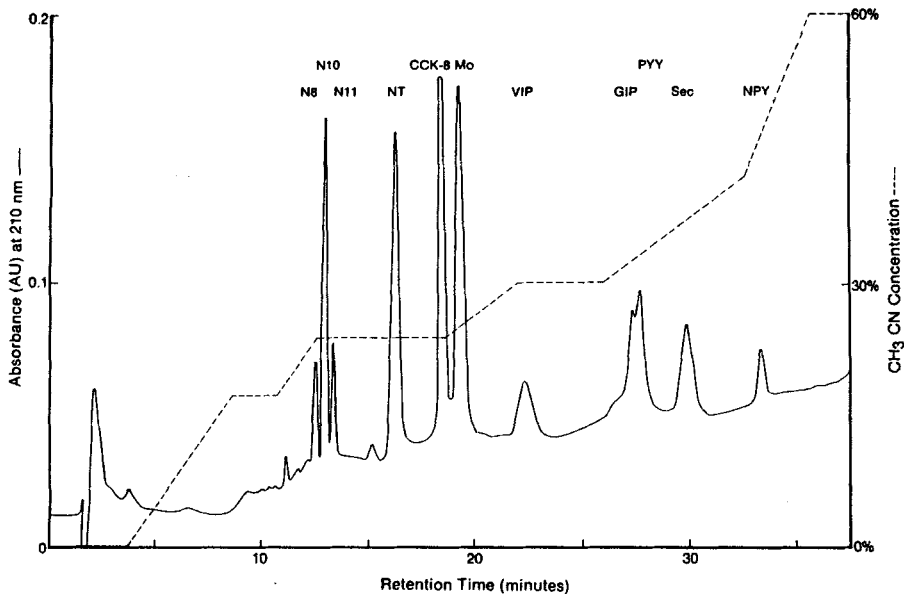


Figure 2. Separation of peptides on the  $\text{CH}_3\text{CN}:0.1\%$  TFA gradient system. Details of the gradient are in the right half of Table 1. Details of the injection technique are the same as in Fig. 1. Abbreviations as in Fig. 1; VIP, vaso-active intestinal peptide. Retention times of the peptides are indicated on the right in Table 2.

chromatogram (not shown), and was felt to be unsuitable as a standard.

These peptides have all been found in pig intestine, and all except motilin have been shown to inhibit gastric acid secretion in experimental models (10-16). Thus, they may all be considered candidate enterogastrones. In a search for new intestinal peptides with enterogastrone (acid inhibitory) activity, these gradients may be useful in separating new substances from these other known peptides.

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## REFERENCES

1. Carraway, R.E., and Leeman, S.E., The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J. Biol. Chem.*, 248. 6854, 1973.
2. Kitabgi, P., Carraway, R.E., Leeman, S.E. Isolation of a tridecapeptide from bovine intestinal tissue and its partial characterization as neurotensin. *J. Biol. Chem.*, 251. 2476, 1976.
3. Hammer, R.A., Leeman, S.E., Carraway, R.E., Williams, R.H., Isolation of human intestinal neurotensin. *J. Biol. Chem.*, 255. 2476, 1980.
4. Rosell, S., Rokaeus, A., Mashford, M.L., Thor, K., Folkers, K., Neurotensin as a hormone in man. In: Marsan, C.A., Traczyk, W.Z., eds. *Neuropeptides and Neural Transmission*. Raven Press, New York, 1980, Pg. 181.
5. Andersson, S., Rosell, S., Sjodin, L., Folkers, K., Inhibition of acid secretion from vagally innervated and denervated gastric pouches by (Gln)<sup>4</sup>-neurotensin. *Scand. J. Gastroenterol.*, 15. 253. 1980.
6. Rosell, S., Burcher, E., Chang, D., Folkers, K., Cardiovascular and metabolic effects of neurotensin and (Gln)<sup>4</sup>-neurotensin. *Acta Physiol. Scand.*, 98. 484, 1976.
7. Carraway, R., Hammer, R.A., Leeman, S.E., Neurotensin in plasma: Immunochemical and chromatographic characterization of acid/acetone-soluble material. *Endocrinol.*, 107. 400, 1980.
8. Ferris, C.F., Hammer, R.A., Leeman, S.E., Elevation of plasma neurotensin during lipid perfusion of the rat small intestine. *Peptides*, 2 (Suppl 2). 263, 1981.
9. Aronin, N., Carraway, R.E., Ferris, C.F., Hammer, R.A., Leeman, S.E., The stability and metabolism of intravenously administered neurotensin in the rat. *Peptides*, 3. 637, 1982.



10. Saito, A., Sankaran, H., Goldfine, I.D., Williams, J.A., Cholecystokinin receptors in the Brain: Characterization and distribution. *Science*, 208. 1155, 1980.
11. Gutierrez, L.V., Baron, J.H., A comparison of Boots and GIH secretin as stimuli of pancreatic secretion in human subjects with or without chronic pancreatitis. *Gut*, 13. 721, 1972.
12. Jornvall, H., Carlquist, M., Kwauk, S., Otte, S.C., McIntosh, C.H.S., Brown, J.C., Mutt, V., Amino acid sequence and heterogeneity of gastric inhibitory polypeptide (GIP). *FEBS Letters*, 123. 205, 1981.
13. Bodanszky, M., Klausner, Y.S., Said, S.I., Biological activities of synthetic peptides corresponding to fragments of and to the entire sequence of the vasoactive intestinal peptide. *Proc. Natl. Acad. Sci. USA*, 70. 382, 1973.
14. Tatemoto, K., Neuropeptide Y: Complete amino acid sequence of the brain peptide. *Proc. Natl. Acad. Sci. USA*, 79. 5485, 1982.
15. Tatenoto, K., Mutt, V., Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature*, 285. 417, 1980.
16. Ikota, N., Takayuki, S., Yamada, S., Amino acids and peptides. XXXI. Phosphorus in organic synthesis. XVIII. Synthesis of porcine motilin by the solid-phase method using diphenyl phosphorazidate (DPPA) and diethyl phosphorocyanidate (DEPC). *Chem. Pharm. Bull.*, 28. 3347, 1980.