Isolation and characterization of neuropeptide Y from porcine intestine

K. Tatemoto[+]*, S. Siimesmaa, H. Jörnvall, J.M. Allen[†], J.M. Polak[°], S.R. Bloom[†] and V. Mutt

Departments of Biochemistry II and [+]Chemistry I, Karolinska Institute, Stockholm, Sweden, and *Departments of [°]Histochemistry and [†]Medicine, Hammersmith Hospital, London, England*

Received 30 October 1984

The isolation and primary structure of intestinal neuropeptide Y (NPY) is described. The peptide was purified from porcine intestinal extracts using a chemical assay and radioimmunoassay for NPY. The amino acid sequence of this peptide is: Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2. This indicates that the structure of intestinal NPY is identical to the NPY of brain origin.

Brain-gut peptide *Gastrointestinal peptide* *Amino acid sequence* *Neuropeptide* *C-terminal amide*

1. INTRODUCTION

Neuropeptide Y (NPY), a 36 amino acids peptide, was originally isolated from porcine brain using a chemical assay method based on the detection of the C-terminal amide structure [1]. NPY has structural similarities to the pancreatic polypeptide and peptide YY (PYY) [2]. The peptide exhibits potent biological activities such as vasoconstriction [3] and inhibition of electrically-stimulated smooth muscle contraction [4,5]. Central administration of NPY induces lowering of blood pressure, respiratory and heart rates [6]. NPY coexists with catecholamines [7,8] and may modulate catecholamine release [9]. NPY is one of the most abundant neuropeptides in mammalian brains [10].

Immunochemical studies suggest that NPY is also present in gut [11], heart [12], pancreas [13], adrenal medulla [14], and reproductive tract [15]. However, chemical structure of the immunoreactive NPY has previously not been identified. The results of a specific immunoassay for NPY revealed that a NPY-like peptide is present in porcine intestinal extracts. We report here the isolation and characterization of this peptide.

2. MATERIALS AND METHODS

Thermolysin was obtained from Daiwa Kasei K.K. Osaka and polyamide thin layer plates from Schleicher & Schüll.

NPY was determined by the amount of tyrosine amide released on treatment of samples with thermolysin using a chemical assay method described earlier [16] and by using a radioimmunoassay for NPY [17]. High-performance liquid chromatography (HPLC) was performed in a Waters instrument using reversed-phase μBondapak C_{18} and phenyl columns under chromatographic conditions described in the legends to fig.1–3. Amino acid analyses were performed with a Beckman 121 amino acid analyser after hydrolysis of samples in 5.7 M NCl containing 0.5% phenol at 110°C for 24 h. Step-wise sequencer degradation of the intact peptide was carried out in a Beckman 690 D liquid-phase sequencer in the presence of glycine-precycled polybrene [18]. Phenylthiohydantoin derivatives were identified by HPLC [19].

* Present address: Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305, USA

Published by Elsevier Science Publishers B.V.
00145793/85/$3.30

3. RESULTS

3.1. *Isolation procedures*

Intestinal NPY was isolated from a starting material obtained as a side fraction during the final purification step for porcine secretin [20]. Briefly, porcine upper intestines (4000 kg) were boiled, frozen, minced and extracted with 0.5 M acetic acid for 18 h. Peptides in the extract were adsorbed onto alginic acid, eluted with 0.2 M HCl and precipitated with NaCl at saturation. The peptide precipitate was further purified by extraction with 66% ethanol, gel chromatography on Sephadex G-25 and extraction with methanol. The methanol-soluble fraction was subjected to ion exchange chromatography on a CM-cellulose column. The secretin-containing fraction (450 mg) was subjected to counter-current distribution (200 transfers) for final purification of secretin. Secretin was found between 60–100 transfers (20) and PYY and NPY were found between 1–59 transfers. Fractions containing PYY and NPY were combined and the phases were coalesced by addition of ethanol. The solution was kept at −20°C overnight. The salts that had precipitated were removed by filtration and the filtrate was diluted with 15 volumes of water and the peptides were adsorbed onto alginic acid at pH 2.7 and eluted from the alginic acid with 0.2 M HCl. The chloride ions in the eluate were exchanged for acetate on a DEAE Sephadex A-25 column and the effluent was lyophilized. This step yielded 223 mg of the starting material for the purification of PYY and NPY.

The starting material was further purified by chromatography on a CM-cellulose column in 0.03 M ammonium bicarbonate (pH 8.0). Fractions containing PYY and NPY were pooled and lyophilized. This material (29 mg) was then subjected to HPLC on a reversed-phase μBondapak C-18 column, first eluted with 36% ethanol containing 0.2% acetic acid and 5 mM ammonium acetate, and then with 80% ethanol containing 0.2% acetic acid and 5 mM ammonium acetate. PYY was eluted with the first buffer in a major HPLC peak and a total of 5 mg of pure PYY was obtained after lyophilization. NPY was found in the fraction eluted with the second buffer and this fraction (8 mg) was subjected to further purification on the same HPLC column using a linear gradient system of 0.12% trifluoroacetic acid/water and 0.1% trifluoroacetic acid/acetonitrile (fig.1). Intestinal NPY was eluted at the position identical to brain NPY in the HPLC. The lyophilized fraction containing NPY was subjected to the final purification on a reversed-phase μBondapak phenyl column (fig.2). This step yielded a total of 0.2 mg pure intestinal NPY.

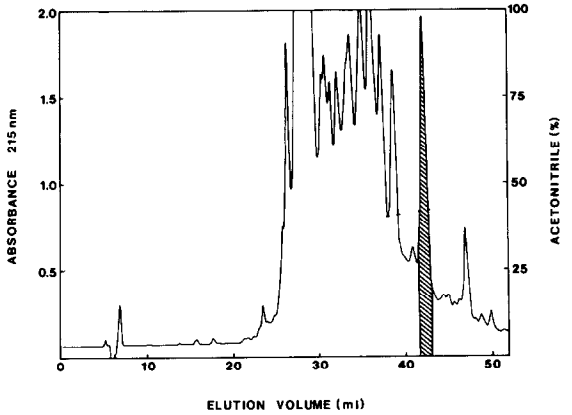

Fig.1. Reversed-phase HPLC profile of the NPY fraction (4 mg) on a μBondapak C_{18} column (7.9 × 300 mm). Solvent system: A, 0.12% CF_3COOH/H_2O; B, 0.1% CF_3COOH/CH_3CN (B: 25–50%). Flow rate, 2 ml/min. The dotted lines indicate the gradient profile. The peak (hatched area) contained NPY.

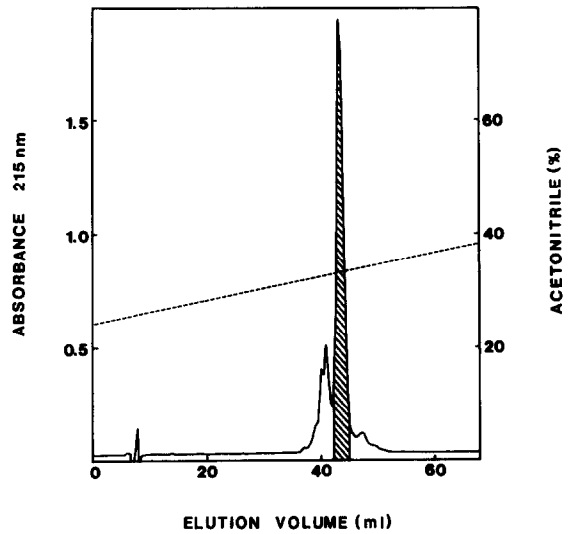

Fig.2. Reversed-phase HPLC profile of the NPY fraction (fig.1) on a μBondapak phenyl column (3.9 × 300 mm). Flow rate, 1 ml/min. Other conditions are as described in fig.1.

3.2. *Structural analysis*

The results of amino acid analysis suggested that the peptide consisted of 36 amino acid residues. The amino acid composition of the intestinal peptide was identical to that of brain NPY. The peptide contained an N-terminal tyrosine and a C-terminal tyrosine amide, which were also identical to those of porcine NPY of brain origin. Further, tryptic fragments of the intestinal peptide were found to be eluted at the positions corresponding to those of tryptic fragments of brain NPY in HPLC. Finally, Edman degradation of the intact peptide in a Beckman 890 D liquid-phase sequencer yielded the amino acid sequence up to the last residue (fig.3), establishing the intestinal peptide to be identical to NPY of brain origin.

4. DISCUSSION

The results of specific radioimmunoassay for NPY indicated that a side fraction obtained during the purification of secretin from porcine intestinal extracts contained a high concentration (1.4 nmol/mg) of immunoreactive NPY. The antibodies used for the assay did not crossreact with PYY known to be present in the same fraction. We therefore decided to isolate the immunoreactive NPY from this fraction. After a successive HPLC step, the product obtained was subjected to structural analysis which revealed the peptide structure to be identical to the NPY of brain origin. Thus, NPY was shown to be a new member of brain-gut peptides.

The physiological roles of NPY in the gastrointestinal tract have not been established. NPY nerves are widely distributed throughout the gut, particularly, in the muscle layers and around blood vessels [11]. NPY reduces blood flow and motility of the colon [21]. These actions are not blocked by adrenergic blocking agents. NPY may therefore act directly on the vascular and intestinal walls and exert biological actions such as vasoconstriction and inhibition of colonic motility. PYY is present in the intestine, in considerably larger amounts than NPY. PYY is confined in the endocrine cells of intestine. This peptide also causes a potent colonic vasoconstriction and inhibition of colonic motility [22]. PYY may thus exert its actions via an endocrine route. Since both peptides are structurally very similar [2], it is possible that NPY and PYY interact with the same receptors in the gut and exert their actions through the two different pathways, one via a neural and the other an endocrine route.

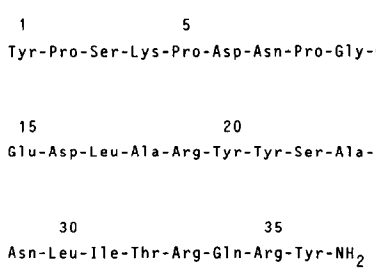

Fig.3. The complete amino acid sequence of neuropeptide Y (porcine intestine).

REFERENCES

[1] Tatemoto, K., Carlquist, M. and Mutt, V. (1982) Nature 296, 659–660.
[2] Tatemoto, K. (1982) Proc. Natl. Acad. Sci. USA 79, 5485–5489.
[3] Lundberg, J.M. and Tatemoto, K. (1982) Acta Physiol. Scand. 116, 393–402.
[4] Lundberg, J.M., Terenius, L., Hökfelt, T., Martling, C.R., Tatemoto, K., Mutt, V., Polak, J.M., Bloom, S.R. and Goldstein, M. (1982) Acta Physiol. Scand. 116, 477–480.
[5] Allen, J.M., Adrian, T.E., Tatemoto, K., Polak, J.M., Hughes, J. and Bloom, S.R. (1982) Neuropeptides 3, 71–77.
[6] Fuxe, K., Agnati, L.F., Härfstrand, A., Zini, I., Tatemoto, K., Pich, E.M., Hökfelt, T., Mutt, V. and Terenius, L. (1983) Acta Physiol. Scand. 118, 189–192.
[7] Hökfelt, T., Lundberg, J.M., Lagercrantz, H., Tatemoto, K., Mutt, V., Terenius, L., Everitt, B., Fuxe, K., Agnati, L. and Goldstein, M. (1982) Neurosci. Lett. 36, 477–480.
[8] Lundberg, J.M., Terenius, L., Hökfelt, T. and Goldstein, M. (1983) Neurosci. Lett. 42, 167–172.
[9] Lundberg, J.M. and Stjärne, L. (1984) Acta Physiol. Scand. 120, 477–479.
[10] Allen, Y.S., Adrian, T.E., Allen, J.M., Tatemoto, K., Crow, T.J., Bloom, S.R. and Polak, J.M. (1983) Science 221, 877–879.
[11] Furness, J.B., Costa, M., Emson, P.C., Håkanson, R., Moghimzadeh, E., Sundler, F., Taylor, I.L. and Chance, R.E. (1983) Cell Tissue Res. 234, 71–92.
[12] Gu, J., Polak, J.M., Adrian, T.E., Allen, J.M., Tatemoto, K. and Bloom, S.R. (1983) Lancet I, 1008–1010.

[13] Sundler, F., Moghimzadeh, E., Håkanson, R., Ekelund, M. and Emson, P. (1983) Cell Tissue Res. 230, 487–493.
[14] Allen, J.M., Adrian, T.E., Polak, J.M. and Bloom, S.R. (1983) J. Autonomic Nervous System 9, 559–563.
[15] Stjernquist, M., Emson, P., Owman, Ch., Sjöberg, N.-O., Sundler, F. and Tatemoto, K. (1983) Neurosci. Lett. 39, 279–284.
[16] Tatemoto, K. and Mutt, V. (1978) Proc. Natl. Acad. Sci. USA 75, 4115–4119.
[17] Allen, J.M., Yeats, J.C., Adrian, T.E. and Bloom, S.R. (1984) Regul. Peptides 8, 61–70.
[18] Jörnvall, H. and Philipson, L. (1980) Eur. J. Biochem. 104, 237–247.
[19] Zimmerman, C.L., Appella, E. and Pisano, J.J. (1977) Anal. Biochem. 77, 569–573.
[20] Jorpes, J.E., Mutt, V., Magnusson, S. and Steele, B. (1962) Biochem. Biophys. Res. Commun. 9, 275–279.
[21] Hellström, P.M. and Tatemoto, K. (1983) in: Gastrointestinal Motility (Roman, C. ed.) pp.433–440, MTP Press, Lancaster.
[22] Lundberg, J.M., Tatemoto, K., Terenius, L., Hellström, P.M., Mutt, V., Hökfelt, T. and Hamberger, B. (1982) Proc. Natl. Acad. Sci. USA 79, 4471–4475.