Volume 258, number 2, 263-265 FEB 07876 December 1989

Sheep neuropeptide Y

A third structural type of a highly conserved peptide

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Received 16 October 1989

Mammalian forms of neuropeptide Y (NPY) for which the amino acid sequences have previously been determined, are the human, pig, ox, rabbit, rat, and guinea-pig polypeptides The only difference among these forms is at position 17, where pig and ox NPY have Leu and the others Met We now show that sheep NPY differs from all the earlier characterized mammalian forms of NPY by having Asp instead of Glu at position 10 At position 17 it has Leu as do both pig and ox NPY Consequently, 3 different structural types of mammalian NPY are now known

Hormonal peptide, Neuropeptide Y, Peptide amide

1. INTRODUCTION

Neuropeptide Y (NPY) is a 36-residue C-terminally amidated peptide, which belongs to a family of peptides also including pancreatic polypeptide (PP) and peptide YY (PYY) (for references see [1]). It was first isolated from pig brain extract [2] using a chemical method for detection of amino acid amides [3] and its primary structure was determined [4]. Since then, the amino acid sequences of the human and rat forms of NPY have been deduced from both cDNA sequences [5,6] and purified peptides [7,8].

Recently, NPY has been isolated from guinea-pig, rabbit [9] and ox brain [10]. The amino acid sequences of human, rat, rabbit and guinea-pig NPY are identical and have Met at position 17 [9]. The only difference between the amino acid sequences of NPY from these species on the one hand and pig and ox NPY on the other is that the latter have Leu-17 instead of Met-17. These findings place NPY among the highly conserved hormonal neuropeptides. Peptides showing structural similarities to NPY have been isolated from the endocrine pancreas of the anglerfish and the Pacific salmon [11,12].

In the present study, we have isolated and characterized sheep brain NPY and show that it has a third type of sequence. It has Leu at position 17 and also a difference at position 10, being Asp instead of Glu.

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The amino acid sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number no Y07516

2. MATERIALS AND METHODS

2.1 *Materials*

Sephadex G-25 fine was from Pharmacia (Uppsala, Sweden), L-(1-tosylamido 2-phenyl) ethyl chloromethyl ketone-treated trypsin from Worthington (Freehold, NJ), pig synthetic NPY from Cambridge Research Biochemicals (Harston, England), and dansyl chloride, pyridine, trifluoroacetic acid, all of sequanal grade from Pierce (Rockford, IL) Ethyl acetate and heptane were of analytical grade and were used after additional distillation Acetonitrile was of HPLC grade from Merck (Darmstadt, FRG) and polyamide TLC sheets were from Schleicher and Schull (Dassel, FRG) Other chemicals were of highest commercial grade

2 2 *Starting material*

Sheep brains (40 kg) were boiled in water for 10 min, frozen, minced and extracted with 0 5 M acetic acid for 24 h Peptides in the extract were adsorbed onto alginic acid, eluted with 0 2 M HCl and precipitated from the eluate with NaCl, essentially as described for a similar concentrate of pig brain peptides [13], with the exception that the eluate was now brought to pH 3 5±0 1, before saturation with NaCl The precipitate (52 g) was dissolved in water and fractionated with isopropanol to produce fraction F2 as described for a similar concentrate of pig intestinal peptides [14] Fraction F2 was the starting material for the further purification as described in section 3

The chemical assay of NPY was performed as described [3] Dansylated tyrosine amide from the digest with trypsin was identified on polyamide TLC sheets by comparisons with the results obtained using pig synthetic NPY

3. RESULTS AND DISCUSSION

Peptide fraction F2 (2.1 g) from a sheep brain preparation (see section 2) was extracted with 105 ml

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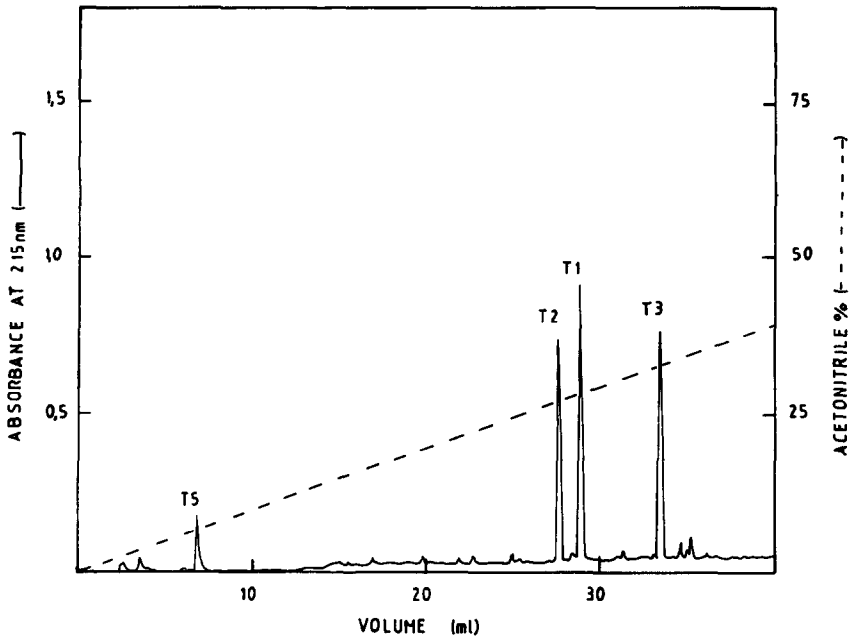

Fig 1 HPLC of fragments obtained by treatment of sheep NPY with trypsin The tryptic fragments were separated on a Vydac 218 TP (5 mm, 4 6×250 mm) in 0 1% trifluoroacetic acid with a gradient of 0–60% acetonitrile at 1 ml/min for 60 min

methanol and the suspension filtered. The filtrate was brought to pH 7.5 ± 0.1 with 0.1 M NaOH in methanol and a precipitate that formed was removed by filtration. The pH of the filtrate was brought to pH 2.7 ± 0.1 with 0.1 M HCl in methanol, after which peptides, and dissolved NaCl, were precipitated with ether (3 volumes). After evaporation of the ether, the precipitate was dissolved in water and peptides were reprecipitated with NaCl. The precipitate (wet weight 0.4 g) was dissolved in 4 ml 0.2 M acetic acid and chromatographed in this solvent on Sephadex G-25 fine (1.5×95 cm). The peptide eluate was divided into two parts. The second part was lyophilized (8 mg) and found to contain peptide(s) with C-terminal tyrosine amide. Final purification was carried out by reverse phase HPLC in a Waters (Millford, MA) system, with dual wavelength detection at 215 and 280 nm, a Vydac 218 TP column (5 μm, 4.6×250 mm), and a gradient (35 min at a flow of 1 ml/min) of 25–60% acetonitrile in 0.1% trifluoroacetic acid.

Table 1

Total composition of the tryptic fragment T1 from sheep NPY

Residue	Mol/Mol
Cys	- (0)
Asx	5 0 (5)
Thr	- (0)
Ser	1 1 (1)
Glx	1 2 (1)
Pro	4 0 (4)
Gly	1 3 (1)
Ala	3 1 (3)
Val	- (0)
Met	- (0)
Ile	- (0)
Leu	1 0 (1)
Tyr	1 0 (1)
Phe	- (0)
Lys	0 9 (1)
His	- (0)
Arg	0 9 (1)

Values shown are molar ratios from acid hydrolysis, and within parentheses, from the sum of the sequence analysis

One fraction was found to have C-terminal tyrosine amide and was essentially pure, as judged by thin layer silica gel chromatography, showing the same R_f value as pig synthetic NPY in butanol/pyridine/acetic acid/water (15:10:3:12 by volume). The peptide thus obtained was degraded in an Applied Biosystems 470A gas-phase sequencer, and phenylthiohydantoin derivatives were analyzed with HPLC on a Nucleosil C18 column with an acetonitrile gradient in sodium acetate as described [15]. The result of the analysis gave a 36-residue amino acid sequence as in pig NPY except for Asp-10 instead of Glu-10. To confirm this structure, the sheep NPY was digested with trypsin and fragments T1–T5 were separated by HPLC on Vydac 218 TP as above (fig.1). The total composition from acid hydrolysis of fraction T1 (table 1) was in complete agreement with the first 19 residues of the sequence (fig.2).

Fig 2 Amino acid sequence of sheep NPY with tryptic fragments T1–T5

That sheep NPY differs from all previously known mammalian NPYs: human, pig, ox, rat, rabbit and guinea-pig is unexpected, having an amino acid residue substitution outside position 17, where the only previously known differences between mammalian NPYs have been found. Sheep NPY is less well conserved evolutionarily than guinea-pig NPY in view of the fact that guinea-pig insulin like the insulins of hystricomorphic rodents is much more different from the insulins of non-hystricomorphic animals than are the latter insulins among themselves [16]. Guinea-pig VIP is the only VIP that differs from the common form of all other known mammalian VIPs and the same applies to guinea-pig C-terminal octapeptide of cholecystokinin [17], although guinea-pig gastrin-releasing polypeptide (GRP) is identical to pig GRP which differs from the dog and human GRPs [18].

Acknowledgements. This work was supported by grants from the Swedish Medical Research Council (projects 13X-1010 and 03X-3532), Karolinska Institutet, KabiGen AB, Skandigen AB, and Tomas Fishers Stiftelse (fellowship R S.)

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