CHARACTERIZATION OF PORCINE MSEL-NEUROPHYSIN

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1. Introduction

Neurophysins, proteins which bind neurohypophysial hormones [1], can be classified in two types, MSEL-neurophysins and VLDV-neurophysins, according to the amino acid residues in position 2,3,6 and 7 [2]. It seems that in mammalian species a protein of each type exists. However the number of neurophysin components disclosed by electrophoresis in a given species is often higher than two because of the presence of truncated forms [3,4]. We have previously suggested, on the basis of structural data, that despite the different electrophoretic mobilities, the so-called neurophysin I of the pig, neurophysin II of the ox and neurophysin-III of the sheep belong to the same MSEL-neurophysin family, and that the porcine neurophysin I may be a C-terminal truncated form of a classical 95-residue MSEL-neurophysin [3,4]. We give now data on the characterization of this 95-residue protein.

2. Results

Purification of porcine MSEL-neurophysin has been carried out as described previously for ovine MSEL-neurophysin [5,6]. The 'crude' neurophysin fraction, obtained from the hormone–protein complex by molecular sieving, is chromatographed onto DEAE–Sephadex A-50 under the conditions described [5]. A fraction A, eluted at pH 5.9 by an ionic strength gradient from 0.2–0.4 M pyridine acetate, is recovered in small amounts when compared with the large peak A observed in the case of ovine MSEL-neurophysin [6]. This fraction contains the 'intact' MSEL-neurophysin (95 residues) and a small part of C-terminal truncated forms of the MSEL-neurophysin which are mainly in peak B. Fraction A is oxidized by performic acid, subjected to tryptic hydrolysis and the resulting peptides are separated by peptide mapping under the conditions previously described [3,4].

Peptides are numbered as for ovine or bovine tryptic peptides since the positions of the basic residues are invariant, so that 8 tryptic peptides (T_1–T_8) are found in the three species with the same number of residues. The amino acid compositions of porcine tryptic peptides are determined (table 1). The amino acid sequences are completely established for the peptides different from the bovine homologous (T_7–T_8) and partially for the peptides with identical amino acid compositions.

The porcine tryptic peptides were arranged in a single polypeptide chain (table 2) by homology with the sequence determined previously for ovine and bovine MSEL-neurophysin [3,4]. On the other hand, a truncated form of peptide T_1, called T'_1, with a 3-residue N-terminal deletion was also found in the tryptic map. This truncated peptide T'_1 was also detected in ovine and bovine MSEL-neurophysin [4].

3. Discussion

The complete sequence of 'native' porcine MSEL-neurophysin comprise 95 residues as in the case of ovine or porcine MSEL-neurophysin (table 2). The amino acid sequence of the first 92 residues is identical to that previously determined by Wuu et al. [7] for a component called neurophysin I. MSEL-neurophysin has 3 additional residues Arg-93–Arg-94–Ala-95 at the C-terminal end. The C-terminal truncated forms

Table 1
Amino acid composition of tryptic peptides of porcine MSEL-neurophysin (values given in residues per mole of peptide)

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Lys		0.90 (1)			0.90 (1)			
His								
Arg	1.18 (1)		1.00 (1)	1.20 (1)	1.00 (1)	1.00 (1)	1.30 (1)	0.60 (1)
Cys[a]		1.60 (2)		3.10 (4)	2.08 (3)	3.60 (5)		
Asp	1.10 (1)			1.00 (1)	1.00 (1)	1.66 (2)		
Thr				0.74 (1)		0.62 (1)		
Ser	1.00 (1)			1.07 (1)	2.80 (3)	1.26 (1)	1.07 (1)	
Glu	1.20 (1)	1.00 (1)		2.10 (2)	4.90 (5)	2.79 (3)	0.83 (1)	
Pro		1.91 (2)		0.65 (1)	2.90 (3)	0.49 (1)		
Gly		3.25 (3)	0.40 (1)	4.10 (4)	3.80 (4)	1.26 (1)	1.30 (1)	
Ala	0.89 (1)			2.23 (2)		2.52 (3)	1.13 (1)	1.00 (1)
Val				1.00 (1)		1.00 (1)		
Met	1.03 (1)							
Ile				0.51 (1)		0.62 (1)		
Leu	2.00 (2)	1.05 (1)		2.00 (2)	1.04 (1)		1.00 (1)	
Tyr					0.60 (1)			
Phe				1.69 (2)			0.70 (1)	
Number of residues	8	10	2	23	23	20	7	2

[a]Determined as cysteic acid; a partial destruction is observed with peptides eluted from paper

Table 2
Comparison of amino acid sequences of MSEL-neurophysins

	1	5	10	15	20
Ovine	Ala–Met–Ser–Asp–Leu–Glu–Leu–Arg–Gln–Cys–Leu–Pro–Cys–Gly–Pro–Gly–Gly–Lys–Gly–Arg				
Bovine	———————————————————				
Porcine	———————————————————				

	21	25	30	35	40
Ovine	Cys–Phe–Gly–Pro–Ser–Ile–Cys–Cys–Gly–Asp–Glu–Leu–Gly–Cys–Phe–Val–Gly–Thr–Ala–Glu				
Bovine	———————————————————				
Porcine	———————————————————				

	41	45	48	50	55	60
Ovine	Ala–Leu–Arg–Cys–Gln–Glu–Glu–Ile–Tyr–Leu–Pro–Ser–Pro–Cys–Gln–Ser–Gly–Gln–Lys–Pro					
Bovine	———————————		–Asn–	———————————		
Porcine	———————————		–Asn–	———————————		

	61	65	70	75	80
Ovine	Cys–Gly–Ser–Gly–Gly–Arg–Cys–Ala–Ala–Ala–Gly–Ile–Cys–Cys–Asn–Asp–Glu–Ser–Cys–Val				
Bovine	———————————————————				
Porcine	———————————————————				

	81	85	89	90	92	95
Ovine	Thr–Glu–Pro–Glu–Cys–Arg–Glu–Gly–Ile–Gly–Phe–Pro–Arg–Arg–Val					
Bovine	———————————		–Ile–	———————		
			–Val–			
Porcine	———————————		–Ala–Ser–	——	–Leu–	—— – Ala

are likely produced by degradation; they are not found in the case of the sheep and the ox probably because leucine-92 of the pig is replaced by a proline residue in the two other species and current proteolytic enzymes cannot split the bonds in which proline is involved. Similar results dealing with the so-called porcine neurophysin III recently published by Wuu and Crumm [8], suggest also that neurophysin I is a C-terminal truncated form of neurophysin III and confirm our proposition to consider a single type of MSEL-neurophysin in mammals [2].

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