IDENTIFICATION OF MSEL AND VLDV NEUROPHYSINS IN HUMAN PITUITARY GLAND

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1. Introduction

Purification of human neurophysin(s) has been attempted [1–4]. Several neurophysin components have been detected by electrophoresis but because of the easy degradation of these proteins [5], the number of authentic neurophysins remains to be determined. Two components have been purified [2] designated neurophysin I and II, according to their electrophoretic mobilities. The N-terminal sequence of neurophysin I has been established [3,6] but there is no structural information about neurophysin II.

A classification of neurophysins by the nature of residues 2, 3, 6 and 7 has been suggested [7] and it is clear from the results now available, that two families, MSEL and VLDV neurophysins, can be distinguished on this sequential basis. To now MSEL neurophysins have fully been characterized in 5 mammalian species (c.f. [8]) and VLDV neurophysins in two species [9]. It seems therefore that each mammalian species has one neurophysin of each type. From the N-terminal sequence of human neurophysin I [3,6] we have deduced [7] that this neurophysin should belong to the VLDV neurophysin family. If so a representative of the second type should exist along with the VLDV neurophysin. Here we report data showing that a second neurophysin, belonging to the MSEl neurophysin family, is present in human pituitary gland.

2. Materials and methods

A 'crude' neurophysin fraction was prepared from acetone-desiccated human posterior pituitaries by the method in [4]. This material was subjected to continuous preparative disc electrophoresis in polyacrylamide gel [4]. Four components called PG I, PG II, PG III and PG IV, following decreasing electrophoretic mobilities to the anode, were isolated. Immunological tests have revealed that PG I, PG II and PG IV show great crossreactivity between them but little with PG III [4]. Chemical investigations have now been performed on PG II, PG IV and on the 'crude' neurophysin fraction.

Neurophysins were oxidized by performic acid and split by trypsin [5]; resulting peptides were separated by peptide mapping as in [10] and characterized by amino acid composition [11]. Amino acid sequence was determined either by a manual Edman degradation [12] for the fragments or by the automated method in [13] in a Socosi model P 110 sequencer for the proteins. Phenylthiohydantoin amino acids were identified by thin-layer chromatography [14].

3. Results

3.1. *PG II*

PG II ~1 mg (100 nmol for mol. wt 10 000 deduced from amino acid composition) after desalting by passage through a Sephadex G-25 column, were oxidized by performic acid and subjected to trypsin hydrolysis. Tryptic fragments (8) were separated by peptide mapping (~15–20 nmol of each peptide were recovered) and analyzed. Peptide maps obtained for MSEL neurophysins of other mammalian species were compared [5,8]. Three peptides T1, T2 and T3 had the same relative positions on the map and the same amino acid compositions (table 1) as those found for

Table 1
Determination of the N-terminal sequence of human MSEL neurophysin

I. Amino acid composition of T1, T2 and T3 isolated from PG II

T1 : (Ala, Met, Ser, Asp, Leu, Glu, Leu, Arg) (18 nmol)

T2 : (Glu, $CySo_3H$, Leu, Pro, $CySO_3H$, Gly, Pro, Gly, Gly, Lys) (16 nmol)

T3 : (Gly, Arg) (18 nmol)

II. Manual degradation on peptides T1 and T2 isolated from unfractionated neurophysins

	1	2	3	4	5	6	7	8	9	10	11	12
T1 :	Ala	Met	Ser	Asp	Leu	Glu	Leu	Arg (64 nmol)				
T2 :									Gln	$CySO_3H$	Leu	Pro ($CySO_3H$, Gly, Pro, Gly, Gly, Lys) (49 nmol)

III. Automated degradation on unfractioned neurophysins

1	2	3	4	5	6	7	8	9	10	11
Ala	Met	Ser	Asp	Leu	Glu	Leu	Arg	Gln	Cys	Leu
	Ala	Pro			Asp	Val		Lys		

the N-terminal peptides T1, T2 and T3 of MSEL neurophysins [5]; it can therefore be assumed that the N-terminal sequence of PG II is identical with the N-terminal sequence found for the MSEL neurophysin family [8].

3.2. *PG IV*

PG IV ~0.8 mg (80 nmol) was subjected to oxidation, trypsin hydrolysis and peptide mapping (~5 nmol of each peptide were recovered). The map was very similar to that given by PG II except that there is an additional peptide. Three peptides having the same relative positions and the same amino acid compositions as T1, T2 and T3 of PG II were identified. It can be inferred that PG IV has the same N-terminal sequence as PG II. However, the additional peptide occupies the position and has the amino acid composition (Arg, Ala) of the C-terminal fragment found in pig, horse and whale MSEL neurophysins [8]. PG IV seems to be the intact MSEL neurophysin and PG II a C-terminal truncated form.

3.3. *Unfractionated neurophysins*

After desalting 3 mg (~300 nmol) was subjected to performic acid oxidation, tryptic hydrolysis and resulting fragments were separated by peptide mapping. A fragment located on the map as peptide T1 of PG II and PG IV was analyzed and completely sequenced by manual Edman procedure (table 1). It shows the N-terminal sequence of a typical MSEL neurophysin. Another fragment located on the map as peptide T2 of PG II and PG IV was also analyzed and partially sequenced (table 1). Its N-terminal sequence Gln–$CySO_3H$–Leu–Pro agrees with the N-terminal sequence of a peptide T2 from a typical MSEL neurophysin.

Automated degradation was done on 2.3 mg (230 nmol) of the 'unfractionated' neurophysin. Results are indicated in table 1. It is clear that this fraction is a mixture of two neurophysins which have identical residues in positions 1, 4, 5, 8, 10 and 11 but different residues in positions 2, 3, 6, 7 and 9. The N-terminal sequence of human neurophysin I is known [3,6]; because this protein has alanine, proline, aspartic acid, valine and lysine, respectively, in positions 2, 3, 6, 7 and 9, by difference the N-terminal sequence Ala–Met–Ser–Asp–Leu–Glu–Leu–Arg–Gln–Cys–Leu can be assigned to the second neurophysin. This agrees with the sequence deduced from peptides T1 and T2.

The results as a whole indicate that a neurophysin belonging to the MSEL neurophysin family is present in human pituitary gland. We assume that PG IV is the intact protein and PG II a C-terminal truncated form, in agreement with immunochemical data [4]. Such a truncation has been observed for the porcine MSEL neurophysin [5].

However, the results obtained by automated Edman degradation on the unfractionated neurophysins reveal that a second neurophysin having the same N-terminal sequence as that found for neurophysin I [3,6] is also present. As suggested [7], neurophysin I probably belongs to the VLDV neurophysin lineage, due to the nature of residues 6, 7 and 9. The Edman degradation carried out on unfractionated neurophysins does not indicate another type of neurophysin so that 2 of the 4 components isolated by electrophoresis are probably derivatives or C-truncated forms of MSEL or VLDV neurophysins.

The two types of neurophysins have been fully characterized in ox and pig [8,9] and we can assume that they will be present in most mammalian species. There is indirect evidence for a specific biological relationship between vasopressin and oxytocin with MSEL and VLDV neurophysin, respectively [15].

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