COMPARISON BETWEEN MSEL- AND VLDV-NEUROPHYSINS

Complete amino acid sequences of porcine and bovine VLDV-neurophysins

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1. Introduction

Neurophysins [1] are small proteins found in the neural lobe of the pituitary gland which give specific and reversible complexes with neurohypophysial hormones [2]. A recent review has been devoted to the molecular and cellular aspects of neurophysins and to their interactions with neurohypophysial hormones [3]. On the basis of sequential data, it has been suggested to classify neurophysins in two types, MSEL-neurophysins and VLDV-neurophysins, according to the amino acid residues in position 2, 3, 6 and 7 [4]. A representative of each type seems to exist in several mammalian species and complete amino acid sequences of MSEL-neurophysins from 5 species, namely ox, sheep, pig, horse and whale, have been determined (c.f. [5]). We report now the complete amino acid sequences of VLDV-neurophysins from two species, pig and ox. The results confirm the existence of two phylogenetic lines of neurophysins in mammals which could be related to oxytocin and vasopressin, respectively.

2. Materials and methods

Purification of bovine [6,7] and porcine [8] MSEL-neurophysins has been described; isolation of VLDV-neurophysins involves essentially the same procedure except that extraction of posterior pituitary glands is carried out with HCl 0.1 N and the material is recovered in peak B after chromatography of 'crude' neurophysin on DEAE–Sephadex A-50 under the conditions described earlier.

VLDV-neurophysins are oxidized by performic acid and split either by trypsin or by *Staphylococcus aureus* protease [9]; the resulting peptides are separated by peptide mapping as in [10]. Peptides are characterized by amino acid composition and partially or completely sequenced with a manual Edman procedure [11]. Long peptides are subjected to hydrolysis by chymotrypsin and/or subtilisin and fragments are isolated by peptide mapping and sequenced.

The intact protein, on the other hand, is reduced by dithiothreitol, alkylated with iodoacetamide [12] and subjected to automatic Edman degradation in a Socosi model P 110 sequencer as in [13]. Phenylthiohydantoin amino acids are identified by thin-layer chromatography [14]. Some tryptic peptides from the reduced-alkylated protein are isolated by peptide mapping and analyzed.

3. Results

Tryptic peptides (T1–T7 for porcine, T1–T6 for bovine) are shown in fig.1. T1, T3 and T4 are identical in both species. There is one substitution in T2 (lysine in porcine, threonine in bovine; position 9 of the complete sequence) and one substitution in T5 (glutamic acid in porcine, glycine in bovine; position 64 of the complete sequence). There is an arginine residue in position 80 in porcine and not in bovine so

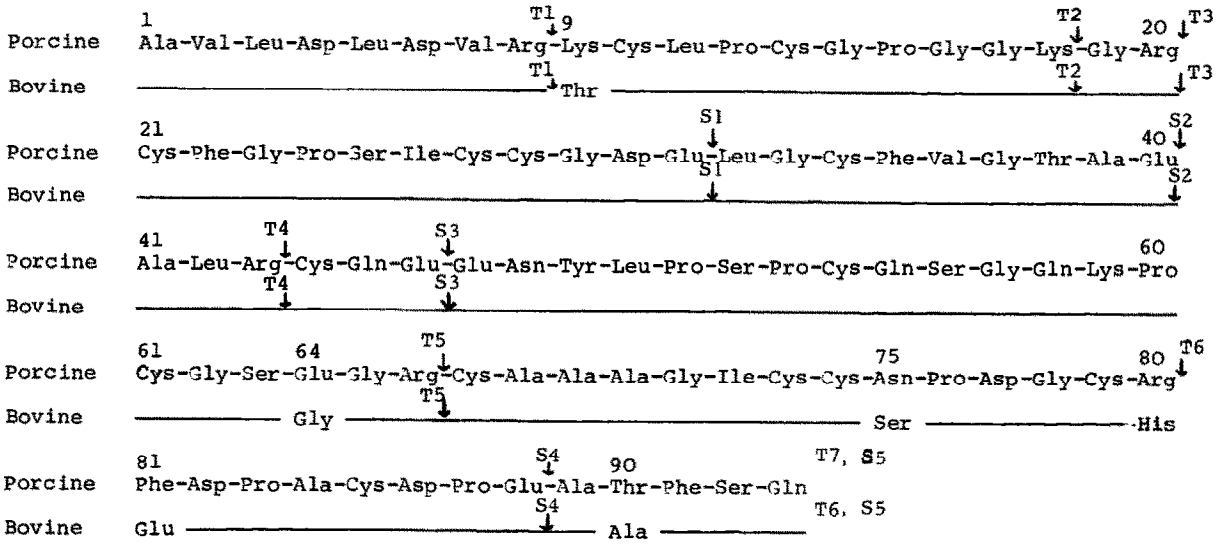

Fig.1. Amino acid sequences of porcine and bovine VLDV-neurophysins. Alignment of tryptic peptides (T1–T7 in porcine, T1–T6 in bovine) has been determined with overlapping peptides produced by *Staphylococcus* protease (S1–S5) and through the sequencer (for N-terminal sequence).

that two tryptic peptides T6 and T7 are found for the first species and a single T6 for the second. Four substitutions are observed in the C-terminal part of the proteins in position 75 (asparagine in porcine, serine in bovine) 80 (arginine in porcine, histidine in bovine) 81 (phenylalanine in porcine, glutamic acid in bovine) and 90 (threonine in porcine, alanine in bovine).

Peptides produced by *Staphylococcus* protease (S1–S5) are indicated in fig.1. They are used to confirm and to order tryptic peptides. Expected cleavages at Glu-64 in porcine and Glu-81 in bovine are not observed. Both proteins have 93 amino acid residues instead of 95 found in the MSEL-neurophysin family [5]. N-terminal sequences are confirmed by automated degradation up to residue 32.

The amino acid sequence of the so-called neurophysin-II of pig [15] has been announced but as far as we know the results have not been published so that a comparison is not possible at present. On the other hand, a sequence has been published for the so-called neurophysin-I of ox [16]; this sequence differs from ours in positions 90 (serine instead of alanine), 92 (leucine instead of serine) and residue 93 is missing.

4. Discussion

The results indicate clearly that pig and ox have two similar neurophysins which only differ by 6 substitutions out of 93 residues. The suggested presence of two types of neurophysins, MSEL- and VLDV-neurophysins, in each mammalian species [4] is confirmed. When porcine and bovine VLDV-neurophysins (93 residues) are compared with porcine and bovine MSEL-neurophysins (95 residues) 19 and 20 substitutions are found, respectively. Porcine and bovine MSEL-neurophysins only differ by 4 substitutions out of 95 residues.

Figure 2 recapitulates the sequences of the known MSEL-neurophysins (5 species) and VLDV-neurophysins (2 species). Substitutions within each family are indicated. It is clear that two regions, the N-terminal (residues 1–9) and the C-terminal (75–93) sequences, display variations. However in the N-terminal sequence, substitutions are very rare within the family so that this sequence can be used as 'marker' to distinguish the two families.

The C-terminal part, from residue 75–88, can also characterize a given family but there is an hypervariable region (residues 89–95) in which the MSEL-family

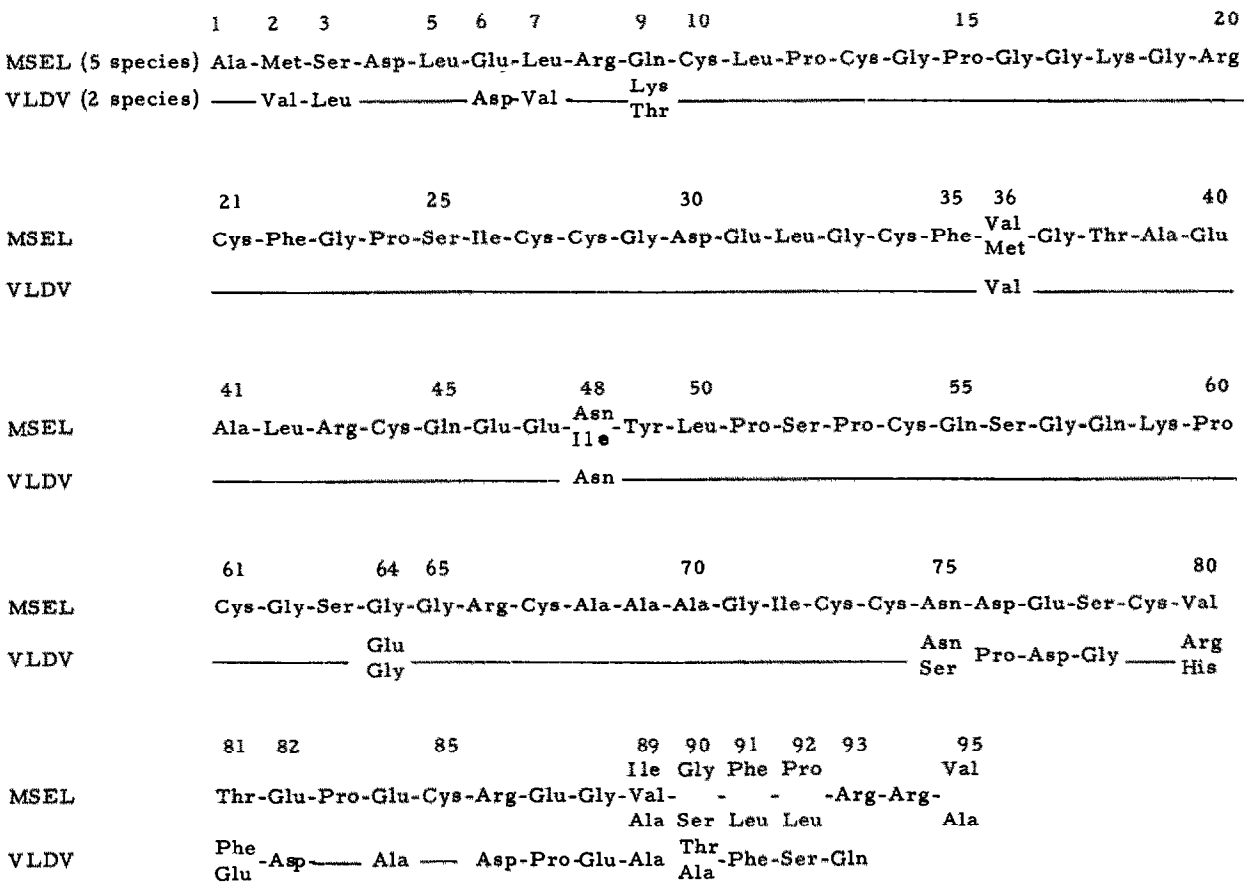

Fig.2. Comparison between MSEL- and VLDV-neurophysins. The upper line shows the sequences of MSEL-neurophysins (ox, sheep, pig, horse and whale); substitutions observed within the family are indicated. The lower line shows the sequences of VLDV-neurophysins, (pig and ox) described in this work; solid lines represent residues identical to those found in MSEL-neurophysins. Substitutions within the family are also indicated.

shows many substitutions. In contrast the central part of the chain (residues 10–74) is nearly invariant within the families and between the families. It is worthy to note that the half-cystine residues are invariant and that probably the 7 disulfide bridges have the same location in the two types of neurophysins. For this reason, and because of the high percentage of homology (~80%), we can assume that the general conformations are very similar.

From an evolutionary point of view, the similarity between the two neurophysins present in each species suggest that they have arisen from a common ancestral neurophysin by gene duplication. Apparently the two types exist in mammals but it remains to be determined whether they are also present in non-mammalian vertebrates and whether two phylogenetic lines can be traced for neurophysins as it has been made for neurohypophysial hormones [17].

It is of interest to point out that there is indirect biological evidence for a specific relationship between one type of neurophysin and one type of hormone (oxytocin or vasopressin) and the two molecules could be cleavage products of a common precursor [3].

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