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IDENTIFICATION OF RAT NEUROPHYSINS : COMPLETE AMINO ACID SEQUENCES OF MSEL- AND VLDV-NEUROPHYSINS

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SUMMARY

Two rat neurophysins have been purified by salt precipitation, molecular sieving and ion-exchange chromatography. The proteins, performic-acid oxidized or reduced-alkylated, have been split either by trypsin or by staphylococcal proteinase and fragments have been separated by peptide mapping. Amino acid sequences of tryptic peptides have been determined either directly or after cleaving the large fragments by subtilisin, chymotrypsin, elastase or staphylococcal proteinase and characterizing the subfragments. Tryptic peptides have been ordered through the fragments given by staphylococcal proteinase. The N-terminal sequences of both proteins have also been established by automated degradation.

The two usual types of mammalian neurophysins have been identified. One neurophysin belongs to the MSEL-neurophysin family and shows 11 substitutions and a 2-residue C-terminal truncation when compared with bovine MSEL-neurophysin. The other belongs to the VLDV-neurophysin family and shows 8 substitutions when compared with bovine VLDV-neurophysin. There are 23 differences between the MSEL- and VLDV-neurophysins of the rat.

INTRODUCTION

Neurophysins (1) are small single-chained proteins (93-95 residues), purified from the neuro-intermediate lobe of the pituitary gland, which give specific and reversible complexes with neurohypophysial hormones (for recent reviews see 2-4). All the mammalian species investigated to date, namely ox, sheep, pig (5-7), horse (8) and man (9), have two types of neurophysins that can be distinguished by their amino acid sequences. The two types are called MSEL- and VLDV-neurophysins, acoording to the nature of residues in positions 2, 3, 6 and 7, by using the one-letter symbols of amino acids usually located in these positions (Ref. 10).

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The number of rat neurophysins has up to now not clearly been determined. Gel electrophoresis often shows three components called neurophysins I, II and III according to the mobilities to the cathode (11-15). N-terminal sequences (14,15) however reveals only two types; moreover the sequence found for neurophysin II by Schlesinger et al. (14) is attributed to the neurophysin I by North and Mitchell (15). We report here the complete amino acid sequences of two rat neurophysins which confirm the existence of the two phylogenetic lines MSEL and VLDV in mammals.

MATERIAL AND METHODS

Purification of rat neurophysins : purification is carried out essentially as previously described (8). Acetone-desiccated posterior pituitary powder (2.15 g, about 5,000 glands) is extracted by 0.1 M HCl (40 ml/g) for 5h at 4°C and the supernatant solution, adjusted to pH 4.0, is subjected to fractionated precipitation with 10% NaCl. The precipitate is dialyzed, freeze-dried (900 mg) and redissolved in 0.1 M formic acid. The solution is clarified by centrifuging and aliquots (200 mg in 5 ml) are subjected to molecular sieving on a column (2.5 X 170 cm) of Sephadex G-75. 3-ml fractions are collected and five peaks are detected by absorbance at 280 nm. The fourth peak corresponds to "crude" neurophysins (total recovery = 118 mg).

Fractions of 40 mg are subjected to ion-exchange chromatography on a column (0.5 X 38 cm) of DEAE-Sephadex A-50 equilibrated with 0.4 M pyridine acetate pH 5.9. A ionic strength gradient (0.4 M to 0.6 M) is applied and 1-ml fractions are collected. MSEL-neurophysin is first recovered (37 mg); after 270 ml, a second gradient (0.6 to M) is used and VLDV-neurophysin is obtained (9.7 mg). The homogeneity is checked by disc polyacrylamide gel electrophoresis.

Amino acid sequences : both neurophysins are oxidized by performic acid, split either with trypsin or with staphylococcal proteinase (16) and resulting peptides are separated by peptide mapping under conditions previously described (17). Peptides are analyzed and amino acid sequences are determined by a manual Edman procedure (18), either directly or after cleavage by subtilisin, chymotrypsin, elastase or staphylococcal proteinase, isolation of subfragments and determination of their sequences.

On the other hand, purified proteins are reduced by dithiothreitol, alkylated with iodoacetamide (19) and subjected to automated degradation (20) in a SOCOSI model P110 sequencer. Phenylthiohydantoin amino acids are identified by thin-layer chromatography (21).

RESULTS

Amino acid sequence of MSEL-neurophysin

Amino acid compositions and sequences of tryptic peptides of MSEL-neurophysin (T1 to T8, Fig. 1) are determined. When the tryptic

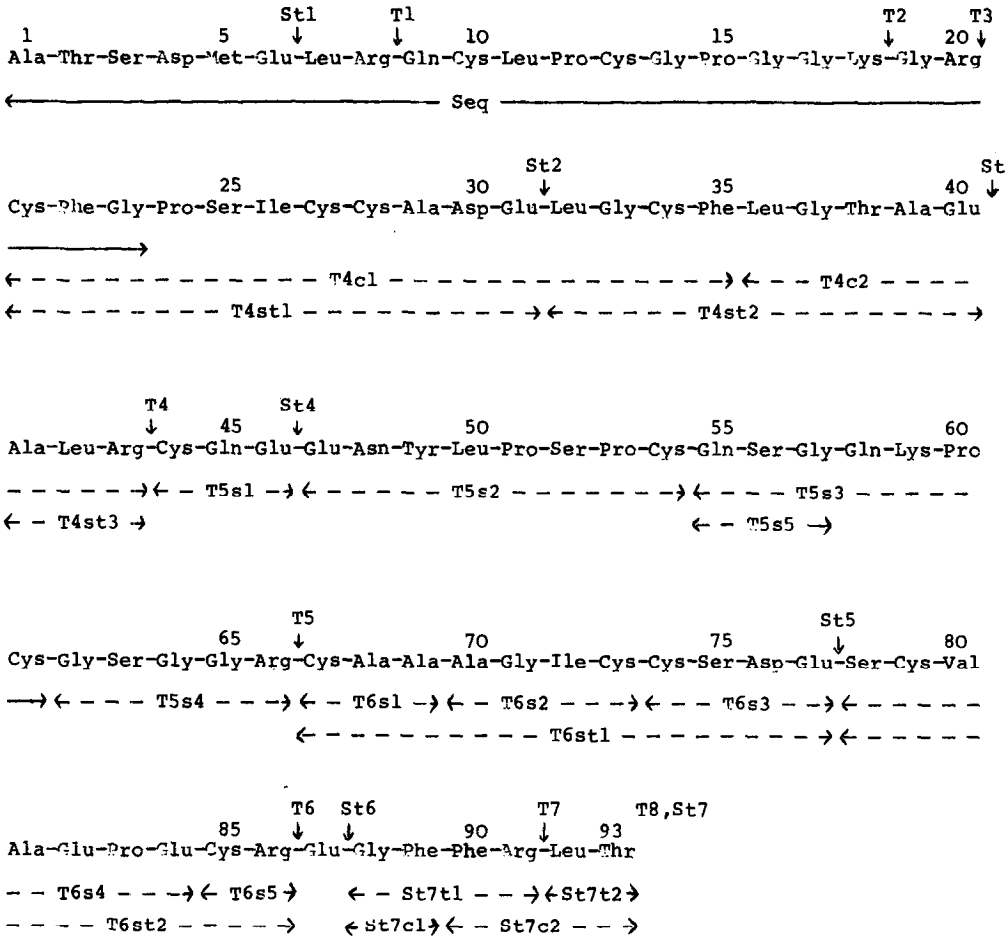

Fig. 1 Amino acid sequence of rat MSEL-neurophysin. Tryptic (T) and staphylococcal proteinase (St) peptides are shown by arrows. Subfragments obtained by chymotrypsin (c), subtilisin (s), staphylococcal proteinase (st) are indicated as T4c1, T4st1, T5s1, etc... Seq. : Sequence determined by automated Edman degradation.

units of rat MSEL-neurophysin are compared with the homologous peptides of bovine MSEL-neurophysin, two substitutions are found in T1 (residues 1-8) in positions n° 2 (Thr in place of Met) and n° 5 (Met in place of Leu). T2 (9-18), T3 (19-20) and T5 (44-66) are identical. Two substitutions are found in T4 (21-43) in positions n° 29 (Ala in place of Gly) and n° 36 (Leu in place of Val) and two substitutions in T6 (67-86) in positions n° 75 (Ser in place of Asn) and

n° 81 (Ala in place of Thr). T7 and T8 are different from bovine T7 and T8 because of an apparent deletion of two residues. T7 (87-91) has five residues instead of 7 and substitutions are observed in positions n° 89 (Phe in place of Ile/Val), 90 (Phe in place of Gly) and 91 (Arg in place of Phe). T8 (92-93) is Leu-Thr instead of Pro-Arg.

Peptides produced by staphylococcal proteinase (St1 to St8) are indicated in Fig. 1. They confirm and order tryptic peptides. St2 gives the alignment T1-T2-T3-T4, St4 gives the alignment T4-T5, St5 the alignment T5-T6, St7 the alignment T6-T7 and St8 the junction T7-T8. N-terminal sequence is confirmed by automated degradation of the reduced-alkylated protein up to residue n° 23.

Amino acid sequence of VLDV-neurophysin

Amino acid compositions and sequences of tryptic peptides of VLDV-neurophysin (T1 to T7) are determined in the same way as for MSEL-neurophysin (Fig. 2). When compared with the homologous tryptic peptides of bovine VLDV-neurophysin, two substitutions are found in T1 (1-8) in positions n° 2 (Ala in place of Val) and n° 7 (Met in place of Val). There is one substitution in T2 (9-18) in position n° 9 (Lys in place of Thr). T3 (19-21) and T5 (44-66) are identical. There is one substitution in T4 (22-43) in position n° 29 (Ala in place of Gly). Because of the substitution in position n° 80 of Arg in place of His, the C-terminal T6 (67-93) of bovine protein corresponds here to two tryptic peptides T6 (67-80) and T7 (81-93). Substitutions are found in positions n° 69 (Thr in place of Gly), n° 80 (Arg in place of His), n° 81 (Thr in place of Glu) and N° 89 (Ser in place of Ala). Cleavage at Arg-80 is not complete so that an overlapping peptide T6-7 is also found.

Peptides produced by staphylococcal proteinase are used to order the 7 tryptic peptides. St2 (7-21) gives the alignment T1-T2-T3-T4,

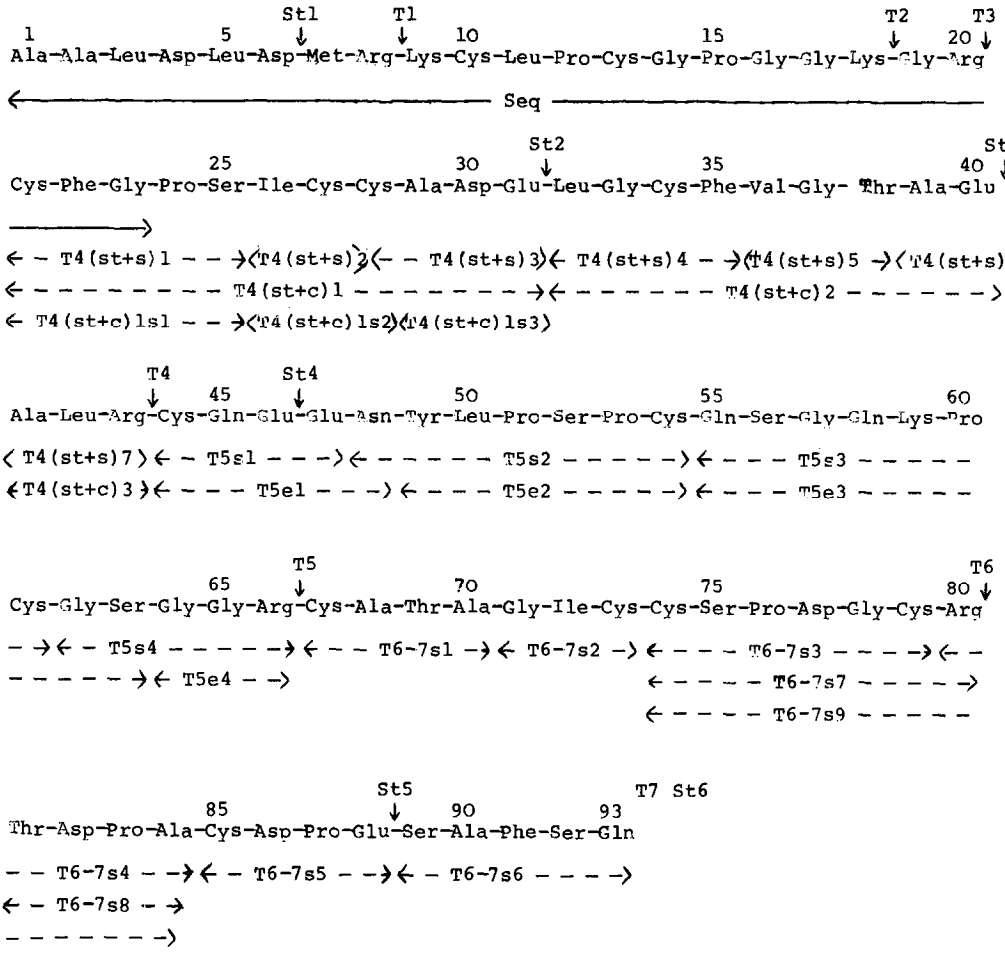

Fig. 2 Amino acid sequence of rat VLDV-neurophysin. Tryptic (T) and staphylococcal proteinase (St) peptides are shown by arrows. Subfragments obtained by chymotrypsin (c), subtilisin (s), elastase (e), staphylococcal proteinase (st) are indicated as T5s1, T5e1, etc... and those obtained by an enzyme mixture by T4(st+s)1, T4(st+c)1, etc... Sub-subfragments are indicated as T4(st+c)1s1, etc... Seq. : Sequence determined by automated Edman degradation.

St4 (41-46) gives the alignment T4-T5 and St5 (47-38) gives the the junction T5-T6-T7. N-terminal sequence is confirmed by automated degradation up to residue n° 23.

DISCUSSION

MSEL-neurophysin family

Fig. 3 shows the comparison between MSEL-neurophysins from six mammalian species. Rat MSEL-neurophysin is peculiar because of the

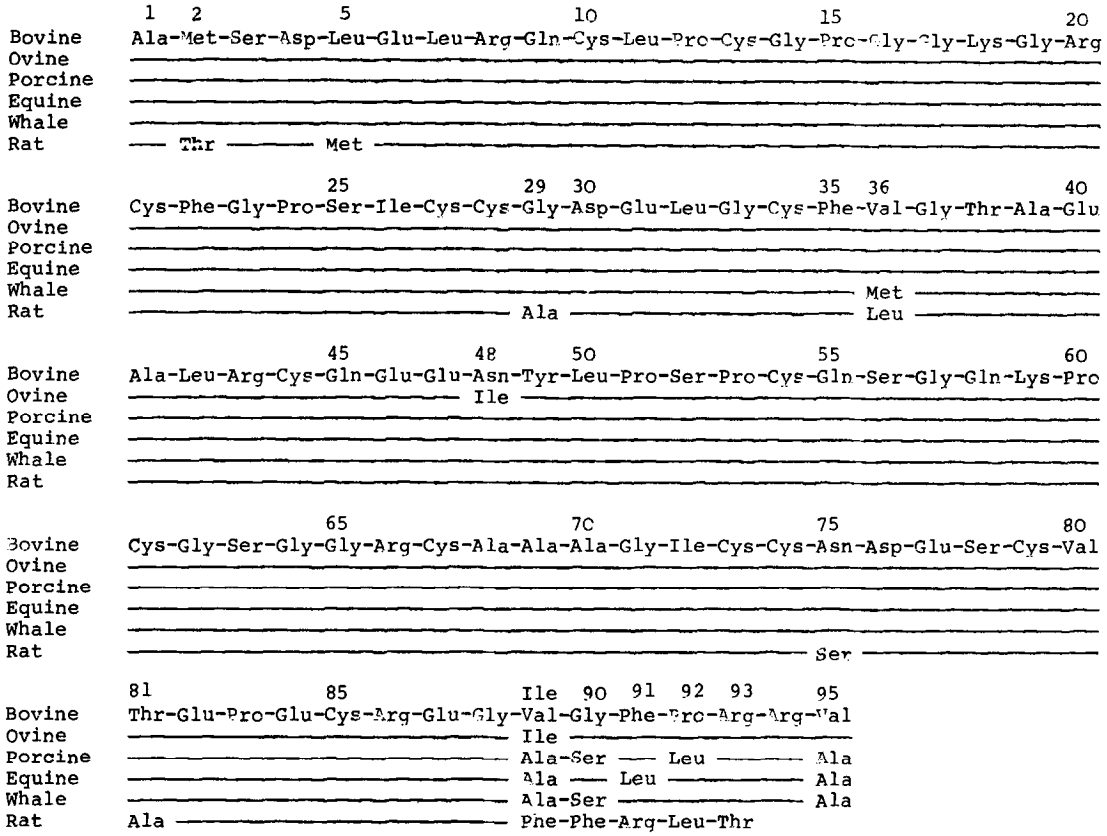

Fig. 3 Comparison of bovine, ovine (5), porcine (6), equine (25), whale (26), and rat MSEL-neurophysins. Solid lines indicate residues identical with those of bovine protein.

variation in position n° 2, Thr instead of the usual Met. However the residues in positions n° 3, 6, 7 and 9 are typical of the MSEL-neurophysin family. Another unusual variation is the shortening from 95 to 93 residues with a peculiar C-terminal sequence 89-93. Two substitutions in the "constant" region 10-75 are observed in positions n° 29 (Ala in place of Gly) and n° 36 (Leu in place of Val). This latter position is also substituted in whale MSEL-neurophysin. Rat MSEL-neurophysin ressembles the so-called rat vasopressin-associated neurophysin whose N-terminal sequence has been determined until residue n° 40 (Ref. 15). However residues 39-40 are here Ala-Glu instead of Leu-Val.

There are 11 substitutions in rat MSEL-neurophysin when compared with bovine protein. Rat belongs to the order Rodentia which is less related to Artiodactyla (ox, sheep, pig) than Perissodactyla (horse) and Cetacea (whale).

VLDV-neurophysin family

Comparison between VLDV-neurophysins from four species is shown in Fig. 4. Again rat VLDV-neurophysin is peculiar. When compared with bovine homologue there are two substitutions in positions n°2 (Ala instead of Val) and n° 7 (Met instead of Val). In position n° 9 there is Lys which seems more frequent than Thr found in ox. In the "constant" region (10-75), two unusual substitutions are found in positions n° 29 (Ala in place of Gly) and n° 69 (Thr in place of Ala). There are three substitutions in the C-terminal part (75-93) when compared with ox, three with pig and six with horse. Rat VLDV-neurophysin ressembles the oxytocin-associated neurophysin whose N-terminal sequence has been determined until residue n° 67 with gaps in positions n° 52, 53, 58, 59, 66 and 67 (Ref. 15). However Gln is found in position n° 55 instead of Gly and Thr in position n° 69 instead of Ala.

There are 8 and 10 substitutions in rat VLDV-neurophysin when compared to bovine and porcine proteins, respectively. Six substitutions are observed between ox and pig which belong to the same order Artiodactyla.

Comparison between rat MSEL- and VLDV-neurophysins

As found in other species, differences are observed essentially in N-terminal (1-10) and C-terminal (75-93) parts of the chains. Because of the shortening or truncation of the MSEL-neurophysin type, both proteins have 93 residues and there are 23 differences (compared with 20, 19 and 20 in ox, pig and horse). Unusual substitutions occur in N-terminal sequences but the two types are recognizable. It is of

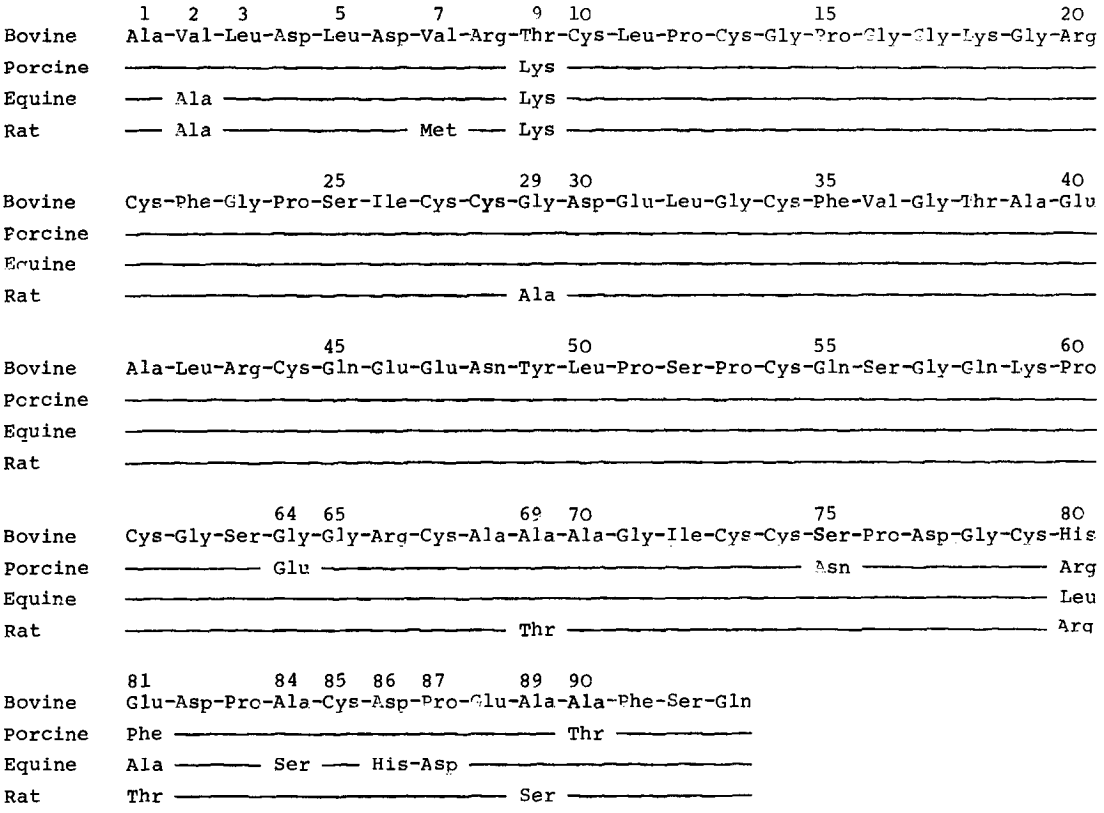

Fig. 4 Comparison of bovine, porcine (7), equine (8) and rat VLDV-neurophysins. Solid lines indicate residues identical with those of bovine protein.

interest to note the common substitution in both proteins in position n° 29 (Ala instead of Gly) located in the common "constant" region, which could suggest a common partial gene for this part of the molecules. However the substitution in position n° 36 (Leu in place of Val) observed in the MSEL type is not seen in the VLDV type.

Two rat pro-neurophysins (Mr = 20,000) have been purified from hypothalamus (22) and rat pre-pro-neurophysins have been synthesized through hypothalamic m-RNA (23). Because oxytocin and arginine vasopressin have been identified in rat (24), and because they are supposed to share common precursors with neurophysins (22), it will be interesting to determine directly the respective locations in the precursors.

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