PHYLOGENY OF NEUROPHYSINS

Complete amino acid sequence of whale (*Balaenoptera physalus*) MSEL-neurophysin

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Received 16 January 1978

1. Introduction

Neurophysins [1] are small proteins which are found associated with neurohypophysial hormones in the neural lobe of the pituitary gland. On the basis of sequential data available up to now, it has been suggested to classify neurophysins in two types, MSEL-neurophysins and VLDV-neurophysins, according to the amino acid residues in positions 2, 3, 6 and 7 [2]. A representative of each type seems to exist in several mammalian species and might be related to vasopressin and oxytocin respectively. A recent review has been devoted to the molecular and cellular aspects of neurophysins and to their interactions with neurohypophysial hormones [3].

Amino acid sequences of sheep [4,5], ox [4,5], pig [5–7] and horse [8] MSEL-neurophysins have been completed. We report now the complete amino acid sequence of a homologous protein isolated from whale posterior pituitary glands. The comparison between 5 species belonging to 3 orders of eutherian mammals (Artiodactyla, Perissodactyla, Cetacea), clearly shows that substitutions have occurred almost exclusively in the last 7 positions of the 95-residue polypeptide chain, the rest of the molecule being nearly invariant.

2. Materials and methods

Isolation of whale MSEL-neurophysin was carried out essentially as in [4–8]. The neurohypophysial hormone–neurophysin complex was purified by fractionated salt precipitation [9,10] and dissociated by molecular sieving in acidic medium [11]. 'Crude' neurophysins were subjected to a chromatography on diethylaminoethyl–Sephadex A-50 and MSEL-neurophysin collected as in [11,12].

MSEL-neurophysin was oxidized by performic acid and split either by trypsin or by *Staphylococcus aureus* protease [13]; resulting peptides were separated by peptide mapping as in [14]. Peptides were characterized by amino acid composition and by partial or complete sequence determined with a manual Edman procedure [15]. The intact protein, on the other hand, was reduced by dithiothreitol, alkylated with iodoacetamide [16] and subjected to automated Edman degradation in a SOCOSI model P 110 sequencer, as in [17]. Phenylthiohydantoin amino acids were identified by thin-layer chromatography [18].

3. Results and discussion

Tryptic peptides (T1–T8, fig.1) were recognized on the map by comparison with ox, sheep, pig and horse tryptic maps [8]. The amino acid compositions and N-terminal sequences confirm the homology. T1, T2, T3, T5 and T6 are identical with those found for ox MSEL-neurophysin. T4 shows 1 substitution, methionine in place of valine (position 36 of the polypeptide chain). T7 has 2 substitutions, alanine in place of isoleucine (position 89) and serine in place of glycine (position 90). T8 displays 1 substitution, alanine in place of valine (position 95). The alignment

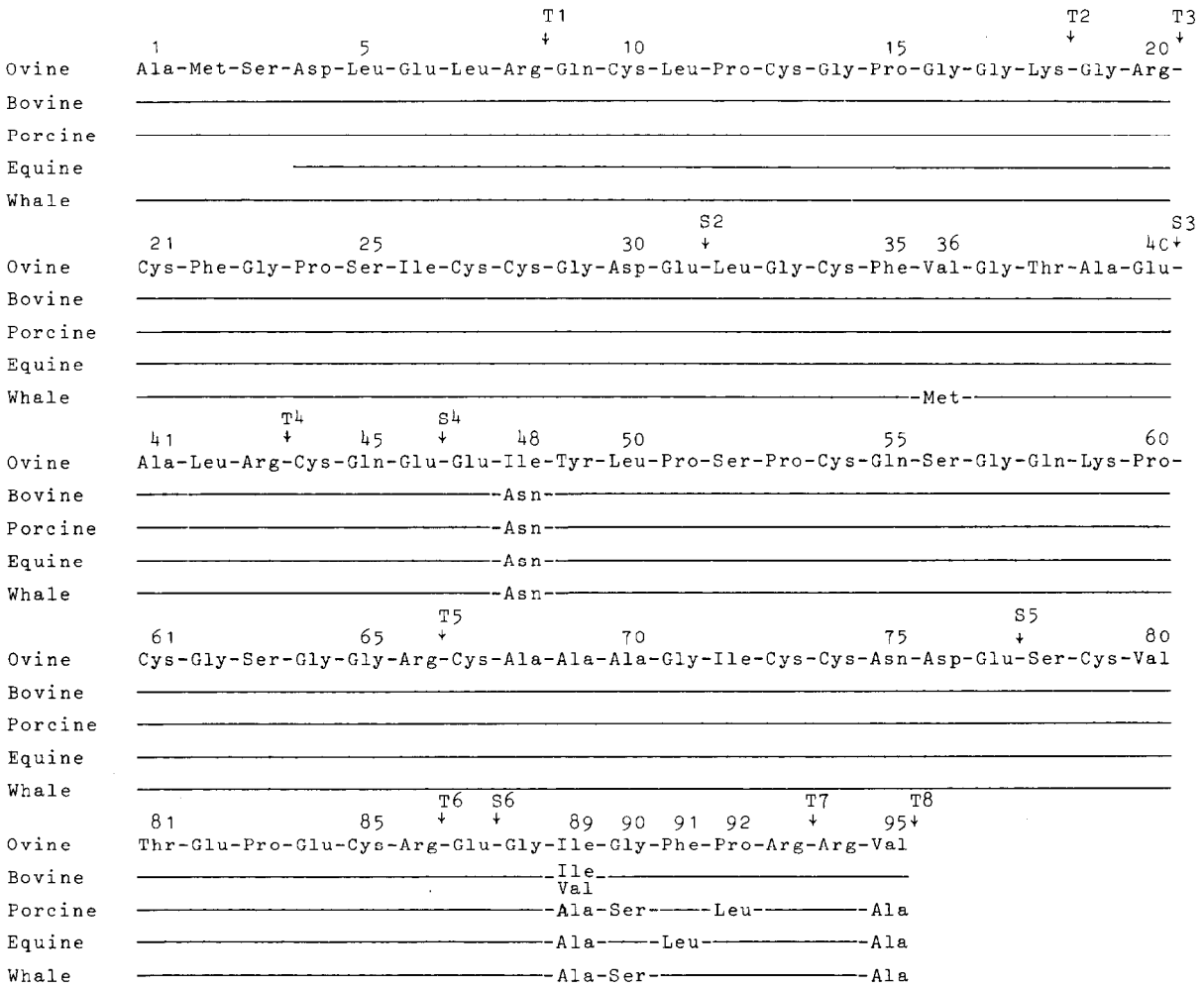

Fig.1. Comparison of sheep, ox, pig, horse and whale MSEL-neurophysins. Sequences identical with those of ovine neurophysin are shown as solid lines. Alignment of tryptic peptides (T) of whale neurophysin has been determined through the sequencer (T1–T4) and by the overlapping peptides produced by staphylococcal protease (S).

of tryptic peptides was determined either through the sequencer (T1–T4) or with overlapping peptides produced by staphylococcal protease (fig.1). These peptides were purified, analyzed and partially or completely sequenced in the same way as for tryptic peptides (fig.1). They prove the alignment which could be deduced by homology with the known MSEL-neurophysins.

Figure 1 shows the amino acid sequences of the MSEL-neurophysins from 5 species which are known to date. The 95-residue pattern seems well preserved, the N-terminal truncation in the horse protein being likely due to a degradation. In this family of neurophysins, there is a large nearly-invariant region for residues 1–88. In this part of the polypeptide chain, substitutions are observed once in position 36 (whale) and in position 48 (sheep).

In contrast the C-terminal part of the chain is variable, since position 89 is occupied either by isoleucine or valine or alanine, position 90 by glycine or serine, position 91 by phenylalanine or leucine, position 92 by proline or leucine and position 95 by

valine or alanine. Curiously positions 89 and 95 seem related since both are occupied either by alanine (pig, horse, whale) or by isoleucine or valine (ox and sheep). If there is a near identity between proteins from two species belonging to Ruminantia (ox and sheep), the pig which belongs to the same order (Artiodactyla) does not appear more akin than species from other orders such as horse (Perissodactyla) or whale (Cetacea).

Because of the N-terminal sequence determined for the so-called neurophysin-II of the dog [19] and the so-called neurophysin-I of the rat [20], we assume that these proteins might belong to the MSEL-neurophysin line.

Acknowledgements

The authors wish to thank Miss Marie-Hélène Simon and Mrs Monique Lidonne for their skilled technical assistance. This study was supported in part by grants from CNRS (ERA No. 563) and Fondation pour la Recherche Médicale.

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