Vol. 100, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1981 Pages 600-605

IDENTIFICATION OF NEUROPHYSINS : COMPLETE AMINOACID SEQUENCE OF HORSE VLDV-NEUROPHYSIN

Marie-Thérèse Chauvet, Jacqueline Chauvet and Roger Acher

Laboratory of Biological Chemistry, University of Paris VI
96, Bd Raspail - 75006 Paris

Received March 3,1981

SUMMARY

Horse VLDV-neurophysin has been purified by salt fractionated precipitation, molecular sieving and ion-exchange chromatography. The protein, performic-acid oxidized or reduced-alkylated, has been split either by trypsin or by Staphylococcus aureus protease and fragments have been separated by peptide mapping. Amino acid sequences have been determined either directly or after cleaving the large fragments by subtilisin, chymotrypsin or elastase and characterizing the subpeptides . The 6 tryptic peptides have been ordered through the 5 fragments given by Staphylococcus protease. The N-terminal sequence of the protein has also been established by automated degradation. The complete sequence of the 93-residue single-chain protein has been determined. When compared with the 95-residue horse MSEL-neurophysin, 20 substitutions are observed exclusively either in N-terminal part (residues 1 to 9) or in the C-terminal region (residues 75 to 93).

INTRODUCTION

Neurophysins (1) are small single-chained (93-95 residues) proteins found in the neural lobe of the pituitary gland which give specific and reversible complexes with neurohypophysial hormones. Recent reviews have been devoted to the molecular and cellular aspects of neurophysins and neurohypophysial hormones, particularly to their possible common precursor (2, 3). All the mammalian species investigated to date (2, 3) have two types of neurophysins that can be identified by their amino acid sequence. It has been suggested to distinguish MSEL- and VLDV-neurophysins according to the nature of residues in positions 2, 3, 6 and 7 by using the one-letter symbols of amino acids located in these positions (Ref. 4).

Complete sequences of MSEL-neurophysins from 5 species, namely ox, sheep, pig, horse and whale (5), and of VLDV-neurophysins from 2

0006-291X/81/100600-06$01.00/0

species, namely ox and pig (6), have already been determined. We report now results obtained with horse VLDV-neurophysin, which confirm the existence of two phylogenetic lines in mammals.

MATERIALS AND METHODS

Purification of horse VLDV-neurophysin : purification has been carried out under the conditions previously described for ox and pig VLDV-neurophysins (6). Acetone-desiccated posterior pituitary powder is extracted by 0.1 M HCl for 4h at 4°C and the supernatant solution, adjusted to pH 4.0, is subjected to fractionated precipitation with 10% NaCl. The precipitate is dialysed, freeze-dried and dissolved in 0.1 M acetic acid (10 mg/ml). The pH is adjusted at 7.0 and precipitated material is removed by centrifuging. The supernatant solution is lyophilized, and the material, dissolved in 0.1 M formic acid (200 mg in 5 ml), is subjected to molecular sieving on a column (2.5 x 170 cm) of Sephadex G-75. The neurophysin fraction is then subjected to ion-exchange chromatography on a column (0.5 x 36 cm or 1.5 x 24 cm if the amount is above 50 mg) of DEAE-Sephadex A-50 equilibrated with 0.4 M pyridine acetate pH 5.9 . A pH gradient from 5.9 to 5.7 is applied and the peak of VLDV-neurophysin is collected. The homogeneity is checked by polyacrylamide gel electrophoresis at pH 9.5. 19 mg (about 2 µmol) of VLDV-neurophysin are recovered from 5 g of posterior pituitary acetonic powder.

Amino acid sequence : VLDV-neurophysin is oxidized by performic acid, split either with trypsin or with Staphylococcus aureus protease (7) and resulting peptides are separated by peptide mapping under conditions previously described (8). Peptides are analysed and amino acid sequences are determined by a manual Edman procedure (9), either directly or after cleavage by subtilisin, chymotrypsin and elastase, isolation of sub-peptides and determination of their sequences.

On the other hand, the purified protein is reduced by dithiothreitol, alkylated with iodoacetamide (10), cleaved by Staphylococcus aureus protease and peptides are separated in the same way. The alkylated protein is also subjected to automated degradation (11) in a SOCOSI model, P 110 sequencer. Phenylthiohydantoin amino acids are identified by thin-layer chromatography (12).

RESULTS AND DISCUSSION

Tryptic peptides (T_1 to T_6, Fig. 1) were recognized on the map by comparison with ox VLDV-neurophysin (6). The amino acid compositions and the sequences confirmed the homology. One substitution is observed in T_1 (residues 1-8), Ala in place of Val in position n° 2, and in T_2 (9-18) Lys in place of Thr in position n° 9 of the protein. T_3 (19-20), T_4 (21-42), T_5 (43-66) are identical with the corresponding peptides of bovine VLDV-neurophysin. Substitutions are more

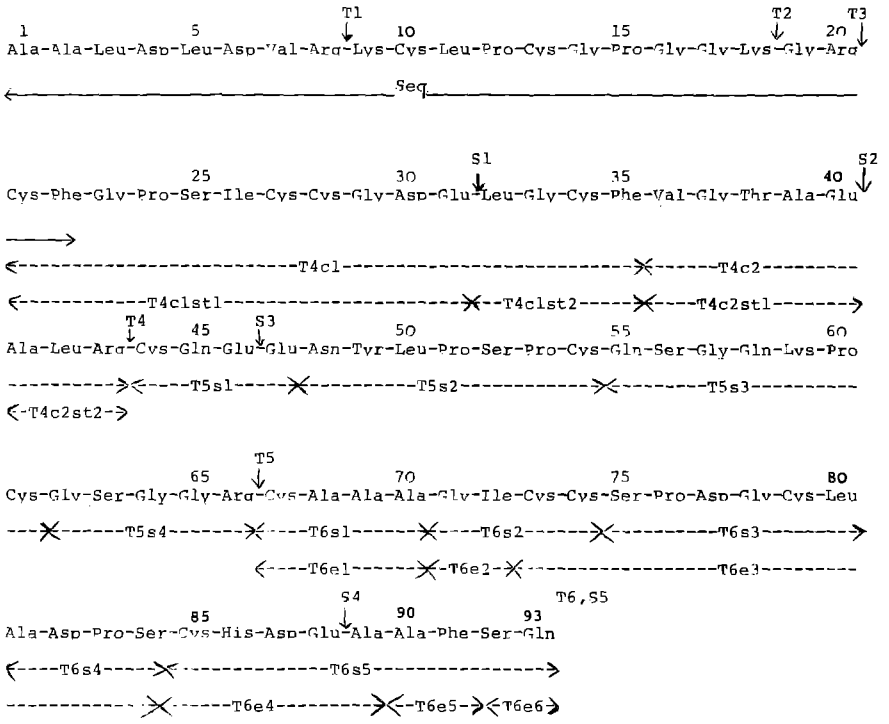

Fig. 1 Sequence of horse VLDV-neurophysin.
Tryptic (T) and Staphylococcus aureus protease (S) peptides are shown by arrows. Sub-peptides obtained by chymotrypsin (c), subtilisin (s), elastase (e), Staphylococcus protease (st) are indicated as T4c1, T5s1, T6e1, etc... and sub-sub-peptide as C4c1stl, etc... Seq. : Sequence determined by automated Edman degradation.

numerous in T_6 (67-93), the C-terminal peptide (5 substitutions in positions n° 80, 81, 84, 86 and 87).

Peptides produced by Staphylococcus protease (S_1 to S_5) are indicated in Fig. 1. They are used to confirm and to order tryptic peptides. S_1 (1-31) gives the alignment T_1 - T_2 - T_3 - T_4, S_3 (41-46) gives the alignment T_4 - T_5 and S_4 (47-88) gives the junction T_5-T_6. Expected cleavages were observed except for Glu-47, but Staphylococcus protease split between Glu-46 and Glu-47. N-terminal sequence was confirmed by automated degradation of the reduced-alkylated protein up to residue 22.

Comparison between porcine, bovine and equine VLDV-neurophysins is shown in Fig. 2. The three proteins have 93 residues. Substitutions are mainly located in the C-terminal part (80-93) of the chain.

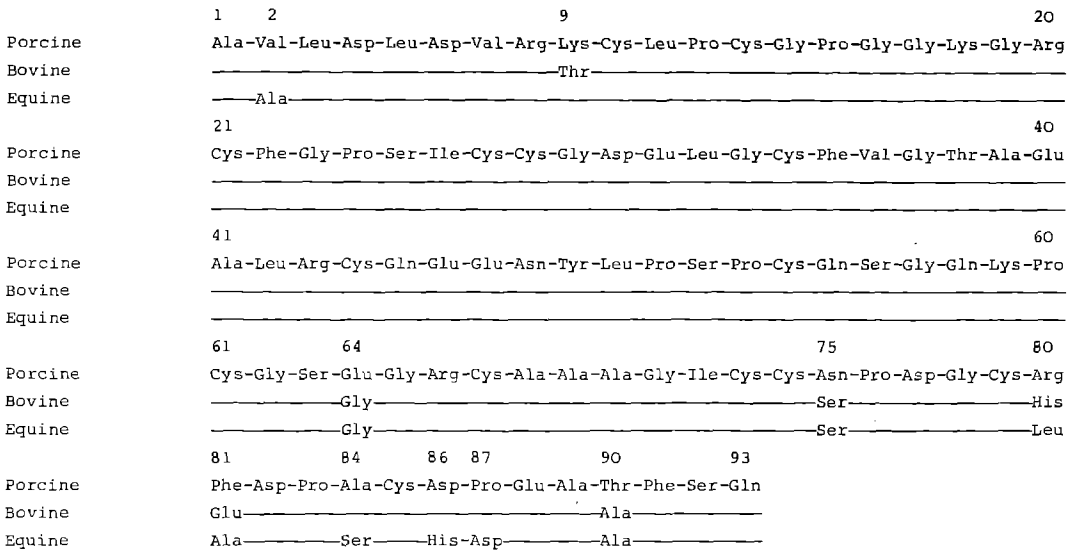

Fig. 2 Comparison of porcine, bovine (ref. 6) and horse VLDV-neurophysins. Solid lines indicate residues identical with those of porcine protein.

In horse protein, there is an alanine residue in position 2 instead of valine found in pig and ox VLDV-neurophysins. In position 9, both porcine and equine proteins have lysine instead of threonine found in bovine molecule, but in positions 64 and 75, both bovine and equine have glycine and serine, respectively, instead of glutamic acid and asparagine found in porcine VLDV-neurophysin. The comparison of horse and ox proteins reveals a difference in 7 amino acid residues, that of horse and pig proteins, a difference in 9 residues. On the other hand, ox and pig, which belong to the same mammalian order, Artiodactyla, differ by 6 substitutions. Apparently, the number of substitutions is greater in the VLDV-neurophysin family than in the MSEL-neurophysin family since in this latter, horse differs from ox and pig by 3 substitutions out of 95 residues (5).

In a given species, MSEL- and VLDV-neurophysins differ by 19-20 residues (20 for the horse). The variations are essentially located in N-terminal and C-terminal parts of the polypeptide chain. Fig. 3 shows the comparison between horse MSEL- and VLDV-neurophysins.

The amino acid sequence of a so-called neurophysin I of ox has been published (13). This neurophysin is composed of 93 residues and is identical with ox VLDV-neurophysin (6) except that the C-terminal

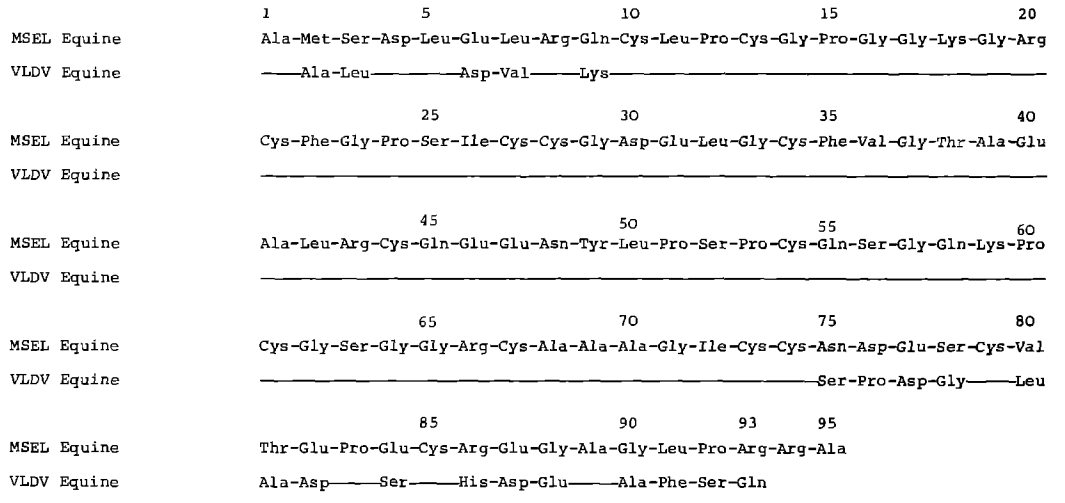

Fig. 3 Comparison between MSEL- and VLDV-neurophysin from the horse. Solid lines indicate residues identical with those of MSEL-neurophysin.

residue is Leu instead of Gln. However a sample of ox neurophysin-I purified by Dr. Hope (Dept. of Pharmacology, Oxford University) and subjected to sequential analysis has been found to have a C-terminal Gln as our VLDV-neurophysin (6). The three VLDV-neurophysins characterized to date have the same C-terminal Gln (Fig. 2). On the other hand the so-called pig neurophysin-I (14) corresponds to a truncated MSEL-neurophysin (15), pig neurophysin-II corresponds to VLDV-neurophysin and pig neurophysin-III to intact MSEL-neurophysin (6). It seems clearer for inter-species comparisons to use the chemical nomenclature (4) rather than the electrophoretic nomenclature which causes confusion because the number of components varies from species to species depending upon the number of truncated forms (6).

Each type of neurophysin shares a common precursor with a neurohypophysial hormone (16) and indirect data suggest that MSEL-neurophysin is bound to vasopressin and VLDV-neurophysin to oxytocin (2, 3, 17). It would be of interest to ascertain whether non-mammalian tetrapods, which have vasotocin and mesotocin instead of vasopressin and oxytocin (3) have also two types of neurophysins, one bound to vasotocin, the other to mesotocin.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs Danielle Thévenet and Miss Christine Jourdain for their skilled technical assistance. This

study was supported in part by grants from the C.N.R.S. (ERA n° 563), D.G.R.S.T. (n° 80-7-294), and the Fondation pour la Recherche Médicale.

REFERENCES

1. Acher, R., Manoussos, G. and Olivry, G. (1955) Biochem. Biophys. Acta 16, 155-156.
2. Acher, R. (1979) Angewandte Chemie, Int. Ed. 18, 846-860.
3. Acher, R. (1980) in "Neuroactive Peptides", Proc. Roy. Soc. B, 210, 21-43.
4. Chauvet, M.T., Chauvet, J. and Acher, R. (1975) FEBS Lett. 52, 212-215.
5. Chauvet, M.T., Codogno, P., Chauvet, J. and Acher, R. (1978) FEBS Lett. 88, 91-93.
6. Chauvet, M.T., Codogno, P., Chauvet, J. and Acher, R. (1979) FEBS Lett. 98, 34-40.
7. Houmard, J. and Drapeau, G.R. (1972) Proc. Natl. Acad. Sci. USA 69, 3506-3509.
8. Chauvet, M.T., Chauvet, J. and Acher, R. (1976) Europ. J. Biochem. 69, 475-485.
9. Chauvet, J.P. and Acher, R. (1972) Biochemistry 11, 916-926.
10. Crestfield, A.M., Moore, S. and Stein, W.H. (1963) J. Biol. Chem. 238, 622.
11. Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80-91.
12. Edman, P. and Henschen, A. (1975) in "Protein Sequence Determination" (Needleman, S.B. ed.) Springer-Verlag, Berlin, pp. 232-279.
13. Schlesinger, D.H., Audhya, T.K. and Walter, R. (1978) J. Biol. Chem. 253, 5019-5024.
14. Wuu, T.C., Crumm, S. and Saffran, M. (1971) J. Biol. Chem. 246, 6043-6063.
15. Chauvet, M.T., Codogno, P., Chauvet, J. and Acher, R. (1976) FEBS Lett. 71, 291-293.
16. Browstein, M.J., Russel, J.T. and Gainer, H. (1980) Science, N.Y. 207, 205-300.
17. Chauvet, M.T., Chauvet, J., Acher, R. and Robinson, A.G. (1979) FEBS Lett. 101, 391-394.