PHYLOGENY OF NEUROPHYSINS: PARTIAL AMINO ACID SEQUENCE OF A SHEEP NEUROPHYSIN

Marie-Thérèse CHAUVET, Jacqueline CHAUVET and Roger ACHER
Laboratory of Biological Chemistry, 96, Bd Raspail, 75006-Paris, France

Received 6 January 1975

1. Introduction

Neurophysins [1] are proteins which are apparently associated with neurohypophysial hormones in the posterior pituitary gland and stable protein-hormone complexes have been isolated [2,3]. The components can be separated and reassociated for giving a complex similar to the native one [3]. In the mammalian species so far investigated, several neurophysin components have often been disclosed by electrophoresis but the real number of native neurophysins can hardly be determined by this procedure because of the possible presence of partially degraded forms [4]. Up to now the complete or partial amino acid sequences of two bovine [5–7], two porcine [8,9] and one human [10,11] neurophysins have been determined.

Our research on sheep neurophysins has revealed that a major neurophysin (about 70% of crude neurophysin) is present in the gland (M. T. Chauvet, G. Coffe, J. Chauvet and R. Acher unpublished results). This report deals with the determination of the N-terminal sequence of the major ovine neurophysin.

2. Materials and methods

Sheep neurophysin-neurohypophysial hormone complex is purified from an acetone-desiccated posterior pituitary powder under conditions previously described by Chauvet et al. [3]. Dissociation is carried out by gel filtration on Sephadex G-25 in 0.2 M acetic acid according to Uttental and Hope [12]. Neurophysin material is then chromatographed on DEAE-Sephadex A-50 using a discontinuous gradient of 0.2–1 M pyridine acetate buffers pH 5.9 under conditions derived from those of Breslow et al. [13]. A single peak is eluted between 0.2 M and 0.4 M pyridine acetate. From 50 mg of crude neurophysin material, 33 mg of a protein are obtained. This protein appears homogeneous by electrophoresis either on cellulose acetate strips (pH 8.8) or on polyacrylamide gel (pH 9.5). N-terminal degradation shows a single amino acid sequence so that the protein can be regarded as proper for structural studies.

Mol. wt of sheep neurophysin, determined by amino acid composition [14] on the basis of a single methionine residue per mol, is near 10 000.

Sheep neurophysin is oxidized by performic acid, split by trypsin and resulting peptides are separated by peptide mapping under conditions previously described [15]. Tryptic peptides are analyzed and partial or complete amino acid sequences are determined by manual Edman procedure [16].

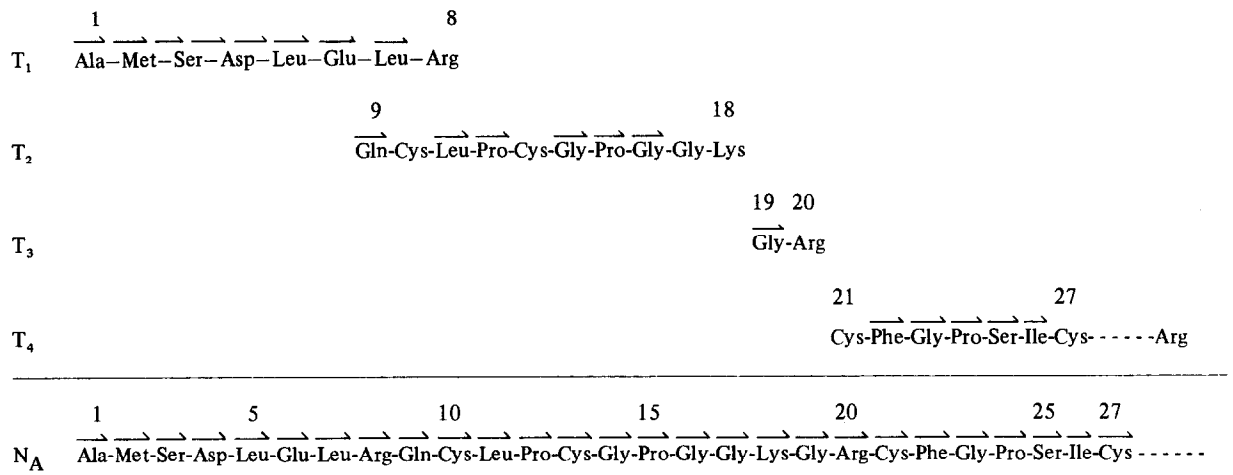
On the other hand, purified protein is reduced with 2-mercaptoethanol and alkylated with iodoacetamide [17]. The derivative is subjected to automated degradation according to Edman and Begg [18] in a SOCOSI model P 110 sequencer. Phenylthiohydantoïn (PTH) amino acids are identified by thin layer chromatography [19].

3. Results and discussion

Results obtained either with tryptic peptides or with alkylated neurophysin permit determination of the amino acid sequence of the first 27 residues of the molecule. There is good agreement between the data given by two procedures (table 1). On the other hand automated sequence analysis gave the alignment

North-Holland Publishing Company – Amsterdam

Table 1
Determination of the N-terminal sequence of ovine neurophysin



T: tryptic peptides of performic acid oxidized neurophysin; N_A: alkylated neurophysin.
→: Edman degradation, manual [16] on peptides and automated [18] on akylated neurophysin.

of the first 4 tryptic peptides of the polypeptide chain.

When ovine neurophysin is compared with other mammalian neurophysins known to date, the N-terminal sequence appears similar to that found for bovine Neurophysin II [5] and porcine Neurophysin I [8]. Apparently the N-terminal part of the molecule has been invariant in homologous proteins of three species of Artiodactyla. However the three proteins are not identical and variations are found in the C-terminal part. Curiously, the reverse is observed in the case of other proteins such as immunoglobulins [20].

In ox and pig, a second class of neurophysins has been found which differs from the first class by having 4 different residues in the N-terminal constant part. In positions 2,3,6 and 7, there are Val, Leu, Asp and Val, in place of Met, Ser, Glu and Leu, respectively. Therefore it might be more proper to distinguish provision

Table 2
Comparison between N-terminal sequences of mammalian neurophysins

Neurophysins	Sequence
MSEL-neurophysins	1 5 10 15 20 25 27
Bovine	Ala-Met-Ser-Asp-Leu-Glu-Leu-Arg-Gln-Cys-Leu-Pro-Cys-Gly-Pro-Gly-Gly-Lys-Gly-Arg-Cys-Phe-Gly-Pro-Ser-Ile-Cys
Porcine	— —
Ovine	— —
VLDV-neurophysins	1 27
Bovine	— Val-Leu — — Asp-Val —Thr —
Porcine	— Val-Leu — — Asp-Val —Lys —
Human	— Ala-Pro — — Asp-Val —Lys — — — — — — — — — — — — — — — — — — Asn— —

The dashes represent residues identical with those of bovine MSEL-neurophysin.

ally, on the basis of structural data, two classes of neurophysins (MSEL-neurophysins and VLDV-neurophysins according to the amino acids in positions 2,3,6 and 7) (table 2) rather than Neurophysins I, II, etc. . . according to the order of the electrophoretic migration towards the anode, which varies from species to species.

In the man, a single neurophysin (Neurophysin I) has so far been isolated and the N-terminal sequence has been determined [10,11]. For the first 27 residues, there are three substitutions in positions 2,3 and 25 when compared with bovine VLDV-neurophysin and six substitutions (2,3,6,7,9 and 25) when compared with MSEL-neurophysins. Furthermore the residue in position 3 (Pro) is hydrophobic as in VLDV-neurophysins and not polar as in MSEL-neurophysins. So human neurophysin I can be regarded as belonging to the first group (table 2).

In the sub-order Ruminantia, the genera Bos (ox) and Ovis (sheep) have diverged some 30 million years ago; in the order Artiodactyla, the two sub-orders Ruminantia and Suiformes (pig) have diverged at the beginning of Eocene times, about 60 million years ago [21]. Consequently ovine proteins are expected to be more similar to bovine than porcine proteins. It is of interest to note that ovine insulin, corticotropin and luteinizing hormone resemble bovine hormones and are relatively different from pig homologues [20]. So a similarity between ovine and bovine neurophysins is logical but complete sequences are necessary for comparison. The two orders of eutherian mammals, Artiodactyla and Primates (man), have separated about 80 million years ago and it is not unexpected to find human neurophysin relatively different from neurophysins of Artiodactyla. However a striking similarity between the amino acid compositions of cod and mammalian neurophysins has been observed [22] and therefore the rate of change of neurophysins in the course of evolution is not very high.

So far two neurophysins have been clearly identified in ox and pig and the question arises whether there is a stoichiometric relationship between the two neurohypophysial hormones, oxytocin and vasopressin, and these two major neurophysins. In the sheep pituitary gland, the amounts of oxytocin and arginine vasopressin, are approximately equal [3] but a major neurophysin accounts for 70% of crude neurophysin. This fact does not support the hypothesis of two protein precursors giving by cleavage a neurohypophysial hormone in one hand and a neurophysin in the other. Moreover in some species such as guinea pig only one neurophysin has been found so far [23] and the multiplicity of neurophysins may not be general.

Acknowledgements

The authors wish to thank Miss Marie-Hélène Simon and Miss Monique Bourdin for their skilled technical assistance. This study was supported in parts by grants from C.N.R.S. and I.N.S.E.R.M.

References

[1] Acher, R., Manoussos, G. and Olivry, O. (1955) Biochim. Biophys. Acta 16, 155–156.
[2] Acher, R., Chauvet, J. and Olivry, O. (1956) Biochim. Biophys. Acta 22, 421–427.
[3] Chauvet, J., Lenci, M. T. and Acher, R. (1960) Biochim. Biophys. Acta 38, 266–272.
[1] Dean, C. R. and Hope, D. B. (1967) Biochem, J. 104, 1082–1088.
[5] Walter, R., Schlesinger, D. H., Schwartz, I. L. and Capra, J. D. (1971) Biochem. Biophys. Research Commun. 44, 293–298.
[6] Capra, J. D., Kehoe, J. M., Kotelchuck, D., Walter, R. and Breslow, E. (1972) Proc. Natl. Acad. Sci. USA 69, 431–434.
[7] North, W. G., Walter, R., Schlesinger, D. H., Breslow, E. and Capra, J. D. (1974) International Conference on Neurophysin Proteins: Carriers of Peptide Hormones. in press.
[8] Wuu, T. C., Crumm, S. and Saffran, M. (1971) J. Biol. Chem. 246, 6043–6063.
[9] Wuu, T. C. and Crumm, S. (1973) 9th International Congress Biochem. Stockholm, Abstract Db 9.
[10] Foss, I., Sletten, K. and Trygstad, O. (1973) FEBS Letters 30, 151–156.
[11] Capra, D., Cheng, K. W., Friesen, H. G., North, W. G. and Walter, R. (1974) FEBS Letters 46, 71–74.
[12] Uttenthal, L. O. and Hope, D. B. (1970) Biochem. J. 116, 899–909.
[13] Breslow, E., Aanning, H. L., Abbrash, L. and Schmir, M. (1971) J. Biol. Chem. 246, 5179–5188.
[14] Spackmann, D. H., Stein, W. H. and Moore, S. (1958) Anal. Chem. 30, 1190–1206.
[15] Chauvet, J., Nouvel, G. and Acher, R. (1966) Biochim. Biophys. Acta 115, 130–140.
[16] Chauvet, J. P. and Acher, R. (1972) Biochemistry 11, 916–926.

[17] Crestfield, A. M., Moore, S. and Stein W. H. (1963) J. Biol. Chem. 238, 622.
[18] Edman, P. and Begg, G. (1967) Eur. J. Bioch. 1, 80–91.
[19] Bailey, J. L. (1967) Techniques in Protein Chemistry, Elsevier Publishing Co. Amsterdam.
[20] Dayhoff, M. O. (1972) Atlas of Protein Sequence and Structure, Vol. 5, The National Biomedical Research Foundation, Silver Spring, Md. USA.
[21] Romer, A. S. (1966) Vertebrate Paleontology. The University of Chicago Press, Chicago, Ill. USA.
[22] Pickering, B. T. (1968) J. Endocr. 42, 143–152.
[23] Sachs, H., Goodman, R., Shin, S., Shainberg, A. and Pearson, D., Excerpta Medica (in press) quoted by Breslow, E. (1974) Adv. in Enzymol. 40, 271–333.