A COMPARATIVE STUDY OF PARTIAL NEUROPHYSIN PROTEIN SEQUENCES OF COD, GUINEA PIG, RAT AND SHEEP

David H. SCHLESINGER, B. T. PICKERING*, W. B. WATKINS**, J. C. PEEK**, L. G. MOORE**, T. K. AUDHYA+ and Roderich WALTER+

*Endocrine Unit, Dept. of Medicine, Mass General Hospital, Boston, MD 02114; *Dept. of Anatomy, Univ. of Bristol, Bristol, U.K.; **Postgraduate School of Obstetrics and Gynaecology, Univ. of Auckland, Auckland, New Zealand; +Department of Physiology and Biophysics, Univ. of Illinois Med. Center, Chicago, IL 60612, USA*

Received 16 June 1977

1. Introduction

The neurohypophyseal hormones, vasopressin and oxytocin, are synthesized in mammals in separate neurosecretory cells of the magnocellular hypothalamo–hypophyseal neurosecretory system together with their respective neurophysin proteins [1–3]. Complete and partial amino acid sequences of neurophysin proteins from a few species have been investigated [4–10], in part, for the purpose of finding a possible structural correlation between the neurophysin and the particular neurohypophyseal hormone associated with in vivo. One of the early insights [5] from these studies of the primary structures of neurophysin proteins, i.e., a great variability of the NH_2-terminal 9 and about the 20 COOH-terminal residues in combination with a strongly conserved internal sequence, has been substantiated by subsequent work [7,11].

In this study the partial sequences of neurophysin-I and -II of sheep (sNP-I and sNP-II) have been determined and compared to the complete sequence of ovine neurophysin-III (sNP-III) [7,8]. Moreover, partial sequences for rat neurophysin-I and -II (rNP-I and -II), for major and minor guinea-pig neurophysin, and for cod neurophysin (cNP) are reported.

2. Material and methods

2.1. *Isolation and purification of neurophysins*

The sNP-I and sNP-II were isolated from whole sheep pituitaries using a method similar to that of Watkins [12]; sNp-II was further purified by Cellex-CM and DEAE-Sephadex chromatography following the method of Audhya and Walter [13]. The separation and isolation of rNP-I and -II on a preparative scale was achieved similarly and has been reported [9]. The crude 'major' gNP mixture [12], when subjected to further purification [13], gave an unresolved major and minor gNP mixture of 7:3 as determined by 'mixture analysis' (vide infra). It should be noted that these major and minor gNP components are both obtained in the 'major' gNP fraction of Watkins and Ellis [14] as well as Sachs et al. [15]. The cNP was isolated by and obtained from Pickering [16].

2.2. *Sequence analysis*

Automated sequence analyses were performed on intact neurophysins by the method of Edman and Begg [17] in which a double-cleavage Quadrol program was used. Samples of neurophysin proteins were dissolved in 0.05 N acetic acid and dried under high vacuum in the spinning cup. Coupling with phenylisothiocyanate was performed twice before initiating automated sequencing. For sequencing of cNP an 'inert carrier' approach was implimented since less than 15 nmol of protein was available. Acetylated, de-heamed myoglobin (4 mg) served as inert carrier, which was applied to the spinning cup of the sequencer in 300 μl of a solution of dimethylformamide/water (1:4) prior to application of the cNP sample. PTH amino acids derived from automated and manual

Edman degradations were determined by gas [18] and thin-layer chromatography [19].

3. Results and discussion

Purified sNP-I and sNP-II each were subjected to 36 cycles of automated Edman degradation and the resulting sequence data are presented in fig.1. Both sequences are identical to that of bovine NP-I [5,20] except that both sNP-I and sNP-II exhibited microheterogeneity in position 18 which contains threonine and lysine; the latter residue is found in bovine NP-I. Microheterogeneity has also been observed with bovine NP-II [6,7]. On the basis of the sequence identity, including the microheterogeneity in position 18 of sNP-I and -II, it appears likely that one of the two proteins is a metabolite of the other. Neurophysin proteins with truncated NH_2- and/or COOH-terminal sequences are known [10,21,22] and it appears that these can form not only in vitro but also in vivo [23–26].

The feasibility of sequence analysis of heterogeneous proteins and peptide mixtures, which are technically difficult or impossible to separate, had been suggested [27,28]. This technique has been successfully applied and discussed in connection with the report of the partial sequences of rNP-I and -II, initially determined by automated sequence analysis of an unresolved mixture of rNP-I and -II [9]. This 'mixture analysis' permitted a tentative assignment of most residues of the rNP-I and -II sequences since these proteins existed in an unequal molar distribution. Definitive sequence assignments for rNP-I and -II for the NH_2-terminal 33-residue sequence have been achieved by separate automated analyses on the chromatographically resolved rNP-I and rNP-II (fig.1).

While it has recently been possible on an analytical level to demonstrate that guinea-pig possesses more than one neurophysin [29,30], the proteins have not yet been separated on a preparative scale. Adopting the approach of a mixture analysis as used for rat neurophysins [9], it was not only possible to demonstrate the presence of a 7:3 ratio of two different neurophysins in the gNP mixture, but moreover, it allowed to determine the tentative sequences of both the major and minor gNP proteins for the first 26 residues. In almost all cases an assignment of residues to either the major or the minor gNP sequence was possible, except at positions 2 and 3 where a third residue appeared; nevertheless, the major residue at positions 2 and 3 was leucine and aspartic acid, respectively.

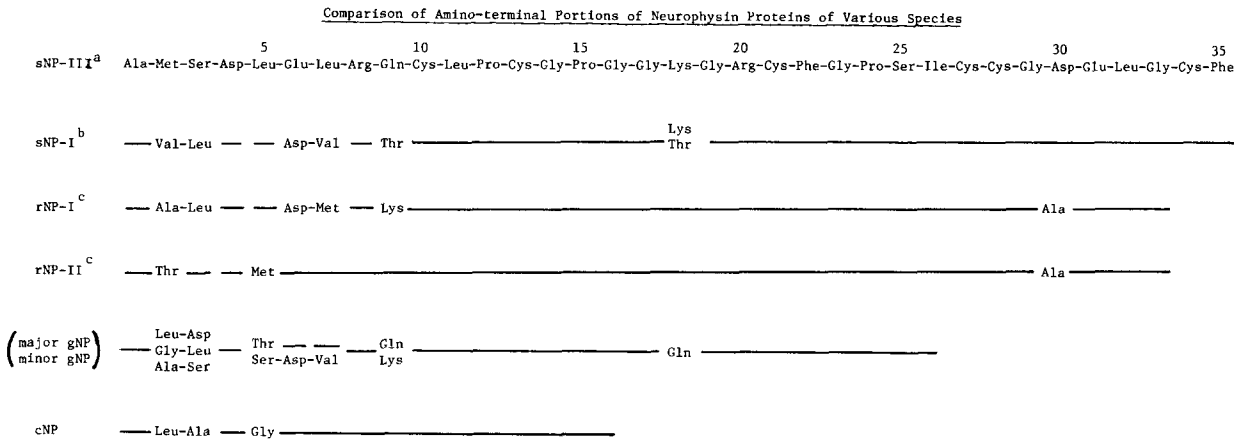

Fig.1. Abbreviations: sNP, sheep neurophysin; rNP, rat neurophysin; gNP, guinea pig neurophysin; cNP, cod neurophysin. Proteins are referred to as NP-I, -II, etc. on the basis of their electrophoretic mobilities on polyacrylamide gel electrophoresis at a running pH of 8.4; NP-I being the most acidic. (a) The complete sequence of sNP-III, reported in refs. [7] and [8], are identical except that Arg^{94} reported in ref. [7] has been confirmed. (b) sNP-I is identical to sNP-II for the NH_2-terminal 35 residues thus far determined, see text. (c) See ref. [9]. All sequence identities with sNP-III are indicated by a solid line.

Cod is the first non-mammalian species for which a partial neurophysin sequence has been reported. Comparison of the NH_2-terminal 16 residues of cNP with those of the mammalian species (fig.1) reveals that considerable variation occurs only in the terminal nonapeptide sequence, which is then followed by sequence identity with mammalian neurophysins. This general pattern has been found so far for all mammalian pituitary neurophysin proteins [5,7,11].

The data presented in this paper and in earlier reports [5,10] would indicate that a classification of neurophysin proteins on the basis of their NH_2-terminal amino acid sequence as suggested by Acher and his coworkers [7,31] may meet with difficulty.

Acknowledgements

This work was supported by contracts N01-CB-53868, N01-CB-64079, USPHS grant AM-18399 the Medical Research Council of New Zealand, and the Auckland Medical Research Foundation. We are grateful for the generous supply of rat posterior pituitary through Dr A. F. Parlow of the NIAMDD Rat Pituitary Hormone Program.

References

[1] Sokol, H. W., Zimmermann, E. A., Sawyer, W. H. and Robinson, A. G. (1976) Endocrinology 98, 1176–1188.
[2] Tasso, F., Picard, D. and Dreifuss, J. J. (1976) Nature 260, 621–622.
[3] Dierickx, K. and Vandesande, F. (1975) Cell Tiss. Res. 164, 153–162.
[4] Wuu, T. C., Crumm, S. E. and Saffran, H. (1971) J. Biol. Chem. 246, 6043–6063.
[5] Capra, J. D., Kehoe, J. M., Kotelchuck, D. and Walter, R. (1972) Proc. Natl. Acad. Sci. USA 69, 431–434.
[6] Wuu, T. C. and Crumm, S. E. (1976) Biochem. Biophys. Res. Commun. 68, 624–539.
[7] Chauvet, M. T., Chauvet, J. and Acher, R. (1976) Eur. J. Biochem. 69, 475–486.
[8] Schlesinger, D. H., Ernst, M., Nicholas, A., Watkins, W. B. and Walter, R. (1975) FEBS Letts. 57, 55–59; ibid. 58, 381.
[9] Schlesinger, D. H., Walter, R. and Audhya, T. K. (1976) in: Proceedings of the 1976 Conference of the Neurohypophysis (Moses, A. M. and Share, L. eds) Karger Press, Basel (in press).
[10] Walter, R., Audhya, T. K., Schlesinger, D. H., Shin, S., Saito, S. and Sachs, H. (1977) Endocrinology 100, 162–175.
[11] Capra, J. D. and Walter, R. (1975) Ann. N.Y. Acad. Sci. 248, 397–407.
[12] Watkins, W. B. (1973) J. Endocrinol. 59, 17–29.
[13] Audhya, T. K. and Walter, R. (1977) Arch. Biochem. Biophys. 180, 150.
[14] Watkins, W. B. and Ellis, H. K. (1973) J. Endocrinol. 59, 31–41.
[15] Sachs, H., Pearson, D. and Nureddin, A. (1975) Ann. N.Y. Acad. Sci. 248, 36–45.
[16] Pickering, B. T. (1968) J. Endocrinol. 42, 143–152.
[17] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–91.
[18] Pisano, J. J., Bronzert, T. J. (1969) J. Biol. Chem. 244, 5597–5607.
[19] Edman, P. (1970) in: Protein Sequence Determination: A Sourcebook of Methods and Techniques (Needleman, S. B., ed), pp. 211–255, Springer-Verlag, New York.
[20] North, W. G., Walter, R., Schlesinger, D. H., Breslow, E. and Capra, J. D. (1975) Ann. N.Y. Acad. Sci. 248, 408–422.
[21] Wuu, T. C. and Crumm, S. E. (1976) J. Biol. Chem. 251, 2735–2739.
[22] Breslow, E., Anning, H. L., Abrash, L. and Schmir, L. (1971) J. Biol. Chem. 246, 5179–5188.
[23] North, N. G., La Rochelle, F. T., Morris, J. F. and Valtin, H. (1976) in: Proceedings of the 1976 Conference of the Neurohypophysis (Moses, A. M. and Share, L. eds) Karger Press, Basel (in press).
[24] Hollenberg, M. D. and Hope, D. B. (1968) Biochem. J. 106, 557–564.
[25] Dean, C. R., Hope, D. B. and Kazic, T. (1968) Brit. J. Pharmacol. 34, 192 P.
[26] Vilhardt, H. and Robinson, I. C. A. F. (1975) J. Neurochem. 24, 1275–1276.
[27] Edman, P. (1960) Ann. N.Y. Acad. Sci. 88, 602–610.
[28] Gray, W. R. (1968) Nature 220, 1300–1304.
[29] See discussion to paper of Watkins, W. B. (1975) Ann N.Y. Acad. Sci. 248, 384–396.
[30] Thorn, N. A., Russell, J. T. and Sunde, D. (1977) in: Proceedings of the Fifth International Congress of Endocrinology, Hamburg, 1976 (James, V. H. T., ed) Excerpta Medica, Amsterdam, in press.
[31] Chauvet, M. T., Chauvet, J. and Acher, R. (1975) FEBS Lett. 52, 212–215.