A COMPARATIVE STUDY OF MAMMALIAN NEUROPHYSIN PROTEIN SEQUENCES

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1. Introduction

Numerous partial [1–5] and complete amino acid sequences [6–13] of the neurophysin proteins have been investigated in an attempt to correlate neurophysin structure with the neurohypophyseal hormones to which they bind in the neural lobe granules. Here, we report a tentative complete amino acid sequence of human neurophysin I (hNP-1) and the N-terminal sequences of hNP-II, ovine neurophysin I and II (oNP-I and II) and extend the guinea pig neurophysin I and II (gNP-I and II). The sequence data presented do not lend support to the generalization of two distinct classes of neurophysin proteins as determined by residues in positions 2,3,6 and 7 in the amino terminal domain [9]. Rather, we conclude that, in view of the high degree of hypervariability in the NH_2 and COOH domains of the neurophysins, no structural generalization can be formed yet relating the amino acid sequence to the hormones that they may bind in the neural lobe granules.

2. Materials and methods

2.1. *Human neurophysin I and II (hNP-I and II) purification*

The human posterior pituitary glands were extracted twice in 0.1 *N* HCl as in [14] and precipitated by slow saturation with 12.5% NaCl at pH 4.0. The precipitate after dialysis was subjected to column chromatography on Sephadex G-100 equilibrated with 0.1 N formic acid. The effluent was monitored by absorption at 220 nm and by radioimmunoassay of neurophysin using bovine neurophysin-II antisera. The immunoactive fractions were lyophilized and further purified on Sephadex G-25 and CM-cellulose column as in [14]. HNP-I was then dissociated from neurophysin-II by anion exchange chromatography on DEAE–Sephadex A-25 using a 0–0.35 M NaCl gradient in 30 mM Tris–HCl buffer (pH 8.1). Neurophysin-I was eluted at 0.31 M NaCl and neurophysin-II was eluted at 0.17 M NaCl. Both HNP-I and II were homogeneous by polyacylamide gel electrophoresis at pH 8.9 and 3.8, immunoelectrophoresis and SDS gel electrophoresis (with 15% gel). Both proteins were devoid of avian vasodepressor activity in the conscious chicken and did not effect electrolyte excretion in hydropenic rats.

The crude GNP mixture [15] was subjected to further purification as above. ONP was purified as in [16].

All neurophysin preparations were reduced and alkylated with reagents and conditions used for bNP-I [12] prior to automated sequence analysis. Reduced and alkylated hNP-I was digested with trypsin (1:100 E/S, 3 h at 37°C) and the resulting fragments purified on DEAE–Sephadex A-25 under conditions for bNP-I in [12].

2.2. *NH_2-terminal sequence analysis*

Automated sequence analyses were performed on reduced and alkylated neurophysins as in [17] using a Beckman Model 890 Sequencer 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. Manual phenylisothiocyanate degradations were performed on tryptic fragments on hNP-I, by the 3-stage method in [18] as modified [19]. Thiazolinone amino acids derived from automated and manual Edman degradations were continued to their more stable PTH amino

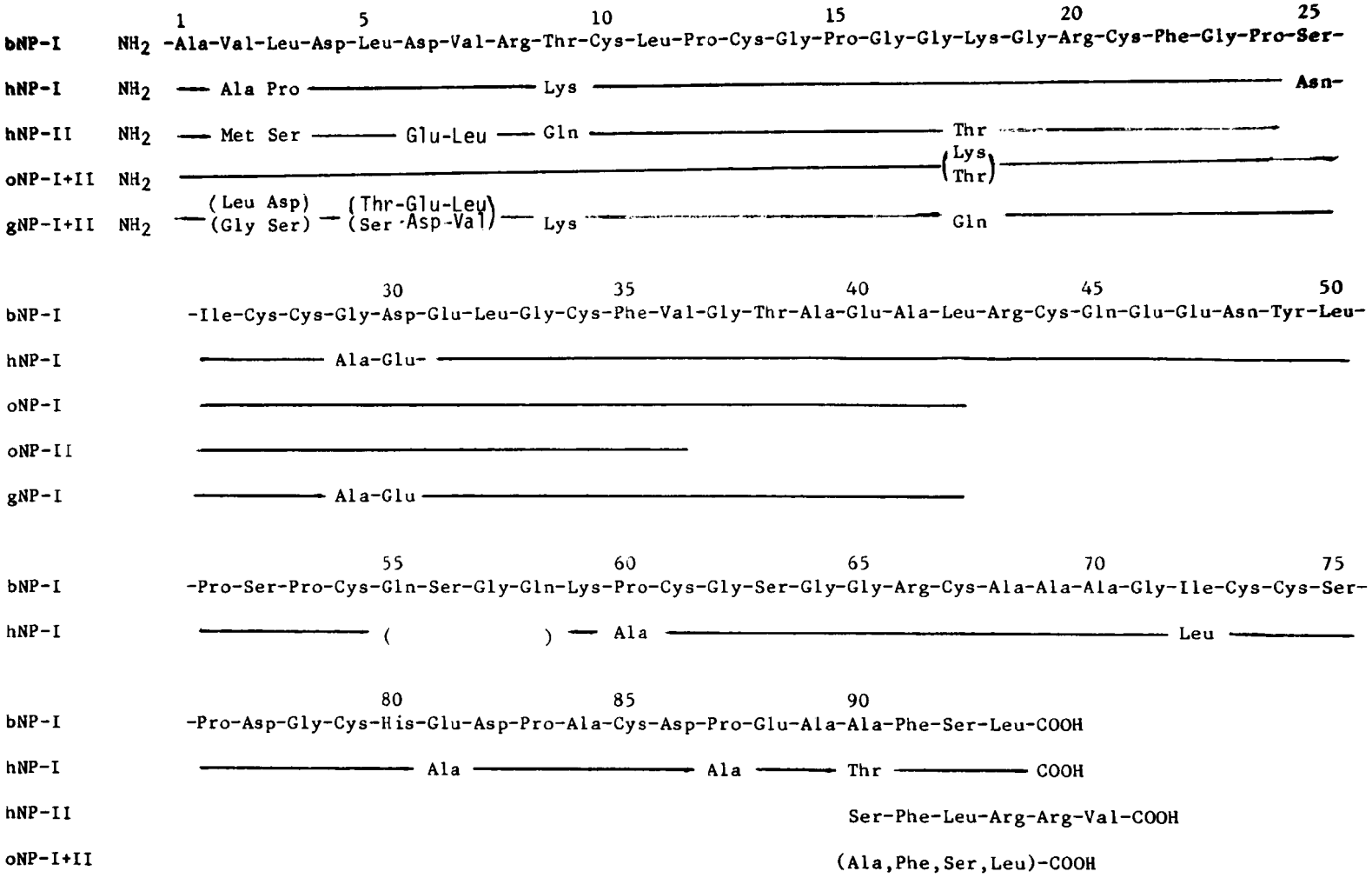

Fig.1. The amino acid sequences of hNP-I and II, oNP-I and II and gNP-I and II as compared to the complete sequence of bNP-I [12].

acids (1 N HCl, 10 min, 80°C) and identified by gas–liquid [20], thin-layer [21] and high-performance liquid chromatography [22,23].

2.3. *COOH-terminal analysis with carboxypeptidase*

Carboxy-terminal analysis of HNP-I and HNP-II was done as in [12] using carboxypeptidase-Y. Enzyme/substrate ratio was 1:200. COOH terminal analyses on oNP were performed using carboxypeptidases A and B from 5 min to 3 h as in [24].

3. Results and discussion

The N-terminal amino acid sequence for hNP-1–51 residues [2] was confirmed here. In addition, purification and sequence analysis of the tryptic peptides of alkylated and reduced hNP-1 resulted in the tentative complete amino acid sequence for this protein (fig.1). The sequence analysis of all tryptic peptides is complete with the exception of residues 55–58 and 67–70 in which no substitutions have yet been found in any neurophysin sequenced to date. Amino acid composition of our preparation and that reported in [25] indicate that hNP-1 is a 93 amino acid protein extremely similar to bNP-I, the first histidine-containing protein for which the complete sequence is known, but possesses a number of amino acid changes with bNP-I (positions 2,3,9,25,29,30,60,72,81,87 and 90).

hNP-II was purified along with hNP-I material by gel filtration and ion-exchange chromatography as above. The NH_2-terminal sequence of hNP-II was obtained through 24 residues and shows identify the amino-terminal sequence of bNP-II sequence except at position 18 in which a threonine is substituted for lysine. Carboxypeptidase Y digestion of native hNP-II reveals a C-terminal sequence of $-Ser_{90}-Phe-Leu-Arg-Arg-Val_{95}-COOH$, which is similar to that of bNP-II.

oNP-I to II were purified with oNP-III, for which the purification and complete sequence has been determined [8,9]. Automated sequence analyses on oNP-I (through 42 residues) and oNP-II (through 36 residues) indicate that these proteins share identity with the bNP-I [12] except at position 18 (fig.1) which reveals microheterogeneity (Thr for Lys), the latter residue being found in bNP-I at that position. Carboxypeptidase A + B digestion of reduced and alkylated oNP-I and II separately revealed that the C-COOH terminal tetrapeptide is composed of (Ala, Phe, Ser, Leu), the sequence of which has yet to be determined. Nevertheless these 4 residues constitute the COOH-terminal tetrapeptide of bNP-II so that one may predict that the tetrapeptide sequence of oNP-I and II may be –Ala–Phe–Ser–Leu–COOH.

A mixture of guinea pig neurophysins I and II was a generous gift of Dr Watkins who purified these proteins as in [15]. The mixture was sequenced through 42 residues and confirms and extends the sequence in [5]. Although two residues are seen at residues 2,3,5,6,7 and 9 the preparation becomes

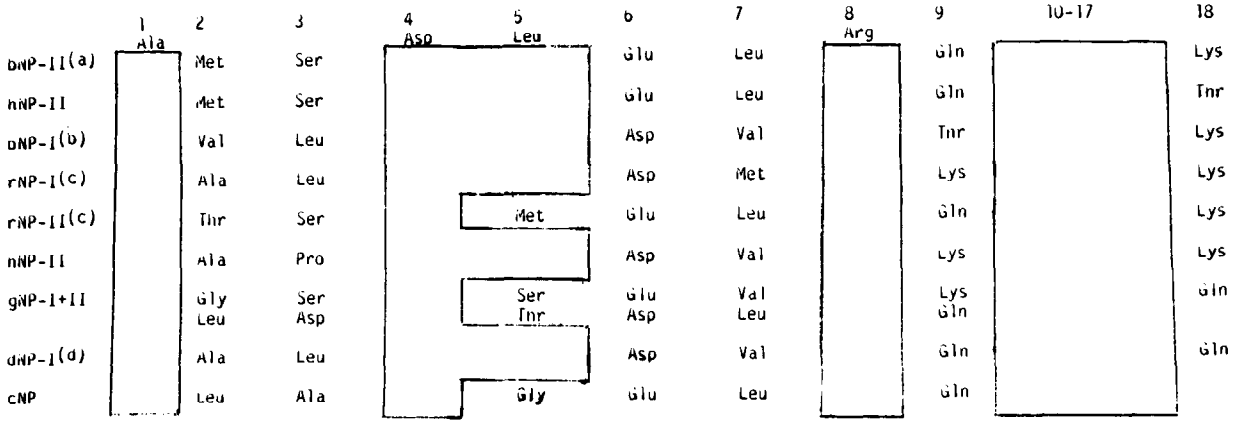

Fig.2. N-Terminal amino acid sequences of neurophysin proteins: (a) the N-terminal sequence is identical for porcine NP-I and III [10,11], dog NP-II [3], equine NP [13] and ovine NP-III [8,9] – regions within the box are invariant positions in all neurophysins studied to date; (b) The complete sequenced bNP-I [12]; (c) partial amino-acid sequences of rat neurophysin I and II [4]; (d) partial sequence of dog NP-I and II [3].

homogeneous from residues 10–42. (For relative merits of mixture analyses, see [26].) These sequences are interesting since they show unusual substitutions not only at positions 2,3,6,7 and 9 (a common feature of the neurophysins) but also substitutions at positions rather conserved in neurophysin previously studied. (The only observed substitution of the Leu at position 5 is in rat neurophysin II [4] and the only other reported substitution of Gln for Lys in position 18 is in dog NP-I [3].)

A comparison of NH_2-terminal sequence of neurophysin proteins from both mammalian and non-mammalian species indicate that positions 2,3,5,6,7,9 and 18 are hypervariable residues in which one of 6 residues can occupy position 2, one of 5 residues can occupy position 3, one of 5 in position 5, one of 2 in position 6, one of 3 in position 7, one of 3 in position 9 and one of 3 in position 18 in neurophysins studied to date (fig.2). These observations do not support the contention that the neurophysins can be divided into two classes (MSEL and VLDV) based solely on one of two residues occupying positions 2,3,6 and 7 [9]. Therefore, the classification of the neurophysins into two distinct structural groups, each biosynthetically related to oxytocin or vasopressin is not supported by this study. Rather, bNP-I and hNP-I seem closely related while bNP-II, oNP-III, pNP-I and III display little variability. This pattern supports the concept that in the evolution of the neurophysin proteins, gene duplication preceded species divergence, a phenomenon cited in [2], since neurophysins within the same species differ more greatly than do similar neurophysins from different species.

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References

[1] Capra, J. D., Kehoe, J. M., Kotelchuck, R., Walter, R. and Breslow, E. (1972) Proc. Natl. Acad. Sci. USA 69, 43.
[2] Capra, J. D., Cheng, K. W., Friesen, H. G., North, W. G. and Walter, R. (1974) FEBS Lett. 46, 71–74.
[3] Walter, R., Audhya, T. K., Schlesinger, D. H., Shin, S., Saito, S. and Sacks, H. (1977) Endocrinology 100, 162–174.
[4] Schlesinger, D. H., Walter, R. and Audhya, T. K. (1976) Conf. Neurohypophysis, International Society of Neuroendocrinology (Moses, A. M. and Share, L. eds) pp. 53–57, Karger, Basel.
[5] Schlesinger, D. H., Pickering, B. T., Watkins, W. B., Peck, J. C., Moore, L. G., Audhya, T. K. and Walter, R. (1977) FEBS Lett. 80, 371–373.
[6] Schlesinger, D. H., Capra, J. D. and Walter, R. (1974) Intl. J. Pept. Prot. Res. 6, 1.
[7] Wuu, T. C. and Crumm, S. E. (1976) Biochem. Biophys. Res. Commun. 68, 634–639.
[8] Schlesinger, D. H., Ernst, M., Nicholas, A., Watkins, W. B. and Walter, R. (1975) FEBS Lett. 57, 55.
[9] Chauvet, M. T., Chauvet, J. and Archer, R. (1975) FEBS Lett. 58, 234–237.
[10] Wuu, T. C., Crumm, S. E. and Saffran, M. (1971) J. Biol. Chem. 246, 6043.
[11] Wuu, T. C. and Crumm, S. E. (1976) J. Biol. Chem. 251, 2735.
[12] Schlesinger, D. H., Audhya, T. K. and Walter, R. (1978) J. Biol. Chem. 253, 5019–5024.
[13] Chauvet, M. T., Cadagno, P., Chauvet, J. and Acher, R. (1977) FEBS Lett. 80, 374–376.
[14] Audhya, T. K. and Walter, R. (1977) Arch. Biochem. Biophys. 180, 130–139.
[15] Watkins, W. B. (1973) J. Endocrinol. 59, 17.
[16] Schaechtelin, G., North, W. G. and Walter, R. (1975) Ann. NY Acad. Sci. 248, 365–377.
[17] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80.
[18] Edman, P. (1960) Ann. NY Acad. Sci. 88, 602.
[19] Boehnert, M. and Schlesinger, D. H. (1979) Anal. Biochem. 96, 464–473.
[20] Pisano, J. J. and Bronzert, T. J. (1969) J. Biol. Chem. 244, 5597.
[21] Edman, P. (1970) in: Protein Sequence Determination: A Sourcebook of Methods and Techniques (Needleman, S. B. ed) p. 211–255, Springer-Verlag, New York.
[22] Zimmerman, C. L., Appella, E. and Pisano, J. J. (1977) Anal. Biochem. 569–573.
[23] Simmons, J. and Schlesinger, D. H. (1980) Anal. Biochem. 30, 1190–1206.
[24] North, W. G., Walter, R., Schlesinger, D. H., Breslow, E. and Capra, J. D. (1975) Ann. NY Acad. Sci. 248, 408.
[25] Cheng, K. W. and Friesen, H. G. (1972) J. Clin. Endocrinol. 34, 165.
[26] Gray, W. R. (1968) Nature 220, 1300–1304.