The neurophysin domain of human vasopressin precursor

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1. INTRODUCTION

Bovine vasopressin precursor is composed of three domains, namely vasopressin, neurophysin II (MSEL-neurophysin) and a glycopeptide, which are cut out by intraneuronal processing [1]. In most placental mammals investigated, on the one hand two neurohypophysial hormones, arginine vasopressin and oxytocin have been identified [2,3], on the other hand two neurophysins, termed MSEL- and VLDV-neurophysins according to the nature of amino acids in positions 2,3,6 and 7, have been characterized [2,4]. In man, the two hormones have been isolated [5] using the neurophysin complex procedure [6] and the two neurophysins have been purified by polyacrylamide gel electrophoresis [7,8]. As a response to nicotine stimulation, arginine vasopressin and MSEL-neurophysin are selectively secreted in blood [7,8] and this co-secretion agrees with the presence of a common precursor similar to the one identified in ox. Furthermore a human neurohypophysial glycopeptide, homologous to the bovine glycopeptide [9], has recently been identified [10]. We report here the complete amino acid sequence of human MSEL-neurophysin, homologous to the neurophysin component of the bovine vasopressin precursor.

2. MATERIALS AND METHODS

Pituitary glands, placed in acetone at the time of post-mortem examination, were collected in various hospitals of Paris through France-Hypophyse. The posterior lobes were separated in the laboratory and pulverized. Four batches of 148, 112, 220 and 230 glands gave 10.5 g material. The third batch titrated at 0.25 U/mg of oxytocic activity and 0.30 U/mg of pressor activity, the fourth 0.34 U/mg of oxytocic activity and 0.36 U/mg of pressor activity.

Extraction, carried out on 1-g samples with 0.1 M HCl (50 ml/g), was followed by molecular sieving on Sephadex G-75 in 0.1 M formic acid and the crude neurophysin fraction was subjected to ion-exchange chromatography on DEAE–Sephadex A-50 using a stepwise ionic-strength gradient made with pyridine acetate (pH 5.9) under the conditions described for bovine neurophysins [11]. MSEL-neurophysin was recovered in the second peak of the fraction eluted over 0.15–0.30 M and VLDV-neurophysin in the third peak. A C-terminal truncated MSEL-neurophysin was found in the peak eluted over 0.4–0.6 M. In a typical experiment, 1 g acetonic posterior pituitary powder gave 33.5 mg crude neurophysins from which 2.7 mg ($\sim$ 300 nmol) of intact MSEL-neurophysin were isolated.

MSEL-neurophysin, intact or truncated, was oxidized by performic acid and split either by trypsin or by staphylococcal proteinase and the resulting peptides were separated by paper chromato-electrophoresis under the conditions in [11]. After elution with 0.25% acetic acid, peptides were hydrolyzed (6 M HCl in vacuo, 48 h, 105°C) and subjected to amino acid analysis in a Spinco model 120 B amino acid analyzer [12]. Amino acid sequences of the peptides were determined by a manual Edman degradation [13] either directly or after cleavage by subtilisin, chymotrypsin or staphylococcal proteinase, isolation of subfragments and determination of their sequences. The two overlapping sets of tryptic and staphylococcal proteinase peptides allowed us to determine the alignment (fig.1). On the other hand MSEL-neurophysin, reduced by dithiothreitol and alkylated with

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iodoacetamide [14] was subjected to automated Edman degradation [15] in a Socosi model P 110 sequencer under the conditions in [11]. Phenylthiohydantoins were identified by thin-layer chromatography [16] and by high-pressure liquid chromatography [17].

3. RESULTS AND DISCUSSION

The complete amino acid sequence of MSEL-neurophysin was deduced from the various results (fig.1). In the C-terminal truncated form, the last 4 residues were missing.

In fig.2 are shown MSEL-neurophysins characterized in ox [18], sheep [18], pig [19,20], horse [21], whale [22], rat [23] and man. In human MSEL-neurophysin, there is an apparent deletion of 2 residues and the number of residues is 93 instead of 95; the C-terminal tripeptide Arg–Arg–Ala found in pig, horse and whale is, however, present, so that residues usually in positions 91 and 92 are supposed deleted. The number of amino acid variations when compared with bovine MSEL-neurophysin [18] is 9 (7 substitutions and 2 deletions). There are 4 substitutions in the 'constant' region (10–74), namely in position 29 (Ala/Gly), 60

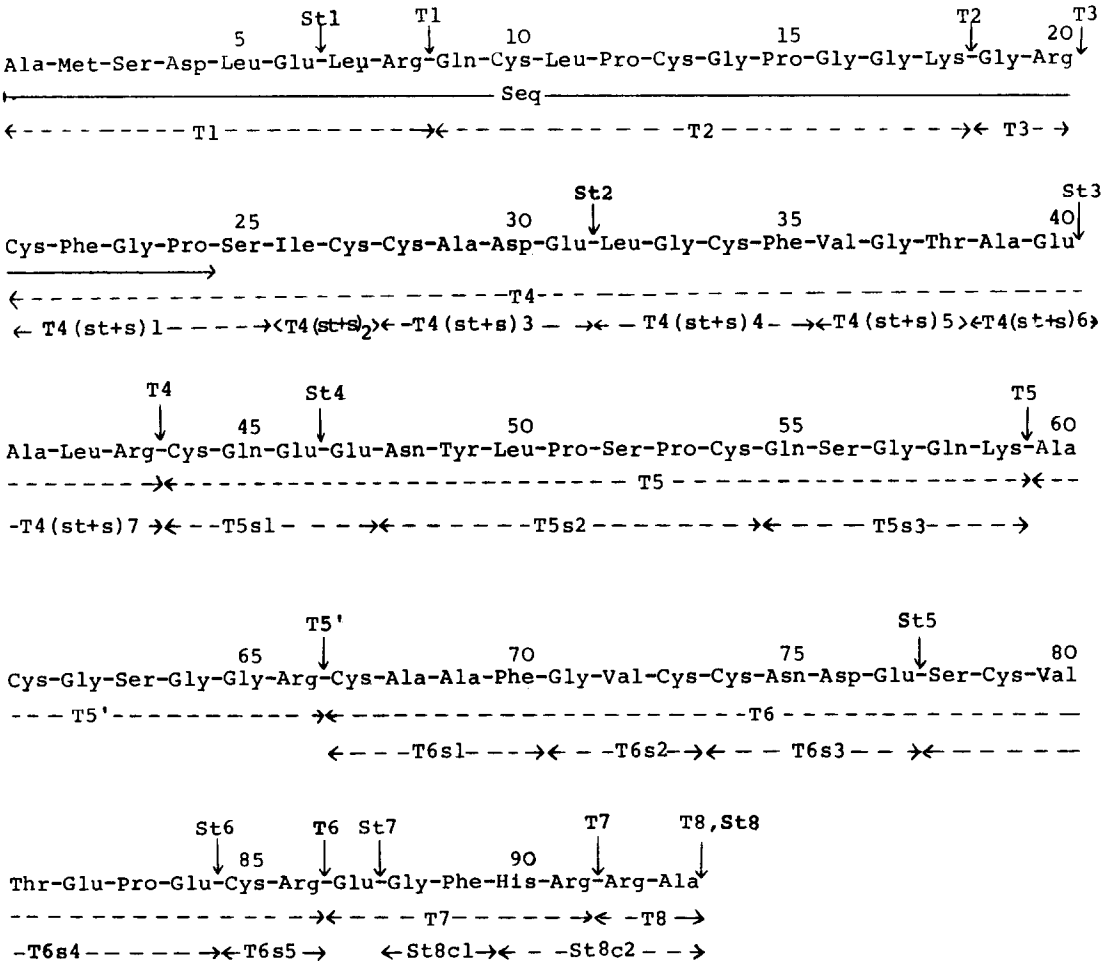

Fig.1. Amino acid sequence of human MSEL-neurophysin. Tryptic (T) and staphylococcal proteinase (St) peptides are shown by arrows. Subfragments obtained by subtilisin (s), chymotrypsin (c), staphylococcal proteinase (st), are indicated as T5sl, St8cl, etc. and those obtained by an enzyme mixture as T4(st + s)l, etc. Seq: sequence determined by automated Edman degradation.

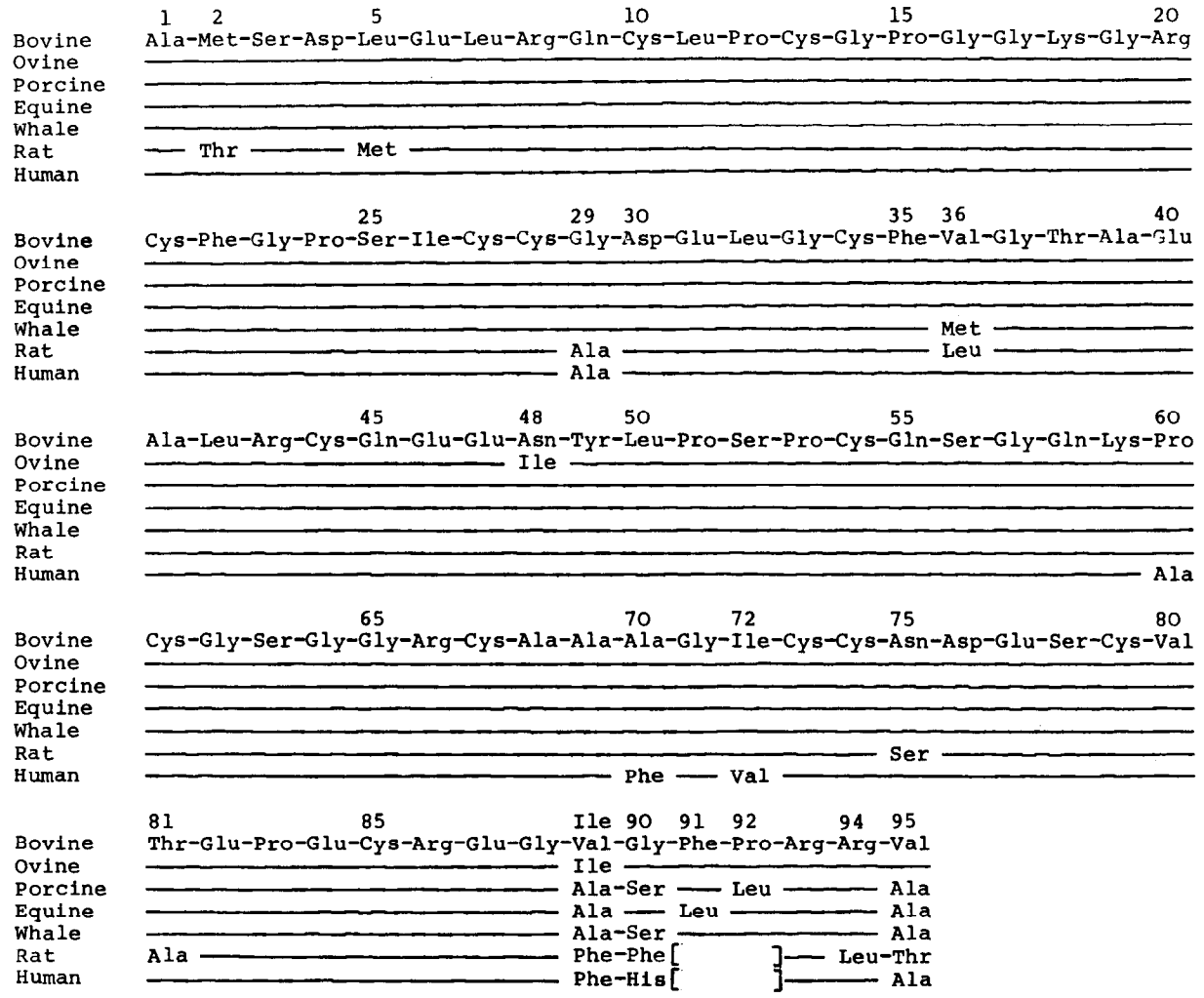

Fig.2. Comparison of bovine [18], ovine [18], porcine [19,20], equine [21], whale [22], rat [23] and human MSEL-neurophysins. Solid lines indicate residues identical with those of bovine protein. [ ] assumed deletion.

(Ala/Pro), 70 (Phe/Ala) and 72 (Val/Ile). In the 'hypervariable' sequence (89–95), there are 5 variations. Human MSEL-neurophysin appears more related to bovine MSEL-neurophysin that is the rat MSEL-neurophysin (12 variations), but of 7 mammals investigated, only human and rat MSEL-neurophysins have 93 residues.

A second human neurophysin, which can easily be classified in the VLDV-family by its N-terminal sequence, has also been purified. This type of neurophysin is co-secreted with oxytocin [7,24] and can be regarded as a fragment of the oxytocin macromolecular precursor [25].

A so-called human neurophysin II has recently been purified and its N-terminal (24 residues) and C-terminal (6 residues) sequences have been determined [26]. The N-terminal sequence resembles that of MSEL-neurophysin but threonine instead of lysine is found in position 18 and the C-terminal sequence is Ser–Phe–Leu–Arg–Arg–Val instead of Gly–Phe–His–Arg–Arg–Ala. The discrepancy for the C-terminal sequence cannot still be explained.

Arginine vasopressin was identified in man 25 years ago by qualitative amino acid composition [5]. The peptide fraction recovered from molecular

Table 1

Amino acid composition of human neurohypophysial hormones

Amino acid	Oxytocin			Arginine vasopressin		
	Reduced (15 nmol)	Oxidized (9 nmol)	Theoretical values	Reduced (10 nmol)	Oxidized (6 nmol)	Theoretical values
Lysine						
Histidine						
Arginine				1.02	1.03	(1)
Half-cystine[a]		1.05	(2)		1.10	(2)
Aspartic acid	1.00	1.00	(1)	1.00	1.00	(1)
Threonine						
Serine	0.11	0.10	–	0.13	0.17	–
Glutamic acid	1.00	1.08	(1)	1.15	1.21	(1)
Proline	1.05	1.00	(1)	1.02	1.14	(1)
Glycine	1.01	1.06	(1)	1.17	1.29	(1)
Alanine		0.08	–		0.16	–
Valine						
Methionine						
Isoleucine	1.04	0.94	(1)			
Leucine	1.08	1.07	(1)			
Tyrosine[a]	0.73		(1)	0.52		(1)
Phenylalanine				1.08	1.14	(1)

[a] Half-cystines are determined as cysteic acid on a separate performic acid-oxidized sample
A partial destruction of cysteic acid is observed when hydrolysis is carried out with paper-eluted peptide. Tyrosine, destroyed in the oxidized sample, is partially protected when hydrolysis is performed under reducing conditions (addition of mercaptoethanol)
Values are in molar ratios, using aspartic acid as reference

sieving of the extract on Sephadex G-75, has been passed on Biogel P_4 in order to separate the neurohypophysial hormones [27] and the pressor hormone has been purified by paper chromatoelectrophoresis under the conditions in [28]. The amino acid composition corresponds to that of arginine vasopressin (table 1).

The human glycopeptide homologous to the glycopeptide component of bovine vasopressin precursor has already been identified [10]. This peptide has 39 residues, 6 of which are substituted in man; it is located at the C-terminal end of the precursor [1,29]. The sizes of the 3 domains are therefore virtually identical in the 2 species, with only a 2-residue deletion in the neurophysin moiety. We may assume that a sequence Gly–(Lys/Arg)–(Lys/Arg), which usually extended in the precursor the C-terminal end of an amide peptide, is also present in the human precursor between arginine vasopressin and MSEL-neurophysin as in bovine precursor [1]. Because of the shortening of human MSEL-neurophysin, the interval between MSEL-neurophysin and the glycopeptide may not be limited to the single arginine found in the bovine precursor.

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REFERENCES

[1] Land, H., Schütz, G., Schmale, H. and Richter, D. (1982) Nature 295, 299–303.
[2] Acher, R. (1980) Proc. R. Soc. Lond. B 210, 21–43.
[3] Acher, R. (1981) Trends NeuroSci. 4, 225–229.
[4] Acher, R. (1979) Angew, Chem. Int. Ed. Engl. 18, 846–860.
[5] Light, A. and Du Vigneaud, V. (1958) Proc. Soc. Exptl. Biol. Med. 98, 692–696.
[6] Acher, R., Light, A. and Du Vigneaud, V. (1958) J. Biol. Chem. 233, 116–120.
[7] Robinson, A.G. (1975) J. Clin. Invest. 55, 360–367.
[8] Chauvet, M.T., Chauvet, J., Acher, R. and Robinson, A.G. (1979) FEBS Lett. 101, 391–394.
[9] Smyth, D.G. and Massey, D.E. (1979) Biochem. Biophys. Res. Commun. 87, 1006–1010.
[10] Seidah, N.G., Benjannet, S. and Chrétien, M. (1981) Biochem. Biophys. Res. Commun. 100, 901–907.
[11] Chauvet, M.T., Chauvet, J. and Acher, R. (1976) Eur. J. Biochem. 69, 475–485.
[12] Spackman, D.H., Stein, W.H. and Moore, S. (1958) Anal. Chem. 30, 1190–1206.
[13] Chauvet, J.P. and Acher, R. (1972) Biochemistry 11, 916–926.
[14] Crestfield, A.M., Moore, S. and Stein, W.H. (1963) J. Biol. Chem. 238, 622.
[15] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–91.
[16] Edman, P. and Henschen, A. (1975) in: Protein Sequence Determination (Needleman, S.B. ed) pp. 232–279, Springer-Verlag, Berlin, New York.
[17] Zeeuws, R. and Strosberg, A.D. (1978) FEBS Lett. 85, 68–72.
[18] Chauvet, M.T., Chauvet, J. and Acher, R. (1975) FEBS Lett. 58, 234–237.
[19] Chauvet, M.T., Codogno, P., Chauvet, J. and Acher, R. (1976) FEBS Lett. 71, 291–293.
[20] Wuu, T.C. and Crumm, S.E. (1976) J. Biol. Chem. 251, 2735–2739.
[21] Chauvet, M.T., Codogno, P., Chauvet, J. and Acher, R. (1977) FEBS Lett. 80, 374–376.
[22] Chauvet, M.T., Codogno, P., Chauvet, J. and Acher, R. (1978) FEBS Lett. 88, 91–93.
[23] Chauvet, M.T., Chauvet, J. and Acher R. (1981) Biochem. Biophys. Res. Commun. 103, 595–603.
[24] Amico, J.A., Seif, S.M. and Robinson, A.G. (1981) J. Clin. Endocrinol. Metab. 56, 1229–1232.
[25] Brownstein, M.J., Russell, J.T. and Gainer, H. (1980) Science 207, 373–378.
[26] Schlesinger, D.H. and Audhya, T.K. (1981) FEBS Lett. 128, 325–328.
[27] Chauvet, M.T., Hurpet, D., Chauvet, J. and Acher, R. (1980) Nature 287, 640–642.
[28] Acher, R., Chauvet, J. and Chauvet, M.T. (1970) Eur. J. Biochem. 17, 509–513.
[29] Brownstein, M.J., Russell, J.T. and Gainer, H. (1982) Front. Neuroendocrinol. in press.