The glycopeptide domain of the rat vasopressin precursor

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The vasopressin precursor is composed of 3 domains, namely vasopressin, MSEL-neurophysin and a glycopeptide. Processing occurs during axonal transport from hypothalamus to neurohypophysis from which the 3 fragments can be isolated. The glycopeptide fragment of the rat vasopressin precursor has been purified and sequenced. Despite the fact that rat MSEL-neurophysin is shortened (93 residues instead of 95 for other mammals), rat glycopeptide has 39 residues, as do the other mammalian glycopeptides, suggesting a similar processing. Fifteen substitutions are however observed when compared to ox glycopeptide. The C-terminal part of MSEL-neurophysin (residues 77–93) and the glycopeptide are encoded by the same exon and the homologies when compared with their bovine counterparts are 58% and 62% respectively. In contrast, the central part of rat MSEL-neurophysin (residues 10–76), which is encoded by a separate exon, displays 96% of homology; vasopressin and the N-terminal part of MSEL-neurophysin (residues 1–9), encoded by a third exon, are nearly invariant.

Neurohypophysial glycopeptide sequence *Vasopressin-neurophysin precursor*
Rat glycopeptide *Exon evolution*

1. INTRODUCTION

Mammalian vasopressin is a fragment of a 3-domain precursor that is usually processed into vasopressin (9 residues), MSEL-neurophysin (93–95 residues) and a glycopeptide (39 residues) [1–3]. Rat vasopressin has been identified as arginine vasopressin [4], current in most placental mammals. Rat MSEL-neurophysin however is somewhat different from other mammalian MSEL-neurophysins, having 93 residues instead of 95 because of an apparent C-terminal deletion [5]; furthermore, a single arginine is found instead of the penultimate Arg–Arg sequence typical of MSEL-neurophysins [6]. It is therefore of interest to characterize the glycopeptide in order to check whether the processing has been modified by these variations.

2. MATERIALS AND METHODS

Freeze-dried posterior pituitary lobes (740 mg, about 1800 glands) are extracted with 0.1 M HCl (25 mg/ml) and the supernatant is subjected to a molecular sieving on Sephadex G-75 under conditions described [5]. The fraction corresponding to 'crude' neurophysins (50 mg) is collected and chromatographed on a column (0.5 × 38 cm) of DEAE–Sephadex A-50 equilibrated with 0.4 M pyridine acetate pH 5.9 [5]. The glycopeptide filters through the column (8.6 mg) whereas neurophysins, retained, are eluted by an ionic strength gradient [5]. The glycopeptide, adsorbed at pH 7.4 on a column of concanavalin A, appears homogeneous. It is split either with trypsin [5] or with staphylococcal proteinase [7] and resulting peptides are separated by peptide mapping [8]. Peptides are analyzed [10] and amino acid sequences are determined by a manual Edman procedure [10] either directly or after cleavage by subtilisin, isolation of sub-fragments and determination of their sequences. Phenylthiohydantoin amino acids are identified by thin-layer chromatography [11].

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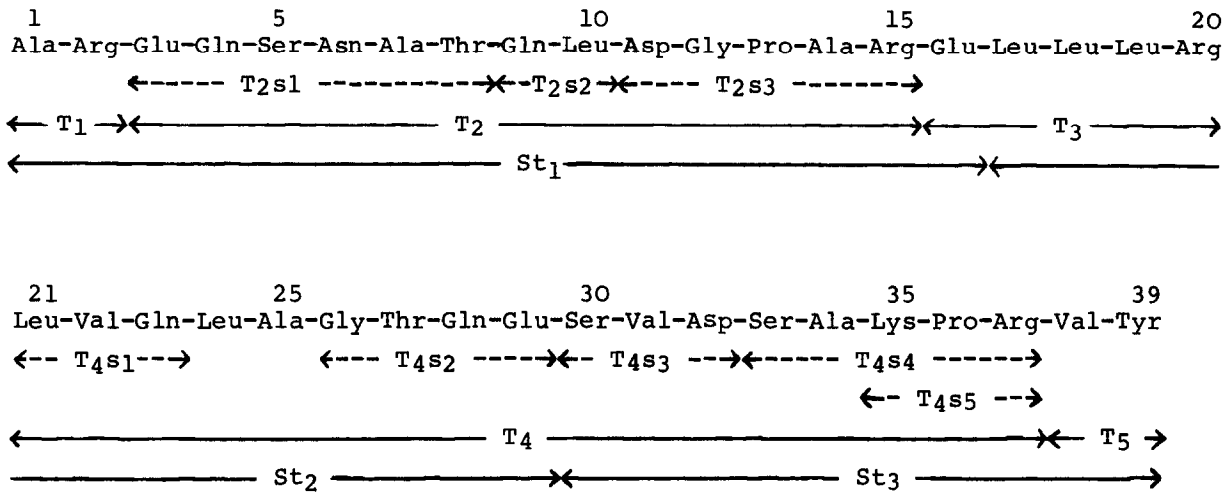

Fig.1. Amino acid sequence of rat glycopeptide. Tryptic peptides (T_1–T_5) are sequenced either directly or through subtilisic subfragments (T_2s_1, etc....). Overlapping staphylococcal proteinase peptides ($St_1$to St_3) give the alignment of the tryptic peptides.

Table 1

Amino acid compositions of tryptic peptides of rat glycopeptide

	T_1 (28 nmol)		T_2 (6.3 nmol)		T_3 (3.6 nmol)		T_4 (4.4 nmol)		T_5 (20 nmol)	
	Analysis	Sequence	Analysis	Sequence	Analysis	Sequence	Analysis	Sequence	Analysis	Sequence
Lys							1.00	(1)		
His										
Arg	1.00	(1)	1.00	(1)	1.00	(1)	1.02	(1)		
Asp			1.98	(2)			1.18	(1)		
Thr			0.89	(1)			1.02	(1)		
Ser			1.16	(1)			2.16	(2)		
Glu			2.87	(3)	0.75	(1)	3.23	(3)		
Pro			1.15	(1)			1.00	(1)		
Gly			1.20	(1)			1.34	(1)		
Ala	0.62	(1)	2.28	(2)			2.45	(2)		
1/2 Cys										
Val							2.18	(2)	1.00	(1)
Met										
Ile										
Leu			1.13	(1)	2.60	(3)	1.84	(2)		
Tyr									0.50	(1)
Phe										
$CysO_3H$										
Glucosamine[a]			2.91							
Number of residues		2		13		5		17		2
Location in the sequence		1–2		3–15		16–20		21–37		38–39

[a] Uncorrected for destruction

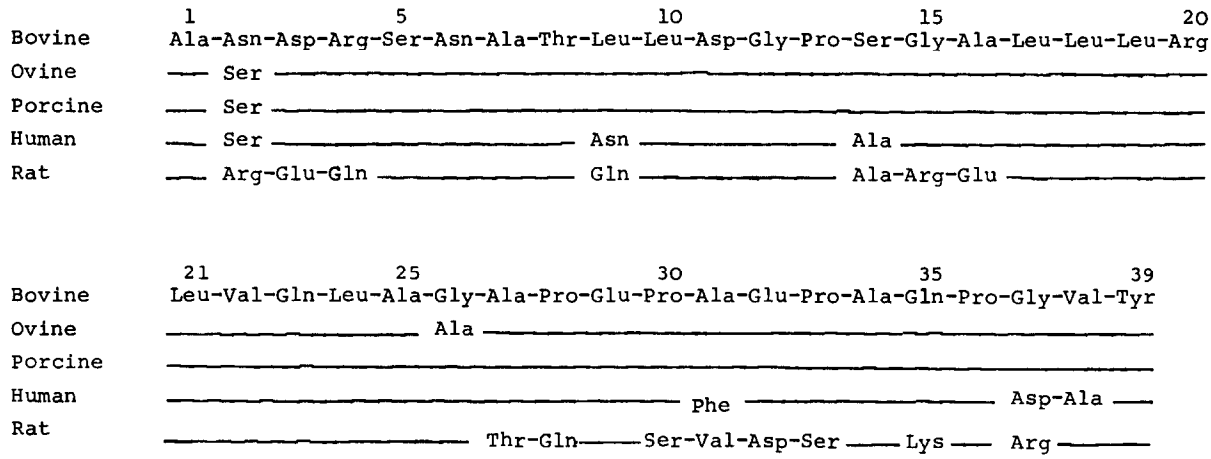

Fig.2. Comparison of bovine [13], ovine [13], porcine [13], human [14] and rat (here, [15]) glycopeptides. Solid lines indicate residues identical with those of bovine glycopeptide.

3. RESULTS

Five tryptic peptides (T_1–T_5) are isolated and analyzed. Amino acid compositions are given in table 1. Peptide T_5 has been detected by a staining reaction specific for tyrosine [12]. Tryptic peptides are sequenced either directly or through subtilisic peptides derived from T_2 (T_2s_1, etc....) and T_4 (T_4s_1, etc....). Amino acid sequences are given in fig.2. The alignment of the tryptic peptides is determined with 3 overlapping peptides (St_1 to St_3) obtained by hydrolysis with staphylococcal proteinase.

4. DISCUSSION

Glycopeptide from vasopressin precursors of ox, sheep, pig [13] and man [14] have already been characterized. All of them, as rat glycopeptide, have 39 residues and display a great homology (fig.2). The MSEL-neurophysins of these species have 95 residues except those of rat and man, which have only 93 residues [6]. In bovine vasopressin precursor, an arginyl residue separates MSEL-neurophysin from glycopeptide [2] and it is assumed that cleavage of the precursor by a trypsin-like enzyme occurs after this arginine, which is subsequently removed from MSEL-neurophysin moiety by a carboxypeptidase B.

Recently the gene for the rat vasopressin-neurophysin precursor has been isolated and the precursor amino acid sequence has been deduced [15]. It appears that the rat vasopressin precursor includes the arginine vasopressin and the MSEL-neurophysin identified in [4,5], the two domains being separated by a sequence Gly–Lys–Arg, usually involved in the mechanism of C-terminal amide formation [16]. On the other hand, MSEL-neurophysin is separated from the glycopeptide by a single arginine as in the bovine vasopressin precursor despite the shortening of rat MSEL-neurophysin. The sequence deduced for the glycopeptide [15] is in complete agreement with that determined directly on the 39-residue fragment.

When compared with bovine vasopressin precursor, rat vasopressin precursor displays 11 substitutions and 2 deletions in the MSEL-neurophysin moiety (about 86% of homology) and 15 substitutions in the glycopeptide moiety (about 62% of homology). However the hyper-variable C-terminal part of MSEL-neurophysin (residues 77–93) and the glycopeptide are encoded by the same exon C [15] and the homology in this part of MSEL-neurophysin (58%) is very similar to that found for the glycopeptide. The 3 exons of the vasopressin gene [15] seem to have different evolutionary drifts since exon B that encodes the central part of MSEL-neurophysin (residues 10–76), is nearly invariant and exon A that encodes the signal peptide, arginine vasopressin and the N-terminal sequence of MSEL-neurophysin (residue 1–9)

displays a few variations in its first and third parts [6,15], but very rarely in the hormonal part [17]. The substitutions observed in the vasopressin precursor are hardly explained by selective pressure but seem rather relevant to the neutral drift [18].

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