Guinea pig copeptin

The glycopeptide domain of the vasopressin precursor

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The vasopressin precursor is composed of 3 domains in line, namely vasopressin, MSEL-neurophysin and a glycopeptide referred to as copeptin, which are separated during the processing. In guinea pig neurohypophysis, the precursor is partially processed so that a two-domain fragment, MSEL-neurophysin – copeptin, can be found along with free MSEL-neurophysin and copeptin. Guinea pig copeptin has been sequenced. It is a glycopeptide composed of 38 amino acid residues rather than the 39 found in other mammalian copeptins. Compared with other copeptins, that from guinea pig shows a few substitutions and the deletion of one acidic residue, probably in position 32. This deletion might be responsible for incomplete cleavage by the trypsin-like processing enzyme.

Vasopressin-neurophysin precursor *Neurohypophysial glycopeptide* *(Guinea pig)* *Copeptin* *Processing enzyme*

1. INTRODUCTION

Vasopressin protein precursor has been shown to be constituted of 3 domains, namely vasopressin (9 residues), MSEL-neurophysin (93/95 residues) and a glycopeptide (39 residues) here referred to as copeptin [1–3]. Processing of this precursor, likely through two distinct enzymic systems, occurs during axonic transport from hypothalamus to neurohypophysis, and leads usually to active C-terminal amidated vasopressin, MSEL-neurophysin and copeptin (review [4]). In guinea pig posterior pituitary, however, a partially processed fragment containing MSEL-neurophysin linked to copeptin by a single arginine residue has been recently discovered [5]. Copeptin has been isolated from a number of mammalian species [5–8] including bovine foetus [9] and in these species, maturation of the precursor is apparently complete. It is therefore of interest to characterize guinea pig copeptin in order to explain the particular incomplete processing.

2. MATERIALS AND METHODS

Frozen posterior pituitary glands were homogenized with 0.1 M HCl (7.5 ml for 100 glands) and extracted at 4°C for 4 h. After centrifugation (10000 rpm, 4°C, 30 min) the supernatant solution was passed through a column (2 × 110 cm) of Sephadex G-75 equilibrated with 0.1 M formic acid. Fractions (1.2 ml) were collected at a rate of 6 ml/h and the absorbance monitored at 280 nm. Materials corresponding to separate peaks were pooled and freeze-dried. In fractions 225–250 a glycopeptide (5.4 mg) was recovered.

The glycopeptide was subjected to trypsin, chymotrypsin and pepsin hydrolyses under the conditions for neurophysin characterization described in [10]. The resulting peptides were separated by paper chromatoelectrophoresis [10], analyzed [11] and sequenced through manual Edman degradation [12]. Large tryptic peptides were submitted to cleavage by subtilisin and subfragments purified and characterized using the

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same techniques. The amino acid sequence of tryptic peptide T_3 (18 residues) has been confirmed using 1 nmol peptide in a gas-phase protein sequencer (Applied Biosystems) under the conditions described by Hewick et al. [13].

3. RESULTS

Three tryptic peptides (T_1–T_3) have been isolated, analyzed (table 1) and sequenced either directly (T_1) or through subtilisic peptides (T_2S_1, etc.). Three chymotryptic peptides (C_1–C_3) and 5 pepsic peptides (P_1–P_5) have been purified through paper chromatoelectrophoresis, analyzed and subjected to Edman sequencing. Alignment of the tryptic peptides has been determined with overlapping chymotryptic or pepsic peptides (fig.1).

Copeptin contains carbohydrates as revealed by periodic acid-Schiff staining carried out after electrophoresis on polyacrylamide gel [14]. This oligosaccharide has been located on Asn^6. The tryptic peptide T_2 contains glucosamine (cf. table 1) as well as its subtilisic sub-peptide T_2S_1. When Edman degradation is performed on this latter tetrapeptide, no phenylthiohydantoin is recognized in position 2 but the presence of aspartyl or asparaginyl is deduced from the amino acid composition.

On the other hand, the amino acid sequence Asn-Val-Thr is compatible with that required for the linking of a polysaccharide on an asparaginyl residue. Alkali treatment (0.5 M NaOH, 48 h, 5°C) of the tryptic peptide T_2, followed by 6 M HCl hydrolysis for amino acid determination, does not

Table 1

Amino acid compositions of tryptic peptides of guinea pig copeptin (residues per mol peptide)

	T1		T2		T3	
	Analysis (11.8 nmol)	Found in the sequence	Analysis (10.8 nmol)	Found in the sequence	Analysis (3.6 nmol)	Found in the sequence
Lys						
His						
Arg	1.00	(1)	1.00	(1)		
Asp	1.09	(1)	1.92	(2)		
Thr			0.92	(1)		
Ser			1.09	(1)	0.43	–
Glu			1.27	(1)	3.02	(3)
Pro			1.11	(1)	3.85	(4)
Gly	1.20	(1)	2.34	(2)	3.24	(3)
Ala	0.81	(1)	2.15	(2)	4.00	(4)
1/2 Cys						
Val			1.16	(1)		
Met					0.82	(1)
Ile						
Leu			2.96	(4)	1.73	(2)
Tyr					0.61	(1)
Phe						
$CysO_3H$						
Glucosamine[a]			1.88			
Number of residues		4		16		18
Location in the sequence		1–4		5–20		21–38

[a] Uncorrected for destruction

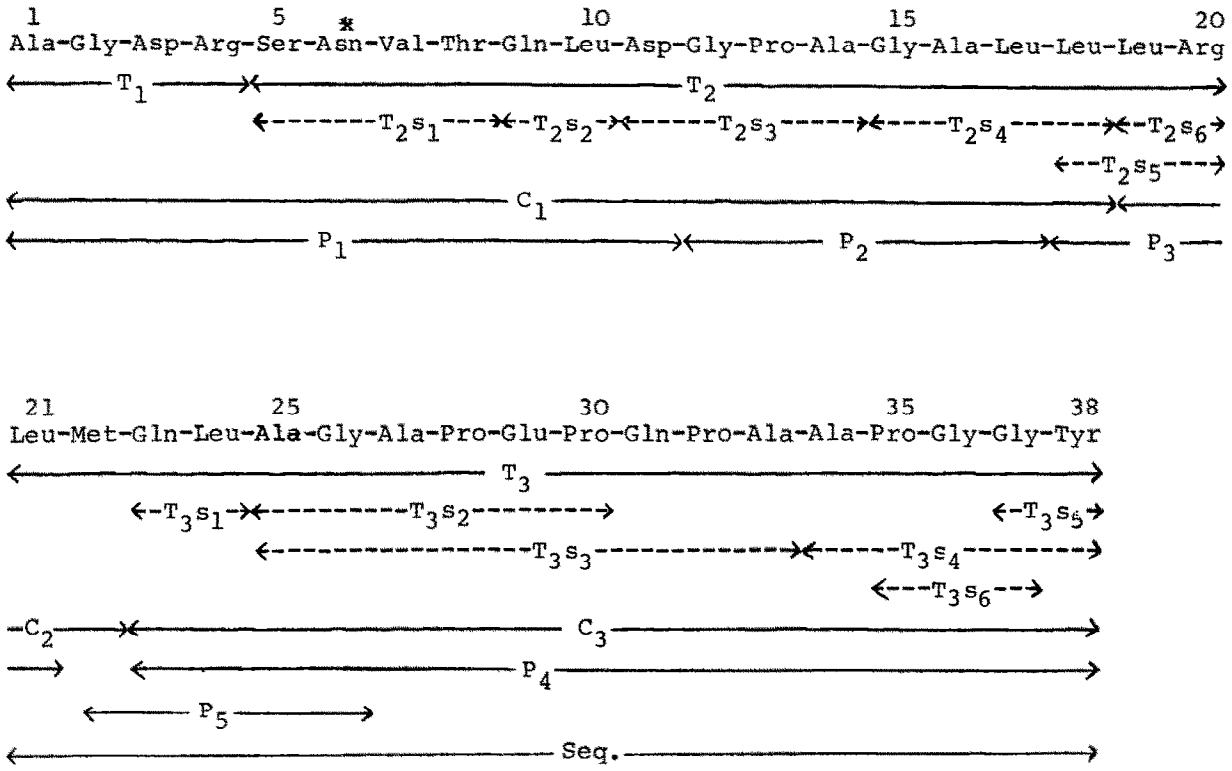

Fig. 1. Amino acid sequence of guinea pig copeptin. Tryptic peptides (T_1–T_3) are sequenced either directly or through subtilisic subfragments (T_2s_1, etc.). Overlapping chymotrypsic (C_1–C_3) and pepsic (P_1–P_5) peptides give the alignment of tryptic peptides. Seq., automated sequencing.

show destruction of serine or threonine as should occur if a polysaccharide were attached via an *O*-glycosidic bond to these residues [15]. Likewise, the carbohydrate has been located on Asn^6 in copeptins from other mammalian species [5–8].

4. DISCUSSION

When guinea pig copeptin is compared with the other known copeptins, a high homology can be noted (fig.2). Eight substitutions and one deletion are observed when comparison is made with bovine copeptin [6,9]. Some positions in copeptin, such as 2, 9, 14, 31, 35, 37 and 38, have been substituted rather frequently. The deletion seems to be located in position 32 and concerns an acidic residue. This might explain the incomplete processing of the precursor since about 20% of copeptin is found linked with MSEL-neurophysin through an arginine residue [5]. Processing involves the successive actions of a trypsin-like enzyme and a carboxypeptidase B so that free MSEL-neurophysin is recovered without a C-terminal arginine [5].

Copeptin and the C-terminal portion of MSEL-neurophysin are encoded by a common exon in rat [1] and ox [16]. Substitutions are rather frequent in this C-terminal part in contrast to the N-terminal and central regions encoded by two separate exons, respectively [1,16]. Cleavage between MSEL-neurophysin and copeptin seems to involve an enzymic system distinct from that splitting between vasopressin and MSEL-neurophysin. Deficiency in the first, due to evolutionary variation, apparently does not affect the second since only free vasopressin is found in the posterior pituitary gland of guinea pig.

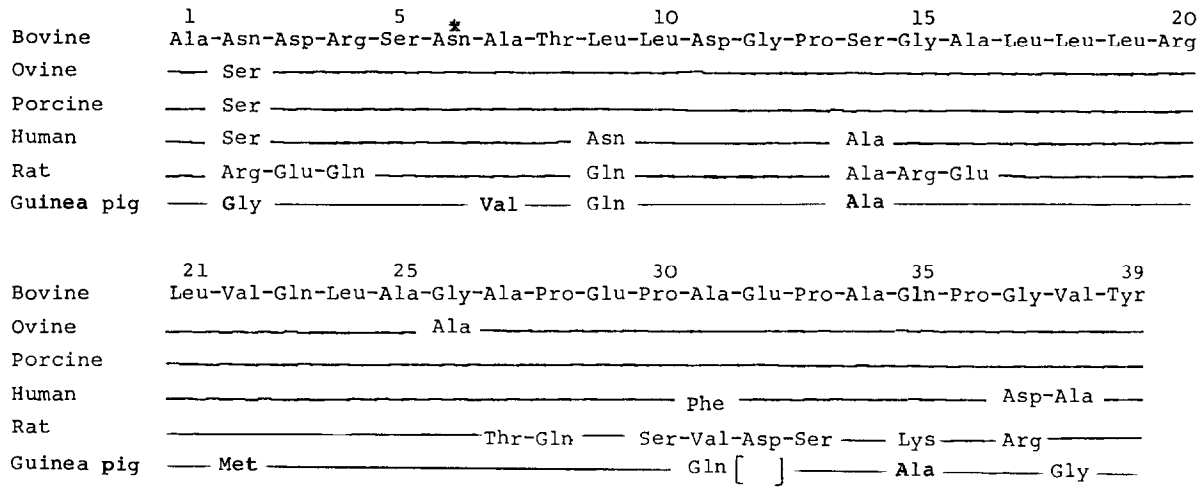

Fig. 2. Comparison of bovine [6], ovine [6], porcine [6], human [7], rat [8] and guinea pig (this work) copeptins. Solid lines, residues identical with those of bovine copeptin; [ ], deletion in guinea pig copeptin; Asn, asparagine linked by an *N*-glycosidic bond to an oligosaccharide.

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