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Guinea pig neurohypophysial hormones

Peculiar processing of the three-domain vasopressin precursor

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Guinea pig neurohypophysial hormones have been purified by two procedures, one involving molecular sieving and paper chromatoelectrophoresis, the other high-pressure reverse-phase liquid chromatography. Arginine vasopressin and oxytocin have been identified by their amino acid compositions and their retention times in HPLC determined through their biological properties. No partially processed precursor, including a neurohormone and a neurophysin, has been detected. Because the cleavage of the three-domain vasopressin-neurophysin-copeptin precursor is apparently complete between the first two domains, whereas it is not between the second and the third, it is supposed that two distinct enzymic systems are involved in the processing.

Oxytocin; Arginine vasopressin; Vasopressin-neurophysin precursor; Neurohypophysial peptide; Copeptin; Processing enzyme

1. INTRODUCTION

The discovery of an incomplete processing of the three-domain vasopressin precursor in guinea pig neurohypophysis and the identification of a MSEL-neurophysin-copeptin fragment [1,2], in contrast to the findings for rat [3–5] and other mammals investigated [6], led us to characterize the neurohypophysial hormones of this species in order to see whether deficiency in processing is related to a structural variation in the first moiety of the precursor.

Comparison between guinea pig and rat concerning MSEL-neurophysin on one hand [1,4] and copeptins on the other [2,5] does not reveal significant differences except a substitution of glycine by glutamic acid in position 88 of guinea pig neurophysin and a deletion of glutamic acid in position 32 in guinea pig copeptin. It is not clear whether processing of the three-domain precursor involves two distinct enzymic systems, one cleaving off vasopressin from the N-terminal end, the other splitting between MSEL-neurophysin and copeptin, or implies a single endopeptidase [6,7]. It seemed therefore of interest to obtain information about the structure and the release of guinea pig vasopressin.

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2. MATERIALS AND METHODS

Purification of neurohypophysial hormones was performed by two procedures, one involving molecular sieving and chromatoelectrophoresis, the second high-pressure reverse-phase liquid chromatography (HPLC). In the first, frozen posterior pituitary lobes (80 glands) were extracted with 0.1 M HCl (5 ml, 4 h at 4°C) and the supernatant solution was subjected to a molecular sieving on a Sephadex G-75 column (1 × 110 cm) in 0.1 M formic acid under conditions described [2]. The active fraction (tubes 161–210; 59 nmol) was concentrated to 2 ml and submitted to a second

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molecular sieving on a Bio-Gel P_4 column (1 × 100 cm) in 0.1 M acetic acid. Oxytocic activity was found in tubes 85–100 and pressor activity in tubes 105–116. Each fraction was concentrated and subjected to paper chromatoelectrophoresis under conditions described [8]. Peptide hormones, detected with dilute ninhydrin, were eluted and samples, reduced (mercaptoethanol) or oxidized (performic acid), were hydrolyzed (6 M HCl, 48 h, 105°C) and analyzed [9].

For the second procedure, frozen posterior pituitary lobes (2 glands) were extracted with 0.1 M HCl and the supernatant (0.130 ml) subjected to HPLC on a μ-Bondapak C-18 column (0.39 × 25 cm) in a Waters chromatograph with a flow rate of 1.5 ml/min. After 5 min with solvent A (5 ml methanol/95 ml of 0.01 M acetate, pH 5.0), a linear gradient with solvent B (70 ml methanol/30 ml of 0.01 M acetate, pH 5.0) was applied for 50 min and then isocratic elution with solvent B was used for 15 min. 0.6-ml fractions were collected. Pressor activity emerges with a retention time of 37.56 min and oxytocic activity with a retention time of 47.73 min. Amino acid analyses were performed on the purified peptides [9].

3. RESULTS AND DISCUSSION

The yields of the purification of guinea pig neurohypophysial hormones are given in table 1 for the two procedures. Amino acid compositions of oxidized and reduced oxytocic and pressor peptides isolated by paper chromatoelectrophoresis are given in table 2. Oxytocin and arginine vasopressin can be identified. Analyses obtained for peptides recovered from HPLC are essentially in agreement but the stoichiometry is not so good, probably because of the smaller amounts used (about 2 nmol). Furthermore, the retention times (RT) in HPLC bring additional arguments for identification: guinea pig oxytocic peptide has a

Table 1

Purification of guinea pig neurohypophysial hormones

	Uterotonic activity			Pressor activity		
	Total amount (U)	Step yield (%)	Overall yield (%)	Total amount (U)	Step yield (%)	Overall yield (%)
A Peptide mapping procedure (80 glands)						
I 0.1 M HCl extract	116	100	100	174	100	100
II molecular sieving on Sephadex G-75						
tubes 161–210	106	94	94	160	92	92
III molecular sieving on Bio-Gel P_4						
tubes 81–91	93	84	79			
tubes 95–110				140	87	80
IV paper chromatoelectrophoresis						
oxytocin	11	12	10			
vasopressin				23.5	17	14
B HPLC procedure (2 glands)						
I 0.1 M HCl extract	2.6	100	100	5.2	100	100
II HPLC on μ-Bondapak C-18						
oxytocin (tubes 3–7)	1.6	61	61			
vasopressin (tubes 23–26)				4.1	79	79

Table 2

Amino acid composition of guinea pig neurohypophysial hormones

Amino acid	Oxytocin			Arginine vasopressin		
	Oxidized (5.4 nmol)	Reduced (6.3 nmol)	Theoretical values	Oxidized (6.7 nmol)	Reduced (13 nmol)	Theoretical values
Lys						
His						
Arg		0.18		0.94	1.02	(1)
Asp	1.00	1.00	(1)	1.00	1.00	(1)
Thr						
Ser	0.11	0.22			0.14	
Glu	1.14	1.05	(1)	1.02	0.96	(1)
Pro	1.03	1.14	(1)	0.90	1.04	(1)
Gly	1.09	1.29	(1)	1.13	1.06	(1)
Ala		0.18				
Cys[a]	0.90		(2)	1.11		(2)
Val						
Met						
Ile	0.91	1.00	(1)			
Leu	1.00	1.09	(1)			
Tyr[b]		0.87	(1)	0.28	0.88	(1)
Phe				0.86	0.90	(1)

[a] Half-cystines are converted into cysteic acid in oxidized samples, partly destroyed after hydrolysis, and into cysteine in reduced samples, entirely destroyed

[b] Tyrosine is largely destroyed in oxidized samples and protected in reduced samples

Values in molar ratios, aspartic acid taken as the reference

RT = 47.71 min compared with 47.45 min for rat oxytocin and guinea pig pressor peptide has a RT = 37.56 min compared with 37.60 min for rat arginine vasopressin (the two chromatographies being carried out one after another).

The only difference found between guinea pig and rat neurohypophysial hormones is the relative ratio of the two hormones, the molar ratio arginine vasopressin to oxytocin, deduced from activities in the crude extracts, being 1.5 for guinea pig and 1.3 for rat. In both cases the release of neurohypophysial hormones from their precursors seems apparently complete since native precursors or fragments including neurohormone and neurophysin have not been detected. Characterization of arginine vasopressin on the one hand, and the fragment MSEL-neurophysin-copeptin on the other [1] allow us to propose a structure for guinea pig vasopressin precursor (fig.1) assuming that the link between the hormone moiety and the neurophysin moiety is identical to the one shown for rat vasopressin precursor through genomic DNA [10]. The processing signal Gly-Lys-Arg is generally involved in the release of an amidated active hormone [11]. This signal is present in both vasopressin and oxytocin precursors of rat [10,12] and because the releases of vasopressin and oxytocin of guinea pig seem similar to those of rat hormones, it probably exists in both guinea pig precursors.

It is possible that the incomplete cleavage between the two last domains of the guinea pig vasopressin precursor is due to a variation in the processing enzyme specificity. The apparent normal release of vasopressin suggests, however, that two distinct enzymic systems are involved in the cleavage of the precursor into the three fragments, vasopressin, MSEL-neurophysin and copeptin.

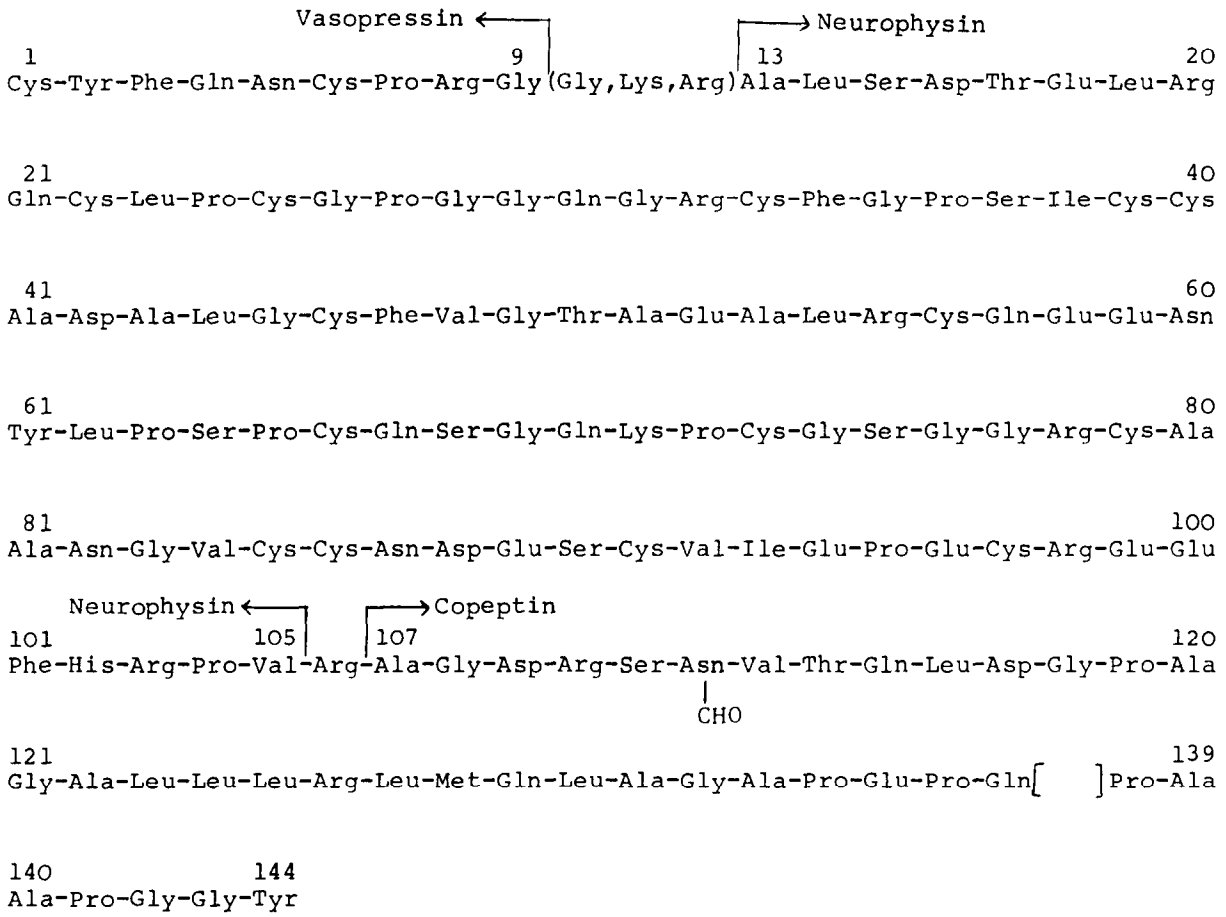

Fig.1. Amino acid sequence proposed for guinea pig vasopressin precursor. Vasopressin, MSEL-neurophysin, copeptin as well as a MSEL-neurophysin-copeptin fragment have been identified. The link Gly-Lys-Arg (in parentheses) between vasopressin and neurophysin has been assumed because of identification of amidated active hormone and by similarity with rat vasopressin precursor. [ ] Deletion observed in guinea pig copeptin when compared with the other known mammalian copeptins. CHO, carbohydrate.

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