FOETAL BOVINE MSEL-NEUROPHYSIN: COMPARISON WITH ADULT HOMOLOGOUS NEUROPHYSIN

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1. Introduction

In the posterior pituitary gland of mammals, two classes of neurophysins [1] have been distinguished and called MSEL-Neurophysins and VLDV-Neurophysins according to the amino acids in positions 2, 3, 6 and 7 [2]. It has recently been shown that within the class of MSEL-Neurophysins, inter-species substitutions are rather rare: 1 out of 95 residues between sheep and ox, and 4 between sheep and pig [3]. Because each type of neurophysin might be related to a specific hormone, oxytocin or vasopressin, either in the biosynthesis or in the carriage [4,5], it was of interest on the one hand to estimate the relative proportions of neurophysins and neurohypophysial hormones in the gland, on the other hand to follow the respective fates of these components during the development. In the adult sheep, the molar ratio of the two neurophysins is about 8 in contrast to a ratio of 1 for the two hormones [6]. This result does not suggest a simple functional relationship between neurophysins and neurohypophysial hormones. To obtain further informations on the physiological role of neurophysins, we have examined the situation in the bovine foetus, and in particular we have compared the foetal MSEL-Neurophysin with its homologous in adult, which has been recently re-investigated [3].

2. Materials and methods

500 posterior pituitary glands collected from foetus (7–9 months) give 4.4 g of acetone-dried powder titrating at 1.0 U mg^{-1} pressor activity and 0.35 U mg^{-1} oxytocic activity. Therefore the ratio of the two activities is 2.8 instead of 1 found usually for acetone-dried powder obtained from the adult.

The neurophysin–neurohypophysial hormone complex is prepared as previously described [7]. From 2.0 g of powder, 58.4 mg of complex titrating at 20.6 U mg^{-1} pressor activity and 9.0 U mg^{-1} oxytocic activity are obtained. At this stage the yields for the two activities are 61% and 67% respectively.

Dissociation of the complex by gel filtration in 0.2 M acetic acid and purification of neurophysins by ion exchange chromatography are conducted as recently described [6]. Fractionation of 'crude' neurophysin on diethylaminoethyl-Sephadex A-50 gives results very similar to those obtained in the case of the adult sheep [6]. A major neurophysin,

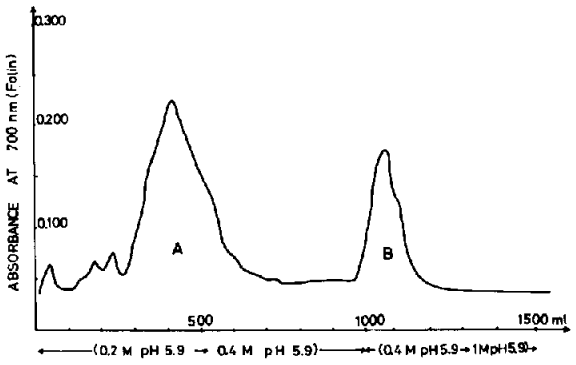

Fig.1. Fractionation of 'crude neurophysin' by ion-exchange chromatography on diethylaminoethyl-Sephadex A-50. Two ionic strength gradients were applied from 0.2 to 0.4 M and from 0.4 M to 1 M pyridine acetate.

Table 1
Amino acid composition of tryptic peptides of foetal (F) and adult (A) bovine MSEL-neurophysins (values given in residues per mole of peptide)

	T_1		T_2		T_3		T_4		T_5		T_6		T_7		T'_7		T_8	
	A (8)	F (13)	A (11)	F (8)	A (12)	F (10)	A (10)	F (20)	A (22)	F (7)	A (13)	F (12)	A (17)	F (8)	A (25)	F (8)	A (4)	F (8)
Lys			0.95	1.10 (1)					0.86	1.10 (1)								
His																		
Arg	1.00	1.10 (1)			1.00	1.00 (1)	0.90	1.10 (1)	0.70	1.00 (1)	0.69	0.75 (1)	1.09	1.30 (1)	1.00	1.20 (1)	0.39	0.60 (1)
Cys[b]			1.50	1.50 (2)			1.94	2.42 (4)	2.40	2.90 (3)	3.30	3.60 (5)						
Asp	0.94	0.65 (1)					1.00	0.89 (1)	0.66	0.80 (1)	1.40	1.70 (2)						
Thr							0.84	0.87 (1)			0.53	0.77 (1)						
Ser	0.59	0.63 (1)					0.64	0.74 (1)	1.57	2.70 (3)	0.62	0.72 (1)						
Glu	0.95	0.81 (1)	1.90	1.20 (1)			2.22	1.80 (2)	4.40	5.40 (5)	3.00	3.00 (3)	0.61	0.63 (1)	0.63	0.70 (1)		
Pro			2.10	1.90 (2)			0.76	0.71 (1)	2.44	2.60 (3)	0.81	0.79 (1)	0.86	1.20 (1)	0.95	1.10 (1)		
Gly			3.30	2.80 (3)	0.38	0.25 (1)	3.76	4.26 (4)	4.00	4.10 (4)	1.05	0.81 (1)	1.96	2.20 (2)	2.40	2.20 (2)		
Ala	0.60	0.67 (1)	0.15	0.30			1.94	1.76 (2)			3.17	2.30 (3)						
Val							0.96	0.89 (1)			0.78	0.88 (1)	1.05	1.10 (1)			1.00	1.00 (1)
Met[b]	0.86	0.65 (1)																
Ile							0.76	0.92 (1)							0.85	1.00 (1)		
Leu	2.00	2.00 (2)	1.00	1.00 (1)			2.00	2.00 (2)	0.78	0.60 (1)	0.71	0.74 (1)						
Tyr										(1)								
Phe							1.63	2.03 (2)					1.00	1.00 (1)	1.00	1.00 (1)		
Number of residues		8		10		2		23		23		20		7		7		2

[a] Amount of peptide subjected to analyses indicated in nmoles, T 1 A(8): 8 nmol, etc.
[b] Determined as cysteic acid and methionine sulfone, respectively.
A destruction, partial for cysteic acid and complete for tyrosine was observed with peptides hydrolyzed after elution from paper.
The integral values, given in parentheses, are those assumed for the sequence.

MSEL-neurophysin, is found in peak A (fig.1). The material is oxidized by perfórmic acid, split by trypsin and the resulting peptides are separated by peptide mapping [8]. Tryptic peptides (T_1 to T_8) are analyzed as previously described for adult ovine or bovine MSEL-neurophysins [2,3]. The same peptides are found in foetal and adult proteins (table 1). Furthermore, the microheterogeneity previously found in position 89 of the adult MSEL-neurophysins [3] is also found in the foetal MSEL-neurophysin since two tryptic peptides, T7 and T'7, (one with Val and the other with Ile) are dtected in approximately equal amounts (table 1).

3. Discussion

The amino acid sequence of adult bovine MSEL-neurophysin (Neurophysin II) recently determined [3] differs considerably from that previously proposed by Walter et al. [9]. From our data, bovine MSEL-neurophysin is nearly identical to ovine MSEL-neurophysin [2,3] with the exception of position 48 (Ile in ovine and Asn in bovine) and a micro-heterogeneity in position 89 (Ile in ovine, Ile and Val in bovine).

Because the same tryptic peptides are found in foetal and adult bovine MSEL-neurophysins, it can be deduced that both proteins are identical (fig.2). Therefore, MSEL-neurophysins is present in the foetus at least at the age of 7–9 months. The second neurophysin, VLDV-neurophysin, may be present in the peak B but this material is not homogeneous and the purification was not undertaken. However from the results of the ion-exchange chromatography it can be assumed that the amount of the possible VLDV-neurophysin should be much lower than that of MSEL-neurophysin.

It is of interest to note that virtually the same ratio of pressor activity to oxytocic activity was found for the acetone-dried posterior pituitary powder and for the complex. Oxytocin and arginine vasopressin have been isolated from the foetal complex (unpublished results) and are responsible for the activities. Therefore the calculated molar ratio vasopressin/oxytocin is approximately 2.8 in the foetus at the age of 7–9 months in contrast to 1 found in the adult. Although this may be a coincidence, in the foetus the major neurophysin, MSEL-neurophysin (Neurophysin II), seems to correspond to the major hormone, arginine vasopressin, as suggested by Dean et al. [10].

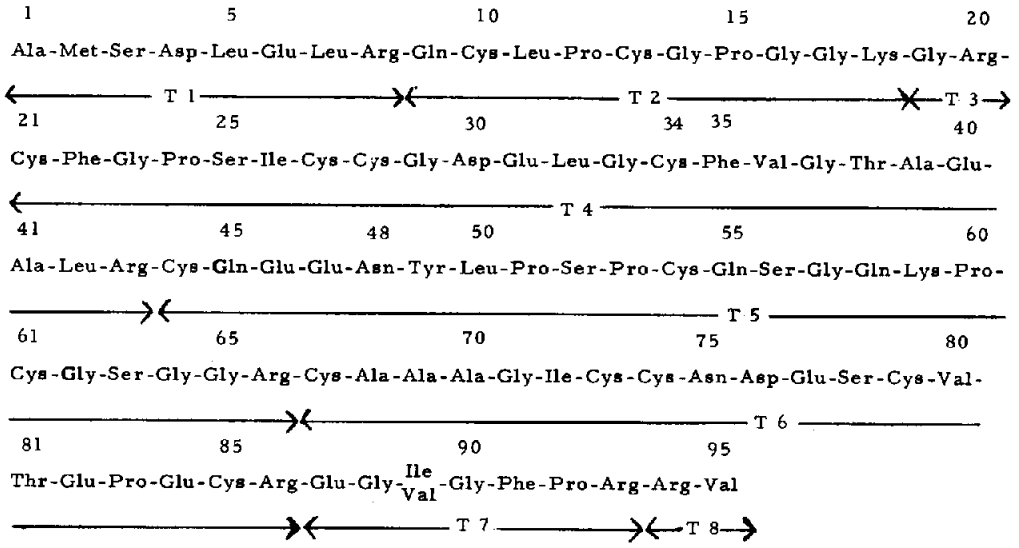

Fig.2. Foetal and adult bovine MSEL-neurophysins. The alignment of the tryptic peptides T1, T2, etc. was deduced by homology with that of ovine MSEL-neurophysin [3].

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