THE COMPLETE AMINO ACID SEQUENCE OF THE MAJOR OVINE NEUROPHYSIN (MSEL-NEUROPHYSIN); COMPARISON WITH A RE-INVESTIGATED BOVINE MSEL-NEUROPHYSIN

Marie-Thérèse CHAUVET, Jacqueline CHAUVET and Roger ACHER
Laboratory of Biological Chemistry, 96, Bd Raspail, 75006 Paris, France

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1. Introduction

Neurophysins [1] are proteins which have been subjected to extensive studies [2] because of their binding properties towards neurohypophysial hormones. Hormone · protein complexes have been purified from posterior pituitary powders of ox [3], pig [4], sheep [5], horse [6], whale [7], man [8] and cross associations between hormones of a species and neurophysins of another have been carried out [5].

The neurophysins of the sheep have been purified through a hormone · protein complex [5] and recently isolated by molecular sieving and ion exchange chromatography [9]. A major neurophysin (MSEL-neurophysin) accounting for about 70% of the neurophysin material, has been partially sequenced [10]. We present now data permitting the determination of the complete amino acid sequence of this protein. On the other hand a comparative investigation was carried out on bovine and porcine MSEL-neurophysins purified under similar conditions.

2. Material and methods

The major ovine neurophysin (MSEL-neurophysin), isolated as previously described [9,10], is oxidized by performic acid, split by trypsin and the resulting peptides are separated by peptide mapping [11]. Tryptic peptides (T1 to T8) are analyzed and partial or complete amino acid sequences are determined by direct manual Edman degradation [12]. Chymotryptic, subtilisic or pepsic fragments are prepared from tryptic peptides and are sequenced in the same way. Sequences of tryptic peptides T1, T2, T3 and a part of T4, have previously been published [10] and table 1 gives the complementary data for T4, T5, T6, T7 and T8.

Overlapping peptides are prepared in the same way by using chymotrypsin, pepsin and subtilisin cleavages on the entire polypeptide chain [12]. These peptides are characterized by amino acid composition. The complete amino acid sequence of ovine MSEL-neurophysin can be deduced from tryptic and overlapping peptides (tables 1 and 2).

On the other hand a similar investigation has been carried out on bovine and porcine MSEL-neurophysins for comparison. The tryptic peptides are virtually the same in bovine than in ovine neurophysin as judged by amino acid compositions and chromatoelectrophoretic migrations. The only differences were found in position 48 (Asn instead of Ile in ovine) and in position 89, which in bovine displays a microheterogeneity : two tryptic peptides T6 in approximately equal amounts were isolated, one with Ile as in ovine T6 peptide, the other with Val. This bovine sequence differs considerably in the C-terminal part (residues 81 to 95) and in positions 34, 59, 71, 75 and 78 from that previously published [13]. In the case of porcine MSEL-neurophysin, the results agree with those previously obtained [14]. When porcine neurophysin is compared to ovine neurophysin, there are 4 substitutions (positions 48, 89, 90 and 92) and an apparent 3-residue C-terminal deletion.

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Table 1
Tryptic peptides of the central and C-terminal parts of ovine MSEL-neurophysin

Peptides	Sequence	Number of residues
T4	21 25 30 35 40 43 Cys-Phe-Gly-Pro-Ser-Ile-Cys-Cys-Gly-Asp Glu Leu-Gly-Cys-Phe-Val-Gly-Thr-Ala-Glu-Ala-Leu-Arg T4s1, T4s2, T4s3, T4s4 T4p1, T4p2, T4p3 C	23
T5	44 50 55 60 65 66 Cys-Gln-Glu-Glu-Ile-Tyr-Leu-Pro-Ser-Pro-Cys-Gln-Ser-Gly-Glu-Lys-Pro-Cys-Gly-Ser-Gly-Gly-Arg Tca1, Tca2 $T5CH_1$ C, T5pl, S	23
T6	67 70 75 80 85 86 Cys-Ala-Ala-Ala-Gly-Ile-Cys-Cys-Asn-Asp-Glu-Ser-Cys-Val-Thr-Glu-Pro-Glu-Cys-Arg T6s2 T6t1, T6s1 S	20
T7	87 93 Glu-Gly-Ile-Gly-Phe-Pro-Arg S P	7
T8	94 95 Arg-Val P	2

⟶ Edman determination on tryptic peptides (upon line) or on sub-fragments (lower line). ⟵ Carboxypeptidase A, T, C, P, S : peptides obtained from the entire chain with trypsin, chymotrypsin, pepsin and subtilisin T4s, T4Ch, T4p, T4t : peptides obtained from the tryptic units with subtilisin, chymotrypsin, pepsin or thermolysin.

Table 2
MSEL – Neurophysins

	1				5					10					15					20
Ovine	H_2N	Ala	Met	Ser	Asp	Leu	Glu	Leu	Arg	Gln	Cys	Leu	Pro	Cys	Gly	Pro	Gly	Gly	Lys	Gly	Arg
Bovine																					
Porcine																					

	21				25					30					34	35					40
Ovine	Cys	Phe	Gly	Pro	Ser	Ile	Cys	Cys	Gly	Asp	Glu	Leu	Gly	Cys		Phe	Val	Gly	Thr	Ala	Glu
Bovine																					
Porcine																					

	41				45			48		50					55					60
Ovine	Ala	Leu	Arg	Cys	Gln	Glu	Glu	Ile	Tyr	Leu	Pro	Ser	Pro	Cys	Gln	Ser	Gly	Gln	Lys	Pro
Bovine								Asn												
Porcine								Asn												

	61				65					70					75					80
Ovine	Cys	Gly	Ser	Gly	Gly	Arg	Cys	Ala	Ala	Ala	Gly	Ile	Cys	Cys	Asn	Asp	Glu	Ser	Cys	Val
Bovine																				
Porcine																				

	81				85					90					95
Ovine	Thr	Glu	Pro	Glu	Cys	Arg	Glu	Gly	Ile	Gly	Phe	Pro	Arg	Arg	Val
Bovine									Ile						
Porcine									Val Ala	Ser	–	–	–	Leu	

3. Discussion

When ovine and bovine MSEL-neurophysins are compared, a nearly identity can be noted (table 2). In fact one out of 95 positions is different in the two species. It may be observed that this nearly identity between ox and sheep has been found for several anterior pituitary hormones : the ovine and bovine somatotropins differ by one residue (position 131, Val and Gly respectively) out of 198 and curiously a microheterogeneity is found for bovine somatotropin in position 128 (Leu as in ovine and Val) [15], the ovine and bovine corticotropins are identical [16] and the α and β chains (96 and 119 residues respectively) of ovine and bovine lutropins are identical [17].

The porcine MSEL-neurophysin is somewhat different from the ovine protein and in fact porcine somatotropin, corticotropin and lutropin are more substituted than bovine homologs when compared with ovine hormones. These results are in agreement with paleontological data since the genera of *Ruminantia* (sheep and ox) are supposed to have diverged some 30 million years ago when *Ruminantia* and *Suiformes* (pig) have diverged some 60 million years ago.

The comparison between the two groups of neurophysins, MSEL-neurophysins and VLDV-neurophysins, is tentative because complete sequence has been only proposed for porcine VLDV-neurophysin (Neurophysin II) [18] and a partial sequence has been suggested for bovine VLDV-neurophysin (Neurophysin I) [19]. However it is clear from the present data that differences are located in the N- and C-terminal parts of the polypeptide chain. There is a large central sequence of about 60 residues which is virtually invariant between the two neurophysins of the same species and between species. From this invariance, from the enzymic accessibility and from studies on the role of the single Tyr-49 [20,21], it has been deduced that the polar sequence 38–58 might be on the surface and might be the hormone binding-site [22]. On the other hand the 14 cysteine residues are in identical positions so that it can be assumed that the 7 disulfide bridges are identical in both group.

In the same group of neurophysins the variations are essentially located in the C-terminal part and two neurophysins from two different species belonging to the same group are more related than two neurophysins of the same species. It seems therefore that at least two lines of related neurophysins exist in mammals and because of the strong structural

similarity they might have arisen by duplication. This multiplicity, not rare in proteins [23], may be related to the possible duplication in the case of the neurohypophysial hormones [24].

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