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A MULTIGENE FAMILY FOR THE VASOPRESSIN-LIKE HORMONES ? IDENTIFICATION OF MESOTOCIN, LYSIPRESSIN AND PHENYPRESSIN IN AUSTRALIAN MACROPODS

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Mesotocin ([Ile^8]-oxytocin), lysipressin ([Lys^8]-vasopressin) and phenypressin ([Phe^8]-vasopressin) have been identified in the western gray kangaroo (Macropus fuliginosus) as well as four other macropodids. Lysipressin and phenypressin, which differ by the amino acids in positions 2 (Tyr/Phe) and 8 (Lys/Arg) are likely products of two separate vasopressin-like genes. It is assumed that arginine vasopressin found in most mammals is the product of two identical genes which can be revealed in some species by differential mutations as seen usually in marsupials. The duality can also be revealed by differential mutations in another domain of the precursors, such as the neurophysin (MSEL-neurophysin), as observed in the ox.

Mesotocin ([Ile^8]-oxytocin), lysipressin (lysine vasopressin, [Lys^8]-vasopressin) and phenypressin ([Phe^2]-vasopressin) have previously been identified in four Australian marsupials belonging to the family Macropodidae, namely the red kangaroo (1-3), the tammar wallaby (1-3), the Eastern gray kangaroo (4) and the quokka wallaby (5). Investigations have now been carried out on a macropodid species living in South-West Australia, the Western gray kangaroo (Macropus fuliginosus). Despite the great geographic dispersion of the Australian macropods investigated, all the five species have the same set of neurohypophysial hormones.

MATERIALS AND METHODS

Posterior pituitary glands : 69 posterior pituitary glands from Western gray kangaroos (Macropus fuliginosus) collected in South-West of Australia, are lyophilized giving 523 mg of dry material (average weight of a desiccated gland : 7,6 mg). The powder titrates at 0.68 U/mg of oxytocic activity (6) and 2.2 U/mg of pressor activity (7). Extraction is carried out with 0.1 N HCl for 4 h at 4°C.

Molecular sieving : The 0.1 N HCl extract of 38.8 mg of posterior pituitary powder (5 glands) is centrifuged and passed through a column (1 x 120 cm) of Biogel P$_4$ equilibrated with 0.1 N acetic acid (8). Fractions (1 ml) are collected.

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Absorbance at 280 nm and biological activities are measured. Tubes 79-95 containing oxytocic activity and tubes 96-105 containing pressor activity are pooled and concentrated for high pressure liquid chromatography.

High pressure liquid chromatography : Purification of peptide hormones by high pressure liquid chromatography is carried out under conditions previously described (5). A Waters liquid chromatograph (Model ALC/GPC 204) equipped with a WISP automatic injector (Model 710 B), a Solvent Programmer (Model 660) and a UV Absorbance Detector (Model 440), is used. A Waters μ-Bondapak C-18 column (3.9 mm ID x 300 mm) is employed with a gradient of methanol (5% to 70%) - 0.01 M sodium acetate buffer pH 5.0 (80 min; flow rate 1.5 ml/mn) (5). Absorbance is measured at 254 and 280 nm. Fractions (0.75 ml) are collected every 30 sec with a collector LKB (Redirac 2112) and bioassays are performed for locating the hormones. Lysipressin is recovered with a retention time (RT) of 41.00 min, mesotocin with a RT of 50.18 min and phenypressin with a RT of 55.72 min.

Amino acid analysis : Peptides samples (5-10 nmol) are hydrolyzed, either after oxidation with performic acid or after addition of dithiothreitol as reducing agent, in sealed evacuated tubes (6 N HCl, 48 h, 100°C). Amino acid analyses are carried out according to Spackman et al. (9) with a Spinco 120 B automatic analyzer fitted with a high-sensitivity cell.

Bioassays : Oxytocic activity is determined on rat uterus without magnesium according to Holton (6). Pressor activity is determined in anesthetized rats following the method of Landgrebe et al (7). Activities are expressed in U.S.P. units.

RESULTS AND DISCUSSION

A typical purification is carried out with 5 dry posterior glands (38.8 mg). The yields in activities at each step of the purification are given in Table 1. The overall yields reach 37% for mesotocin and about 60% for the vasopressin-like peptides.

The amino acid compositions (Table 2) and the retention times in HPLC permit the identification of mesotocin, lysipressin ([Lys^8]-vasopressin) and phenypressin ([Phe^2]-vasopressin). These peptides have previously been characterized in four species of the family Macropodidae. If mesotocin is present in all the Australian marsupials investigated, in contrast lysipressin and phenypressin seem typical for the macropodids. These two peptides have been found in all the individuals examined to date. They differ by two amino acid residues in positions 2 (Tyr or Phe) and 8 (Lys or Arg) and are likely products of two separate genes.

Oxytocin and arginine vasopressin found in placental mammals are fragments of protein precursors in which they are

TABLE I

Purification of Macropus fuliginosus neurohypophysial hormones
(5 dry posterior glands : 38.8 mg)

	Step	Oxytocic activity			Pressor activity		
		Total U	Step-yield %	Overall yield %	Total U	Step-yield %	Overall yield %
I	0.1 N HCl extract	27	100	100	86	100	100
II	Molecular sieving on Biogel P_4						
	oxytocic peak	16	59				
	intermediate peak	1	4	69	1.6	2	92
	pressor peak	1.7	6		78	90	
III	High pressure liquid chromatography	5	100		22	100	
	mesotocin	2.7	54	37			
	$[Lys^8]$-vasopressin				12.7	57	60
	$[Phe^2]$-vasopressin				1.9	8.6	

associated to VLDV-neurophysin and MSEL-neurophysin (10) respectively, as shown by direct isolation of the precursors (11) or inferred from the cDNA corresponding sequences (12,13). Recently the structural organization of the rat gene for the arginine vasopressin-neurophysin precursor has been elucided (14). It appears that vasopressin and the variable N-terminus (residues 1-9) of MSEL-neurophysin belong to the same exon, the nearly invariant central part (residues 10-76) of MSEL-neurophysin to a second exon and the hypervariable C-terminal part (residues 77-93) to a third exon. Vasopressin gene is already present in Prototherian mammals such as the egg-laying echidna (15) and could have duplicated in Therian mammals in the course of evolution. The two genes might give identical protein products as far as their exons are not substituted as it occurs for the two human α-globin genes (16,17). They are disclosed if one or both are subjected to mutations in the exons. It can be assumed that in macropodids, one vasopressin gene has mutated in the neurohormone moiety, giving $[Phe^2]$-vasopressin instead of the usual vasopressin and the second gene also giving $[Lys^8]$-

TABLE II

AMINO ACID COMPOSITIONS OF THE WESTERN GRAY KANGAROO NEUROHYPOPHYSIAL HORMONES [a]

Amino acid	Mesotocin			Lysine vasopressin			Phenypressin		
	Oxidized (2.6 nmol)	Reduced (3.4 nmol)	Theoretical values	Oxidized (4.3 nmol)	Reduced (10 nmol)	Theoretical values	Oxidized (2.1 nmol)	Reduced (6.5 nmol)	Theoretical values
Lys				0.97	0.88	(1)			
Arg							0.95	0.73	(1)
Asp	1.00	1.00	(1)	1.00	1.00	(1)	1.00	1.00	(1)
Thr	0.26	0.29	-		0.12	-		0.26	-
Ser	0.91	0.20	-	0.22	0.33	-	0.63		-
Glu	1.24	1.09	(1)	1.09	1.01	(1)	1.21	0.97	(1)
Pro	1.06	1.08	(1)	1.00	0.98	(1)	0.88	0.85	(1)
Gly	1.70	1.04	(1)	1.20	1.19	(1)	1.53	1.35	(1)
Ala	0.61	0.41	-		0.22	-	0.31	0.26	-
Val					0.13	-		0.09	-
Ile	1.48	1.59	(2)		0.10	-	0.28	0.23	-
Leu	0.31	0.26	-		0.14	-			
Tyr [b]		0.68	(1)		0.80	(1)		0.34	-
Phe				0.95	0.85	(1)	1.74	1.60	(2)
Cys [b]	1.10		(2)	1.63		(2)	1.28		(2)

[a] Values in molar ratios ; aspartic acid taken as the reference.

[b] Half-cystine is determined as cysteic acid on a separate performic acid-oxidized sample. A partial destruction of cysteic acid is observed giving serine or alanine as by-products.

vasopressin. The molar ratios of these two peptides is usually 1:2 in macropodids and they are present in all the individuals of the five species investigated. Mutations could also have occurred in the neurophysin moieties of the genes and in this case two different MSEL-neurophysins should be isolated. Although macropodid neurophysins are not yet characterized, it is of interest that in ox, a "microheterogeneity" has been found in MSEL-neurophysin, one protein having Val^{89} and the other Ile^{89} (18). These two proteins have also been identified in bovine foetus (19). They may be products of two separate vasopressin genes. However a single vasopressin, arginine vasopressin, has been characterized in ox (20,21) and so far a single glyco-peptide (22), the third fragment of the vasopressin precursor (12), has been found. So if there are two vasopressin genes in ox, their exons only differ in the expression of a single amino acid. It may be recalled that such a situation exists for the two human γ-globin chains (146 residues) which differ by only one residue (Gly^{136} or Ala^{136}) (23,24) and are products of two distinct genes, G_γ and A_γ, separated by 3.5 kpb on chromosome 11 (25,26).

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