A REPTILIAN NEUROHYPOPHYSIAL HORMONE, MESOTOCIN (Ile^8-OXYTOCIN), IN AUSTRALIAN MARSUPIALS

M. T. CHAUVET, D. HURPET, J. CHAUVET and R. ACHER
Laboratory of Biological Chemistry, University of Paris VI, 96, boulevard Raspail, 75006 Paris, France

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1. Introduction

Mammals are supposed to be derived from primitive mammal-like reptiles constituting the sub-class Synapsida [1]. However if modern reptiles, namely viper, cobra, elaphe and iguana, living in France, China, Japan and South America, respectively, have a neurohypophysial hormone mesotocin (Ile^8-Oxytocin), in contrast present-day eutherian mammals have oxytocin instead and this replacement is usually regarded as related to lactation [2]. Some Australian marsupial species have been found to differ from eutherians in that they possess 2 vasopressin-like peptides, lysine vasopressin (Lys^8-vasopressin) and phenypressin (Phe^2-Arg^8-vasopressin) instead of the single arginine vasopressin [3,4]. We report now the identification of mesotocin in 3 Australian marsupial species, namely the red kangaroo (*Macropus rufus*) and the tammar (*Macropus eugenii*) belonging to the family *Macropodidae* and the possum (*Trichosurus vulpecula*) belonging to the family *Phalangeridae*. Mesotocin has also been detected in the euro (*Macropus robustus*) and the quokka (*Setonix brachyurus*). This finding raises the question of the role of mesotocin in lactation of non-placental mammals on the one hand and on the other reveals the intermediate position of Australian Metatheria in mammal evolution.

2. Methods

Posterior pituitary glands were removed rapidly after death and desiccated in dry and cool acetone. Rat oxytocic activity [5] of the acetone powder varied from 0.66–1.12 U/mg and rat pressor activity [6] from 2.20–3.54 U/mg (table 1). The ratio pressor

Table 1
Biological activities of posterior pituitary powder of some Australian marsupials

Species	No. glands in the sample	Oxytocic act. (U/mg)	Pressor act. (U/mg)	Ratio P/O
I: Macropodidae				
M. rufus	(4)	1.12	2.85	2.54
	(9)	0.66	2.98	4.42
	(14)	0.88	2.70	3.07
M. eugenii	(24)	0.80	2.30	2.88
	(33)	0.83	2.20	2.65
M. robustus	(1)	1.14	3.54	3.10
	(1)	0.78	2.25	2.88
M. giganteus	(1)	1.85	3.00	1.62
S. brachyurus[a]	(23)	0.15	0.35	2.33
II: Phalangeridae				
T. vulpecula	(10)	2.00	6.30	3.15

[a] Entire pituitary

activity to oxytocic activity ranges from 1.62–4.42 instead of ~1 found frequently in placental mammals. Purification of the peptide hormones regarding red kangaroo, tammar and brush-tailed possum was conducted as described, through molecular sieving on Biogel P4 and paper chromatoelectrophoresis [4]. In each species, a single spot (electrophoretic migration, 11–15 cm; chromatographic migration, 19–22 cm) giving after elution (0.1 M acetic acid) a peptide endowed with oxytocic activity was found. Mesotocin and oxytocin have the same location on the peptide map and further characterization is necessary for identification. Amino acid analysis and sequence determination were performed on material purified by paper chromatoelectrophoresis.

3. Results

The amino acid analysis was carried out after hydrolysis (6 N HCl 48 h, 105°C, in vacuo) in a Spinco 120 B automatic analyser fitted with a high-sensitivity cell [7]. The results are given in table 2. It is clear that leucine is absent in the peptide and that there are 2 residues of isoleucine instead of 1 as in oxytocin. The amino acid composition corresponds to that of mesotocin.

The amino acid sequence has been determined by using Edman recurrent degradation in the paper-strip adaptation in [8]. Identification of phenylthiohydantoin (PTH) amino acids was carried out by thin-layer chromatography according to [9]. Spots were located by fluorescence and by ninhydrin staining. For comparison, ~50 nmol ox oxytocin or frog mesotocin were subjected to Edman degradation under the same conditions.

Sequence analysis has been performed on the oxytocin-like peptide of the red kangaroo. Tyr, Ile, Gln, Asn and Pro were identified in positions 2–5 and 7 (Cys in positions 1 and 6 could not be seen because of the disulphide bridge). Residues in positions 8 and 9 were not identified because of the small amount of material but from the amino acid composition and the homology, it is deduced that these residues are Ile and Gly respectively (table 3).

Partition chromatography of the oxytocin-like hormones was performed on Sephadex G-25 by using the solvent system *n*-butanol:3.5% acetic acid in 1.5% aqueous pyridine (1:1) described [10]. This solvent system clearly separates oxytocin and mesotocin that can be recognized by their respective positions, as shown [11]. Taking the elution volume of ox oxytocin (V_o) as the reference, the ratio of the mesotocin elution volume (V_m) to that of oxytocin (V_m/V_o) was found to be 1.9–1.26. The ratios found for the oxytocin-like peptide of tammar, euro and possum

Table 3
Amino acid sequence of mesotocin

	1	2	3	4	5	6	7	8	9	
Mesotocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Ile	Gly	(NH_2)
Oxytocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	Gly	(NH_2)

Table 2
Amino acid composition of tammar, red kangaroo and possum mesotocins (values in molar ratios, using aspartic acid as reference)

Amino acid	Tammar (5.5 nmol)	Red kangaroo (9.5 nmol)	Possum (7.9 nmol)	Mesotocin (theoretical)	Oxytocin (theoretical)
Asp	1.00	1.00	1.00	1	1
Ser	–	0.18	0.41	–	–
Glu	0.95	1.01	1.05	1	1
Pro	0.98	1.07	0.95	1	1
Gly	1.18	1.06	1.32	1	1
Ile	1.96	1.83	1.85	2	1
Leu	–	–	–	–	1
Tyr	0.73	0.95	0.99	1	1
Cys[a]	1.38	0.90	1.07	2	2

[a] Half-cystine are determined as cysteic acid on a separate performic acid-oxidized sample

A partial destruction of cysteic acid is observed when hydrolysis is carried out with paper-eluted peptide

were 1.23, 1.19 and 1.11, respectively, suggesting the presence of mesotocin in the 3 species. For the red kangaroo, large fractions were collected so that the peak position could not be precisely determined but the oxytocic material was recovered in the distinctive mesotocin region. These chromatographic results are in agreement with the chemical data.

Purification of the oxytocin-like peptide of the quokka (*Setonix brachyurus*) was initially attempted by ion-exchange chromatography on Amberlite CG-50 as in [3]. The amino acid composition was not satisfactory probably because of some contamination but the virtual absence of leucine (not shown) suggested that the active peptide was not oxytocin. A pharmacological characterization was therefore carried out by determining the ratio of the fowl vasodepressor activity [12] to the rat oxytocic activity [5]. This ratio is 1.03 ± 0.16 for oxytocin and 2.06 ± 0.08 for mesotocin [13]. A value of 1.91 was found for the quokka peptide suggesting the presence of mesotocin.

4. Discussion

Mesotocin has therefore been detected in the 5 species so far examined and seems to be a typical hormone of the Australian marsupials. This finding can be interpreted in 2 ways. Because oxytocin had been identified in the echidna [14], a primitive prototherian, it was assumed that the substitution of mesotocin for oxytocin occurred very early in mammalian evolution. If so, the presence of mesotocin in Australian marsupials would be the result of a reverse mutation. The codons for isoleucine and leucine differ only by one nucleotide and the interchange can be determined by a point mutation. In this case mesotocin and oxytocin might be functionally equivalent and the passage of the first to the second would be a 'neutral' evolution. However, the long evolutionary stability of mesotocin, which appeared in early tetrapods such as lung-fishes and remained until modern reptiles [2] and on the other hand the broad distribution of oxytocin in eutherians, are better explained by adaptation to the function. It seems therefore that Australian marsupials remained relatively closer to ancestor mammalian reptiles than eutherians at least regarding the neurohypophysial hormone involved in reproduction.

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