Vol. 127, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1985 Pages 277-282

THE NEUROHYPOPHYSIAL HORMONES OF THE EGG-LAYING MAMMALS : IDENTIFICATION OF ARGININE VASOPRESSIN IN THE PLATYPUS (ORNITHORHYNCHUS ANATINUS)

Jacqueline Chauvet, Dominique Hurpet, Gilles Michel, Marie-Thérèse Chauvet, Franck N. Carrick[+] and Roger Acher

Laboratory of Biological Chemistry, University of Paris VI 96, Bd Raspail, 75006 Paris, France

[+]Department of Veterinary Anatomy, University of Queensland, St Lucia Queensland, Australia 4067

Received January 14, 1985

Two neurohypophysial peptides have been purified from acetone desiccated posterior pituitary glands of the platypus (Ornithorhynchus anatinus) by molecular sieving and high-pressure liquid chromatography. A single pressor peptide, having an amino acid composition and a chromatographic retention time identical to those of arginine vasopressin, has been identified. A single oxytocic peptide has been isolated that ressembles oxytocin by its chromatographic retention time, but lack of material has prevented to obtain a correct amino acid composition. The pressor peptide is roughly four times more abundant than the oxytocic peptide. Neurohypophysial hormones of platypus seem similar to those of echidna, the other living prototherian, and to those of most placental mammals.

The egg-laying monotremes are the only survivors of the prototherian mammals ; two genera are known, Tachyglossus (the echidna) and Ornithorhynchus (the platypus) both confined to the Australian region. Echidnas are relatively more abundant and easier to catch because they are terrestrial. The neurohypophysial hormones of the echidna (Tachyglossus aculeatus) have previously been characterized by their amino acid compositions and their pharmacological properties. Oxytocin and arginine vasopressin have been identified, vasopressin structure being moreover confirmed by amino acid sequence determination (1). These hormones are known to be present in placental mammals (Eutherians) (2). Recent research on marsupials (Metatherians) has however revealed that these species, although belonging to the same sub-class Theria as placental mammals, are endowed with different sets of hormones. Australian Phalangeridae have mesotocin and arginine vasopressin (3) whereas Australian Macropodidae have mesotocin, lysipressin and pheny-

0006-291X/85 $1.50

pressin (4). In contrast South American Didelphidae have oxytocin, lysipressin and arginine vasopressin (5) whereas the North American opossum (Didelphis virginiana) has oxytocin, mesotocin, lysipressin and arginine vasopressin (6). Hormone gene duplication in marsupials has therefore been suggested (3-6). The present work deals with the neurohypophysial hormones of the second living species of the sub-class Prototheria, the platypus.

MATERIALS AND METHODS

Posterior pituitary glands : 20 posterior pituitary glands from animals caught in Queensland (Australia) were freeze-dried giving 22.4 mg of material. 19 glands (21.7 mg) were extracted with 0.1 M HCl (2 ml), first during 15 min at 4°C in a homogeneizer, then by stiring for 4 h at 4°C. The oxytocic (7) and pressor (8) activities were determined on the clear supernatant solution obtained by centrifugation.

Molecular sieving : The clear solution (1,7 ml) was passed through a column (0.6 x 120 cm) of Bio-Gel P4 equilibrated with 0.1 M acetic acid. 1.1-ml fractions were collected and bioassays performed. Tubes containing either oxytocic activity (30 to 37) or pressor activities (38-42) were subjected to high pressure liquid chromatography by pooling tubes 31-32 on one hand, tubes 39-40 on the other.

High pressure liquid chromatography : Purification of peptide hormones by HPLC has been carried out under conditions previously described (9) using a Waters liquid chromatograph (Model ALC/GPC 204) equipped with a WISP automatic injector (Model 710 B) and a Waters μ-Bondapak C-18 column. Absorbance has been measured at 254 and 280 nm and fractions (0.75 ml) have been collected every 30 sec with a collector LKB (Redirac 2112). Bioassays have been performed for locating the hormones. The two active fractions from Bio-Gel P4 have been passed separately. Oxytocic activity was recovered as a single peak in fraction 97-99 and pressor activity also as a single peak in tubes 90-94 (Table 1).

Amino acid analysis : Peptides samples (0.8 to 3.3 nmol) were hydrolyzed, either after performic acid oxidation, or dithiothreitol reduction, in sealed evacuated tubes (6 M HCl 48 h, 100°C). Amino acid analyses have been carried out according to Spackman et al. (10) with a Spinco 120 B automatic analyzer fitted with a high-sensitivity cell (Table 2).

RESULTS AND DISCUSSION

Two peptides have been isolated from the posterior pituitary gland of platypus. Because of the scarcity of the material, characterization has been limited to amino acid composition, retention time in HPLC and molecular activity. Identification of arginine vasopressin seems safe. The amino

TABLE I

Purification of Platypus neurohypophysial hormones
(19 dry posterior pituitaries : 21.7 mg)

	Step	Oxytocic activity			Pressor activity		
		Total	Step-yield	Overall yield	Total	Step yield	Overall yield
		U	%	%	U	%	%
I	0.1 N HCl extract	2.4	100	100	7.8	100	100
II	Molecular sieving on Bio-Gel P_4						
	oxytocic peak (tubes 30-37)	0.90	45	45			
	pressor peak * (tubes 38-42)	0.27	13	13	6.95	89	89
III	High pressure liquid chromatography **						
	oxytocin	0.61	68	31			
	Arg-vasopressin				4.25	61	54

*Arginine vasopressin has a secondary oxytocic activity, about 5% of the pressor activity

**Values calculated from an aliquot

acid composition is in agreement with theoretical values. The value for cysteic acid residues derived from performic acid-oxidized cystine is low because of a destruction during hydrolysis, which is currently observed (9). A by-product of this destruction is serine. On the other hand the retention time in HPLC was 47.96 min in an analytical chromatography made with 320 mU (a single gland) and 47.00 min in the preparative chromatography carried out with 2 500 mU. The average retention time is therefore 47.48 min. For ovine arginine vasopressin, retention times of 47.15 and 46.86 min were found for 80 and 200 mU, respectively, giving an average of 47.06 min, value close to that observed for platypus pressor peptide. A molecular rat pressor activity of 505 U μmol^{-1} is determined compared to 435 ± 45 U reported for synthetic arginine vasopressin (11).

Characterization of the platypus oxytocin-like peptide as oxytocin is less satisfactory. The amino acid composition shows nearly equal amounts of leucine and isoleucine, ratio

TABLE II

AMINO ACID COMPOSITIONS OF THE PLATYPUS NEUROHYPOPHYSIAL HORMONES [a]

Amino acid	Oxytocin-like peptide		Arginine vasopressin		
	Reduced (0,8 nmol)	Theoretical values	Reduced (3,3 nmol)	Oxidized (1,1 nmol)	Theoretical values
Lys					
Arg			1.06	1.09	(1)
Asp	1.00	(1)	1.00	1.00	(1)
Thr					
Ser[b]	1.16	-	1.09	1.30	-
Glu	1.17	(1)	1.36	1.27	(1)
Pro		(1)	0.85		(1)
Gly	1.27	(1)	1.39	2.10	(1)
Ala					
1/2 Cys					
Val					
Met					
Ile	0.70	(1)			
Leu	0.81	(1)			
Tyr[b]	0.15	(1)	0.73		(1)
Phe			0.70		(1)
$CysO_3H$[b]		(2)		1.10	(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 destruction, partial for cysteic acid and total for cysteine, is observed giving serine or alanine as by-products. Tyrosine, destroyed in oxidized sample, is protected in reduced sample.

expected for oxytocin. However, because of insufficient amount of material subjected to hydrolysis (0.8 nmol), results are incomplete : proline and cysteic acid are not seen and tyrosine is very weak. The retention times for the platypus oxytocin-like peptide were 50.40 min in analytical HPLC with 80 mU (a single gland) and 49.95 min in the preparative HPLC with 460 mU, giving an average of 50.18 min. The control with sheep oxytocin gave 51.98 and 50.30 min for 80 and 200 mU, respectively, giving an average of 51.14 min. A mixture of oxytocin and platypus peptide gave a single peak. A molecular rat uterotonic activity of 475 U μmol^{-1} is found compared with 450±30 U reported for synthetic oxytocin (11).

Comparison between the two living species of egg-laying mammals is of great interest. The average weight of the acetone

desiccated posterior pituitary gland of platypus is 1.14 mg compared with 2.9-3.3 mg found for echidna. The oxytocic activity of platypus posterior pituitary powder was much lower than that found for echidna powder prepared apparently under the same conditions : 0.11-0.18 U mg^{-1} compared with 1.4-2.2 U mg^{-1} (ref. 1). From 21.7 mg of platypus powder only 1 nmol of the oxytocin-like peptide have been recovered for analysis whereas from 28.6 mg of echidna powder 8 nmol of oxytocin were used for amino acid composition. In the same way, pressor activity of platypus powder was 0.42-0.70 U mg^{-1} compared with 6.1-7.9 U mg^{-1} found for echidna powder. About 5 nmol of arginine vasopressin were recovered from 21.7 mg of platypus powder instead of about 70 nmol from 28.6 mg of echidna powder. The low contents in peptide hormones may be related to the conditions of life, echidna living in dry environment and feeding on ants and termites, whereas platypus is found in the freshwater systems of eastern Australia and eats molluscs, crustacea and aquatic insects (12). The ratio of pressor activity to oxytocic activity is 3.8-3.9 for platypus and 3.6-4.3 for echidna, compared with roughly 1 found for many placental mammals except whales and rabbit (13). In these latter species, lack of freshwater might explain the great excess of arginine vasopressin acting as antidiuretic hormone but this reason is certainly not valid for platypus. A similar high ratio is also found for marsupials (3-6).

The presence of the same antidiuretic hormone, arginine vasopressin, in prototherians and in eutherians, suggests that the change of the reptilian vasotocin into vasopressin occurred very early in mammals, perhaps in mammal-like reptiles. The dimorphism of the vasopressin peptide in most of Australian and American marsupials (4-6), corresponding probably to a gene duplication, seems mainly limited to metatherians.

ACKNOWLEDGMENTS

The authors thank Mrs Danielle Thevenet and Miss Christine Jourdain for their skilled technical assistance. The investigation was supported in part by CNRS (ERA 070563 and ATP 950037), the "Mission à la Recherche Universitaire", the "Fondation pour la Recherche Médicale" and the "Fondation pour la Recherche en Hormonologie".

REFERENCES

1. Acher, R., Chauvet, J. and Chauvet, M.T. (1973) Nature New Biol. 244, 124-126.

2. Acher, R. (1980) Proc. R. Soc. Lond. B 210 21-43
3. Hurpet, D., Chauvet, M.T., Chauvet, J. and Acher, R. (1982) Int. J. Peptide Protein Res. 19, 366-371.
4. Chauvet, M.T., Colne, T., Hurpet, D., Chauvet, J. and Acher, R. (1983) Biochem. Biophys. Res. Commun. 116, 258-263.
5. Chauvet, J., Hurpet, D., Chauvet, M.T. and Acher, R. (1984) Bioscience Rep. 4, 245-252.
6. Chauvet, J., Hurpet, D., Michel, G., Chauvet, M.T. and Acher, R. (1984) Biochem. Biophys. Res. Commun. 123, 306-311.
7. Holton, P. (1938) Brit. J. Pharmacol. 3, 328-334.
8. Landgrebe, F.W., Macaulay, M.H. and Waring, H. (1946) Proc. R. Soc. Edinburgh, Sect. B, 62, 202-210.
9. Chauvet, M.T., Colne, T., Hurpet, D., Chauvet, J. and Acher, R. (1983) Gen. Comp. Endocrinol. 52, 309-315.
10. Spackman, D.H., Stein, W.H., and Moore, S. (1958) Anal. Chem. 30, 1190-1206.
11. Berde, B and Boissonnas, R.A. (1968) Handbook of Experimental Pharmacology, 23, 802-870.
12. Griffiths, M. (1978) The Biology of the Monotremes, Academic Press, Inc. New York.
13. Valtin, H., Stewart, J. and Sokol, H.W. (1974) in Handbook of Physiology, Section 7 Endocrinology, Vol. IV part 1 (Knobil, E. and Sawyer, W.H. Eds) American Physiological Society, pp 131-171.