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DUAL DUPLICATION OF NEUROHYPOPHYSIAL HORMONES IN AN AUSTRALIAN MARSUPIAL : MESOTOCIN, OXYTOCIN, LYSINE VASOPRESSIN AND ARGININE VASOPRESSIN IN A SINGLE GLAND OF THE NORTHERN BANDICOOT (ISOODON MACROURUS)

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Neurohypophysial hormones of an Australian marsupial, the Northern bandicoot (Isoodon macrourus), have been identified by their retention times in high-pressure reverse-phase liquid chromatography using two solvent systems and by their molar pressor or uterotonic activities. Two pressor peptides, arginine vasopressin and lysipressin, and two uterotonic peptides, mesotocin and oxytocin, have been characterized. Because mesotocin and arginine vasopressin have been identified in three other Autralian marsupial families, it is assumed that a duplication of each ancestral gene occurred in Peramelidae and subsequent mutations in one copy led to the additional oxytocin and lysipressin. A similar dual duplication of neurohypophysial hormones has previously been discovered in the North-American opossum (Didelphis virginiana) so that the duplication propensity seems peculiar to marsupials in contrast to placental mammals.

Neurohypophysial hormones have revealed themselves as good markers to trace vertebrate evolutionary lineages (1,2). In particular, striking differences have been found between the two extant groups of the mammalian sub-class Theria, placentals and marsupials, both diverged from common ancestors, about 130 million years ago (3). Whereas Eutheria have always oxytocin and, except pig, arginine vasopressin, Metatheria appear virtually always endowed with the reptilian mesotocin in place of oxytocin. The four families of Australian marsupials that have been examined up to now, namely Phalangeridae (4), Dasyuridae (5), Phascolarctidae (5) and Macropodidae (6), own mesotocin. In addition, the first three have arginine vasopressin (4,5) whereas the fourth possesses two pressor peptides : lysipressin ([Lys^8]-vasopressin) and phenypressin ([Phe^2]-vasopressin) (6). The apparent duplication of the pressor peptide is not limited to Australian marsupials since American opossums have also two pressor peptides : arginine vasopressin and lysipressin (7). The duplication propensity of marsupials has now been confirmed by examining a fifth Australian family,

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Peramelidae. In the Northern bandicoot (Isoodon macrourus), four neurohypophysial peptides have been identified, revealing a dual duplication dealing with both the oxytocic and the pressor hormones.

MATERIALS AND METHODS

Posterior pituitary gland : A freeze-dried posterior pituitary gland (0.9 mg) of a male Northern bandicoot (Isoodon macrourus) has been ground and homogenized with 0.1 M HCl (0.1 ml) in a Potter-Eveljhem for 4 min at 0°, then stirred for 4 h at 4°. After centrifugation, the pellet has been washed with 0.03 ml and centrifuged again. The two supernatants have been pooled (0.110 ml). 20 µl have been used for bioassays. The solution contained 2.75 U pressor activity and 1.2 U uterotonic activity per mg of dry tissue. The remaining 90 µl have been used for two analyses by high-pressure reverse-phase liquid chromatography (5 and 80 µl).

High-pressure reverse-phase liquid chromatography (HPLC) : Neurohypophysial hormones have been separated using two HPLC systems with a WATERS Model 204 chromatograph equipped with a Model U6K manual injector, a Model 730 data module, a Model 660 solvent programmer and a Model 441 absorbance detector. A Nucleosil C-18 column (4.6 x 250 mm) has been employed. In the first system a solvent A made with 13% acetonitrile - 87% 0.1 M phosphate pH 3.0 was used first, then a linear gradient with a solvent B : 45% acetonitrile - 55% 0.1 M phosphate pH 3.0 was applied for 30 min. The flow rate was 0.6 ml/min and absorbance was monitored at 214 and 280 nm.

In the second system, solvent A was made with 15% acetonitrile - 85% 0.015 M sodium acetate pH 5.0 and a linear gradient was applied with solvent B : 50% acetonitrile - 50% 0.024 M sodium acetate pH 5.0 (0% to 29% of solvent B) for 30 min . The flow rate was 0.6 ml/min and absorbance measured as for the first system.

Authentic hormones prepared either by purification such as goose mesotocin and vasotocin or by synthesis such as oxytocin, arginine vasopressin and lysipressin, were chromatographed under identical conditions for determining specific retention times. Extracts of posterior pituitary glands of Eastern native cat (Dasyurus viverrinus) and brush-tailed possum (Trichosurus vulpecula) were also chromatographed for comparison. These species possess mesotocin and arginine vasopressin (4,5).

Bioassays : Uterotonic activity on rat uterus was determined without magnesium according to Holton (8). Pressor activity was measured on anesthetized rat according to Dekanski (9).

RESULTS AND DISCUSSION

In the first experiment, 5 µl of hydrochloric acid extract were injected using the acetonitrile-phosphate pH 3 solvent system. Two peaks with pressor activity and two peaks with uterotonic activity were detected (Fig. 1). Table I gives the retention times for authentic hormones, bandicoot peptides, eastern native cat peptides and possum peptides.

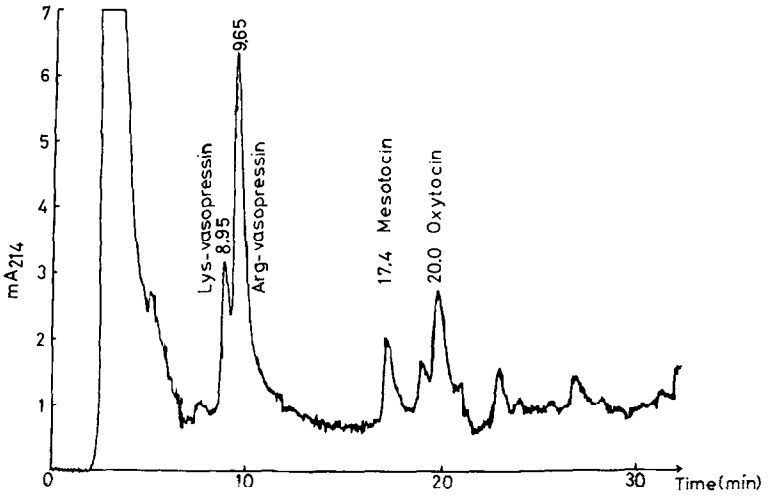

Fig. 1 :

Separation of the four neurohypophysial hormones of the Northern bandicoot (Isoodon macrourus) by HPLC using an acetonitrile-phosphate pH 3.0 gradient system.

For the second experiment 80 µl of hydrochloric extract were employed using the acetonitrile-acetate pH 5 solvent system. Two pressor peaks and two oxytocic peaks were again detected (Fig. 2). Authentic hormones were used as controls. Table II gives the results.

Identification of lysipressin, arginine vasopressin, mesotocin and oxytocin has been made by comparing in two HPLC

TABLE I

RETENTION TIMES OF AUTHENTIC HORMONES AND MARSUPIAL NEUROHYPOPHYSIAL PEPTIDES IN THE ACETONITRILE-PHOSPHATE pH 3.0 SYSTEM (RT in min)

Control		Northern bandicoot	Eastern native cat	Brush-tailed possum
Vasotocin	6.1			
Lysipressin	9.1	8.95		
Arg-vasopressin	9.8	9.65	9.65	10.10
Mesotocin	17.6	17.40	17.55	17.75
Phenypressin	18.7	(19.20)*		
Oxytocin	20.1	20.00		

* An inactive small peak was observed

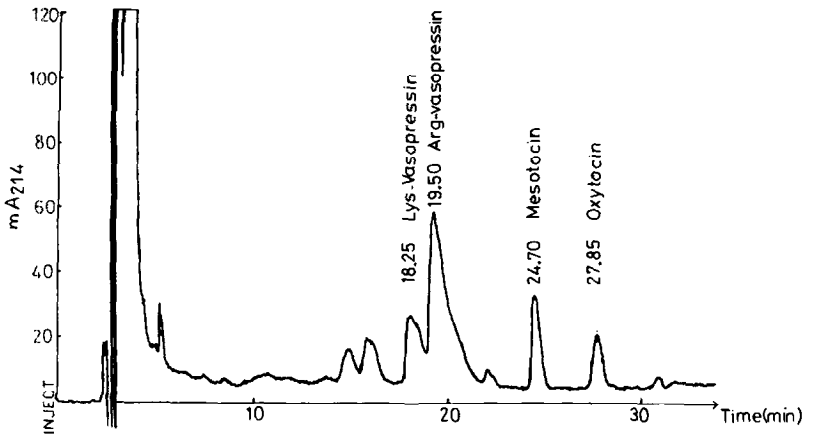

Fig. 2 :
Separation of the four neurohypophysial hormones of the Northern bandicoot (*Isoodon macrourus*) by HPLC using an acetonitrile-acetate pH. 5.0 gradient system. Yields in activities are given in Table II.

solvent system their retention times with those of authentic hormones and by comparing their molar activities with those of synthetic peptides (10). The amount in nmol of each hormone has been evaluated by comparing its peak area with that of a known amount of the corresponding authentic hormone. The calculated molar activities are for bandicoot lysipressin : 230 mU/nmol on rat blood pressure (synthetic : 285 mU), for arginine vasopressin 450 mU/nmol on rat blood pressure (synthetic : 412 mU), for mesotocin 290 mU/nmol on rat uterus (synthetic : 291 mU) and for oxytocin 430 mU/nmol on rat uterus (synthetic : 450 mU).

TABLE II

IDENTIFICATION OF THE NORTHERN BANDICOOT NEUROHYPOPHYSIAL PEPTIDES IN THE ACETONITRILE-ACETATE pH 5.0 SYSTEM

(RT : Retention times in min)

Control	Northern bandicoot								
	RT	RT	Fractions	Pressor activity		Uterotonic activity		Amount	Molar activity
	(min)	(min)	(tubes)	(U)	(yield %)	(U)	(yield %)	(nmol)	(mU/nmol)
Vasotocin	13.30								
Lysipressin	18.95	18.25	31-32	0.25	88			0.9	230
Arg-vasopressin	20.25	19.50	34-35	1.50				3.3	450
Mesotocin	25.10	24.70	41-43			0.35	74	1.2	290
Oxytocin	28.30	27.85	46-48			0.30		0.7	430

It is of great interest to note that the dual duplication of neurohypophysial hormones has already been observed in the North-American opossum (Didelphis virginiana) which possesses the same four peptides that the Australian Northern bandicoot (11). However although the molar ratio of arginine vasopressin to lysipressin is about 1 in the North-American opossum, it is about 3 in the bandicoot. In contrast the molar ratio of oxytocin to mesotocin is roughly 1 in the two species. It is difficult to allow a peculiar function to each peptide. If we assume that the dual duplication of neurohypophysial hormones is the result of two gene duplications, and if we postulate that the common ancestor of marsupials was endowed with mesotocin and arginine vasopressin, as suggested by the presence of these peptides in three Australian families, it seems likely that duplications occurred independently in Australian and American marsupials lineages. If so, the mutations giving oxytocin from a mesotocin copy or lysipressin from an arginine vasopressin copy would not be random. It should be instructive to compare on one hand the activities of oxytocin and mesotocin on marsupial mammary gland, on the other hand the activities of lysipressin and arginine vasopressin on the marsupial kidney in order to see whether the mutations have been selective or not.

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