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SOME REFLECTIONS ON THE EVOLUTION OF THE VERTEBRATE NEUROHYPOPHYSEAL HORMONES

BY

J. F. G. VLIEGENTHART AND D. H. G. VERSTEEG

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Abstract

Literature data about the vertebrate neurohypophyseal hormones are summarized. Two evolutionary routes will be proposed which bring these closely related peptides into a logical system. The hypotheses are based on the premises that a) these peptides are products of the normal protein biosynthesis; b) the genetic code is universal; c) each change in the structure of these peptides is caused by one mutation at a time.

The comparative investigation of the neurohypophyseal hormones of the various classes of vertebrates started with the discovery by POPENOE *et al.* [22], that the antidiuretic hormone of the ox and the pig are chemically different. The neurohypophyseal principles of a large number of animals have now been chemically identified, and those of several other animals were characterized by deduction from pharmacological data. In total the structures of the following six polypeptides have been established.

number	position 1 2 3 4 5 6 7 8 9
1	cys–tyr–ileu–gluNH$_2$–aspNH$_2$–cys–pro–leu–glyNH$_2$ oxytocin
2	cys–tyr–phe–gluNH$_2$–aspNH$_2$–cys–pro–arg–glyNH$_2$ arginine vasopressin
3	cys–tyr–phe–gluNH$_2$–aspNH$_2$–cys–pro–lys–glyNH$_2$ lysine vasopressin
4	cys–tyr–ileu–gluNH$_2$–aspNH$_2$–cys–pro–arg–glyNH$_2$ vasotocin = 8-arg. oxytocin
5	cys–tyr–ileu——ser——aspNH$_2$–cys–pro–ileu–glyNH$_2$ isotocin = ichthyotocin = 4-ser, 8-ileu, oxytocin
6	cys–tyr–ileu–gluNH$_2$–aspNH$_2$–cys–pro–ileu–glyNH$_2$ mesotocin = 8-ileu, oxytocin

The numerous investigations are extensively reviewed by HELLER [13]. The following picture is based on this review and later published reports.

Nr.	Animal groups	Hormones		Literature
1	Mammalia: Suina	lys. vasopressin (arg. vasopressin)	oxytocin	13
	other mammals	arg. vasopressin	oxytocin	13, 5, 30, 31
2	Aves	vasotocin	oxytocin	13, 17
3	Reptilia: Chelonia	vasotocin	oxytocin	13
	Crocodilia			13
4	Amphibia: Anura	vasotocin	mesotocin	13, 3, 11
	Urodela			13
5	Dipnoi	vasotocin	mesotocin	13,9, 11
6	Actinopterygii: Teleostei	vasotocin	isotocin	13, 10
	Holostei	vasotocin	isotocin	10
	Chondrostei	vasotocin		10
7	Chondrichtyes: Squaliformes	?	?	13
	Rajiformes			13
8	Cyclostomata	vasotocin	—	13, 10

Some comments on this table:

1. All mammals have oxytocin in common. Most mammals synthesize arginine vasopressin. However, the pig, the Hippopotamus and some other Suina form lysine vasopressin. In the posterior pituitary lobe of the white-lipped peccary, and probably of the warthog, arginine vasopressin occurs together with lysine vasopressin (FERGUSON *et al.* [8]).

2. Avian pituitary glands contain oxytocin and vasotocin. The claim of CHAUVET *et al.* [6], that a third avian, arginine vasopressin-like, hormone should exist, has not been confirmed [17, 18].

3. The neurohypophyseal principles of the reptiles have been examined only scarcely. The presence of two oxytocic substances in pituitary extracts has been shown by HELLER and PICKERING [14] by paper chromatography and pharmacological experiments. One substance behaved identical to vasotocin.

On the basis of pharmacological assays, SAWYER *et al.* [29] hold the view that the other peptide is oxytocin. These experiments do not clearly discriminate between oxytocin and the oxytocin-like peptides of the lower vertebrates. The phylogenetic position of the reptiles, however, makes it highly probable that they elaborate oxytocin.

4. ACHER *et al.* [3] demonstrated recently the presence of mesotocin besides vasotocin in the amphibian pituitary gland. It remains unclear

whether a third amphibian neurohypophyseal principle is existing. The occurrence of an arginine vasopressin-like polypeptide in pituitary extracts [2] may possibly be a consequence of the used isolation procedure (compare avian material).

The natriferic activity [16, 3], however, which is associated with (a part of?) the oxytocic fraction needs to be clarified. In their investigation of amphibian pituitary extracts FOLLETT and HELLER [11] did not give indications for the existence of a third hormone.

5. The lungfishes are closely related to the amphibians. In pituitary extracts vasotocin is demonstrated by FOLLETT and HELLER [9, 11] with paperchromatographical and pharmacological techniques. The same authors found the oxytocin-like principle in these extracts to be identical to the principle of the amphibians. We may conclude that the lungfishes synthesize vasotocin and mesotocin.

6. The bony fishes elaborate vasotocin and isotocin. In the Chondrostei, however, the occurrence of isotocin besides vasotocin is doubtful [10].

7. From the cartilagenous fishes only incomplete and sometimes contradictory data are available. HELLER and PICKERING [14] have shown the existence of two oxytocic principles in pituitary extracts of Squalus. One peptide should be related to oxytocin and the other one should differ from vasotocin.

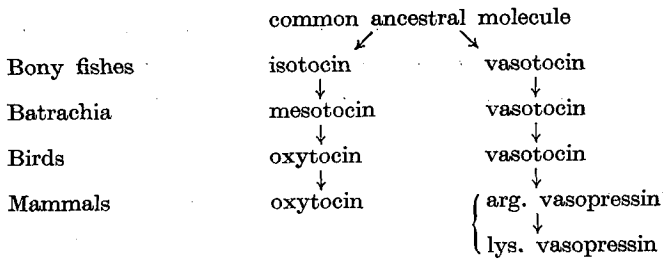
Oxytocic, milk ejection and antidiuretic activities were found in extracts of the neuro-intermediate pituitary lobes of Squalus and Raja specimen in the remarkable ratio 3:1:0,05 (PERKS *et al.* [19, 20, 21]). SAWYER *et al.* [28] estimated the pharmacological properties of two samples of Squalus neurointermediate lobe extracts.

The divergency of the assay results makes it hardly possible to find an acceptable interpretation by deduction. The relatively high activity in the frog bladder assay makes the occurrence of a vasotocin-like principle probable.

8. The results of the investigations of the most primitive vertebrates are in close harmony. The biological activities of pituitary extracts of Petromyzon are in full consistence with the occurrence of vasotocin only [28]. In Lampetra and Myxine FOLLETT and HELLER [10] could demonstrate the presence of vasotocin. With the aid of paper chromatography they could obtain no real evidence for the existence of a second polypeptide hormone in these animals. The conclusion must be, that the Cyclostomata synthesize only vasotocin.

In the Urochordata [25] such peptides could not be demonstrated, so it is obvious to regard the various chemical structures of these hormones as an aspect of the vertebrate evolution.

In the literature some attempts to outline the peptide evolution are recorded. ACHER [1] e.g. has given the following scheme:



In ACHER's view isotocin and vasotocin are the most ancient peptides of two molecular phyla, one ending in oxytocin, the other in vasopressin. The common ancestral peptide can be chemically different from the two most ancient peptides or identical with one of them. An objection to this scheme is that it does not give expression of the time scale in the evolution process.

On the ground of the exclusive occurrence of vasotocin in the Cyclostomata, SAWYER [26, 27] is of the opinion that this peptide is the most primitive one. The rest of his evolution scheme for a great part became obsolete by recent findings.

We want to suggest two alternative routes for the evolution of the neurohypophyseal hormones. In accordance to Sawyer, our starting-point is, that vasotocin is the primeval peptide. After gene doubling two peptide series are derived from vasotocin by mutation. A mutation may be either a step in the evolution as a whole, or may lead to a side-branch specific for an animal group. As framework we used a simplified vertebrate evolution scheme according to ROMER [24].

Comment on scheme A.

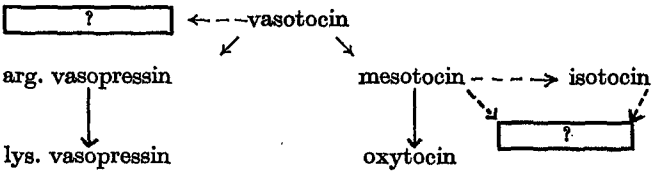
In this proposal mesotocin is the first oxytocin-like hormone. After gene doubling this peptide has been arisen from vasotocin by the mutation arg → ileu.

From the class of the Chondrichtyes we have very little information viz. only about the Elasmobranchs. We expect now that in this subclass a vasotocin analogue is occurring, which is derived from vasotocin by a point mutation. This vasotocin analogue is accompanied by mesotocin or by a mesotocin analogue. On the basis of the report of RASMUSSEN *et al.* [23] about the relation between peptide structure and amphibian urinary bladder activity, we suggest, that for the vasotocin analogue conversions on the 4 or 8 place can be taken in consideration. It would be interesting to investigate the biological properties of 4-ser, 8-arg oxytocin (compare 4, 12, 23).

Isotocin is an oxytocin-like hormone specific for the Actinopterygii. In the present scheme it is concluded to be derived from mesotocin by the mutation gluNH$_2$ → ser.

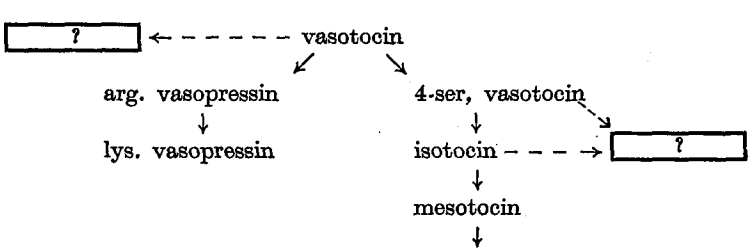
SCHEME A

Fossil		Recent	
Animal groups	Hormones	Animal groups	Hormones
Agnatha	vasotocin	*Cyclostomata*	vasotocin
Ostracodermi	vasotocin ?		
Placodermi	vasotocin mesotocin	*Chondrichtyes*	? ?
Osteichtyes	vasotocin mesotocin	*Actinopterygii*	vasotocin isotocin
Sarcopterygii	vasotocin mesotocin	*Dipnoi*	vasotocin mesotocin
Prim. Amphibians	vasotocin mesotocin	*Amphibia*	vasotocin mesotocin
Prim. Reptiles	vasotocin oxytocin	*Reptilia* *Aves*	vasotocin oxytocin vasotocin oxytocin
Prim. Mammals	arg. vasopressin oxytocin	*Suina* *other Mammalia*	lys. vasopressin oxytocin arg. vasopressin oxytocin



SCHEME B

Fossil		Recent	
Animal groups	Hormones	Animal groups	Hormones
Agnatha	vasotocin	*Cyclostomata*	vasotocin
Ostracodermi	vasotocin 4-ser-vasotocin (glu NH_2 → ser, 4)		
Placodermi	vasotocin isotocin (arg → ile, 8)	*Chondrichtyes*	? ?
Osteichtyes	vasotocin isotocin	*Actinopterygii*	vasotocin isotocin
Sarcopterygii	vasotocin mesotocin (ser → glu NH_2, 4)	*Dipnoi*	vasotocin mesotocin
Prim. Amphibians	vasotocin mesotocin	*Amphibia*	vasotocin mesotocin
Prim. Reptiles	vasotocin oxytocin (ile → leu, 8; ile → phe, 3)	*Reptilia* *Aves*	vasotocin oxytocin vasotocin oxytocin
Prim. Mammals	arg. vasopressin oxytocin	*Suina* (arg → lys, 8) *Other Mammalia*	lys. vasopressin oxytocin arg. vasopressin oxytocin



Comment on scheme B.

Here we consider isotocin to be the most ancient oxytocin-like peptide. It differs from vasotocin on two positions. The supposition, that 4-ser, vasotocin (= 4-ser, 8-arg, oxytocin) is an intermediate, seems indispensable. After gene doubling gluNH$_2$ → ser is the first mutation.

In this case we should expect in the Elasmobranchs a vasotocin analogue together with isotocin or an isotocin analogue. The mentioned analogues are thought to be derived from the corresponding ancestors by single mutations.

A modification of this scheme can be obtained by supposing that vasotocin together with 4-ser, vasotocin have still been present in the Placodermi. In the Elasmobranchs a vasotocin analogue and 4-ser, vasotocin or a derivative thereof could then occur.

A possible objection to our scheme B may be that the mutation gluNH$_2$ → ser must be turned back later in the development.

Information about the neurohypophyseal hormones of representatives of a large number of animal groups is necessary to corroborate our hypotheses. We have some preference for the scheme A.

It is worthy to note that the structural evolution of these hormones seems to be the consequence of single base conversions in the amino acid code. Accepting the genetic code is universal; we can demonstrate this e.g. with the symmetrically degenerate triplet code of Eck [7], which has been modified according to the findings of Matthei *et al.* [15]. The conversions in the neurohypophyseal hormone evolution must have been the following:

Nr.	Position of altered amino acids in the peptide	Amino acid change	Code change	Base change
1	3	ileu → phe	UUA → UUU UCA → UCU	A → U
2	4	gluNH$_2$ → ser	UAC → GAC UGC → GGC	U → G
3	8	arg → ileu	GAA → UAA GGA → UGA	G → U
4	8	ileu → leu	UAA → UAU UGA → UGU	A → U
5	8	arg → lys	GAA → AAA GGA → AGA	G → A

In the cases 1, 2, 3, and 4 we meet the conversion of a purine into a pyrimidine or vice-versa. Only in the case 5 the change is from a purine

into another purine. Perhaps data from the genetic code shall be useful to find out the relationship between new peptides and those already known.

Addendum

After preparing the manuscript, some new data are presented by W. H. SAWYER, Endocrinology **75**, 981 (1964) and by A. M. PERKS and W. H. SAWYER, Nature **205**, 154 (1965).

a.) SAWYER has examined the neurohypophyseal hormones of representatives of the Cyclostoma, the cartilogenous fishes, the bony fishes and the lungfishes. His conclusions are summarized in the following table.

Animal groups	Species	Hormones	
Cyclostomes	river lamprey	vasotocin	
Chondrichtyes: Elasmobranchs	Squalus	unknown	
	Raja ocellata	unknown but different from Squalus	
Holocephalians	Hydrolagus collei	vasotocin	oxytocin
Actinopterygians	Polypterus (a very primitive bony fish)	vasotocin	mesotocin
Dipnoi	Protopterus	vasotocin	oxytocin

SAWYER's conclusion about the occurrence of oxytocin in the lungfishes is contradictory to the opinion of FOLLETT and HELLER [11], that mesotocin is present in these animals. His arguments for the presence of either oxytocin or mesotocin are based on the biological activity ratio's rat uterus with Mg^{++}: rat uterus without Mg^{++}, and milk ejection: rat uterus without Mg^{++}. FOLLETT and HELLER [11] have shown that these ratio's for mesotocin are not significantly different from oxytocin. These authors found, however, that the ratio chicken bloodpressure: rat uterus without Mg^{++} differs significantly for these peptides. For convincing evidence, that oxytocin occurs in lower vertebrates this ratio from oxytocic fractions free of vasotocin is necessary; it has not been determined, however.

SAWYER interpretes the presence of oxytocin in Hydrolagus collei by suggesting that oxytocin as well as mesotocin descend from vasotocin directly. This means that the conversion arg → leu *and* the conversion arg → ileu should have occurred. This suggestion does not fit into the present state of knowledge of the genetic code. In Eck's model the code for arg can be [GAA] or [GGA]. By the single base change G → U the code for ileu: [UAA] or [UGA] can be obtained, but no single base change is sufficient to reach the code for leu. In this view oxytocin cannot be derived from vasotocin directly.

Considering [GCC] or [GUC] as code for arg, the single base change

G → U can give rise to the code [UCC] or [UUC] for leu. The code for ileu cannot be obtained by a single base conversion in [GCC] or [GUC]. As this code for arg cannot be converted to the code for lys by a point mutation, we prefer to reject SAWYER'S suggestion.

b.) PERKS and SAWYER have isolated the oxytocic principle of the skate Raja ocellata and have found the amino acid composition: asp, $(cys)_2$, glu, gly, ileu, pro, ser and tyr. They consider for this peptide the two following structures:

1. cys–tyr–ser–gluNH_2–aspNH_2–cys–pro–ileu–glyNH_2
   3–ser, 8–ileu, oxytocin

2. cys–tyr–ileu–gluNH_2–aspNH_2–cys–pro–ser–glyNH_2
   8–ser, oxytocin

We want to suggest two other possible structures:

3. cys–tyr–gluNH_2–ser–aspNH_2–cys–pro–ileu–glyNH_2
   3–gluNH_2, 4–ser, 8–ileu oxytocin

4. cys–tyr–ileu–ser–aspNH_2–cys–pro–gluNH_2–glyNH_2
   4–ser, 8–gluNH_2 oxytocin

It is unclear whether vasotocin, mesotocin or isotocin is the ancestor of the Raja ocellata peptide. We desist from giving the derivation of the four alternative peptide structures from their possible ancestors, as this is complicated and speculative. We only want to propose that

cys–tyr–ileu–gluNH_2–aspNH_2–cys–pro–gluNH_2–glyNH_2, (8–gluNH_2 oxytocin) is an interesting intermediate for structure 4.

Laboratory of Organic Chemistry,
University Utrecht,
Croesestraat 79, Utrecht
The Netherlands

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