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THE EVOLUTION OF THE VERTEBRATE NEUROHYPOPHYSIAL HORMONES IN RELATION TO THE GENETIC CODE

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SUMMARY

An outline is presented of the phyletic distribution of the neurohypophysial hormones and of the genetic code. The evolution of these peptides is proposed to have proceeded along the known lines of protein evolution. Based on this premise a scheme is elaborated for the evolution of the structures of the neurohypophysial hormones. Structures for some unknown intermediates are suggested.

INTRODUCTION

The evolution of the neurohypophysial hormones has been the subject of several papers (Acher, 1963, 1965; Follett & Heller, 1964*b*; Sawyer, 1964, 1965; Vliegenthart & Versteeg, 1965*a*). The phyletic distribution of the hormones forms the principal basis of these reports. The evidence provided by Sachs & Takabatake (1964), that the precursor of arginine vasopressin is probably a product of protein biosynthesis, leads to a new approach to the problem and suggests that the observations concerning the biosynthetic origin of arginine vasopressin should be extended to the related peptides. Thus the evolution of the neurohypophysial hormones may have taken place along the same lines as the evolution of proteins [e.g. haemoglobins (Ingram, 1962; Hill, Buettner-Janush & Buettner-Janush, 1963)]. The identity of the precursor of arginine vasopressin is not exactly known but Sachs & Takabatake (1964) suggested that it is a protein, from which the hormone is split off. It is also possible, however, that the precursor is a peptide, which needs to be enzymically converted to become (fully) active.

When dealing with protein evolution, the genetic code has to be considered. Recent advances in this field of study (Nirenberg, Leder, Brimacombe, Trupin, Rottman & O'Neil, 1965; Brimacombe, Trupin, Nirenberg, Leder, Bernfield & Jaouni, 1965; Söll, Ohtsuka, Jones, Lohrmann, Hayatsu, Nishimura & Khorana, 1965; Matthaei, Heller, Voigt, Kleinkauf, Küntzel, Vogt & Matthaei, 1965) have made the connexion between a defined codeword and an amino acid considerably less hypothetical. The substitution of one amino acid for another in a polypeptide chain may be the result of a single base change in the corresponding codeword. An important part of the evolutionary alterations in proteins can be referred to such point

mutations. Since all neurohypophysial hormones have the same chain length, only this type of mutation needs to be considered.

The identity of the neurohypophysial hormones of a large number of species is still uncertain and so are some points of the genetic code. However, integration of the data now available may be of value in the tracing of unknown evolutionary links. A preliminary discussion of this subject has already been presented (Vliegenthart & Versteeg, 1965*b*).

THE GENETIC CODE

The linear sequence of bases in deoxyribonucleic acid (DNA) forms the information for the specific amino acid sequence of polypeptide chains. Translation of the information is mediated through messenger ribonucleic acid (m-RNA), whose nucleotide sequence is a complementary transcription of that in the DNA. In m-RNA the pyrimidine bases uracil (U) and cytosine (C) and the purine bases adenine (A) and guanine (G) occur. A unit of three consecutive bases (= triplet = codon) forms the codeword for an amino acid. With four different bases 64 ($= 4^3$) triplets are possible.

The first steps in the elucidation of the genetic code, i.e. the establishment of the coding properties of the 64 m-RNA triplets, were performed with synthetic homo- and copolynucleotides in a cell-free system from *Escherichia coli* (Nirenberg & Matthaei, 1961; Nirenberg & Jones, 1963; Ochoa, 1963). The results made it highly probable that the code is degenerate, i.e. that one amino acid may be represented by more than one codon. The sequences of 56 of the possible 64 codons have been reported by Nirenberg and his associates (Leder & Nirenberg, 1964; Nirenberg *et al.* 1965; Brimacombe *et al.* 1965) and these findings support the postulate of Eck (1963) that the code is degenerate according to a systematic pattern. The 64 triplets can be divided into two series of 16 pairs. The bases in the first and second position of the triplets of a pair are identical. In 16 pairs the third position in one triplet of every pair is occupied by A, in the other triplet by G. In the remaining 16 pairs C and U are in the third position. In general, the triplets of a given pair are coding for the same amino acid (see Fig. 1; Versteeg & Vliegenthart, 1965).

The results of Khorana and co-workers (Söll *et al.* 1965), Salas, Smith, Stanley, Jr., Wahba & Ochoa, (1965) and of Matthaei *et al.* (1965), confirm in general the findings of Nirenberg, that all the possible codons have been ascribed to amino acids with two exceptions. Evidence is accumulating that the triplets UAA and UAG are 'nonsense' codons, i.e. codons that are not coding for any amino acid, at least in *E. coli* (Brenner, Stretton & Kaplan, 1965; Weigert & Garen, 1965). Mutants of *E. coli* have been isolated in which the nonsense mutations are suppressed, with the effect that serine, glutamine, tyrosine and probably leucine, tryptophan, lysine or glutamic acid (see Fig. 1) are incorporated in the nonsense position (Capecchi & Gussin, 1965; Kaplan, Stretton & Brenner, 1965; Galluci & Garen, 1966).

Results obtained with cell-free systems from bacterial and non-bacterial origin leave little doubt that the genetic code is universal (Basilio, Bravo & Allende, 1966). Furthermore, all the known amino acid substitutions in the abnormal human haemoglobins can be explained in terms of single base conversions in the engaged codewords (Beale & Lehmann, 1965).

Chemical structures and phyletic distribution of the neurohypophysial hormones

The structures of seven naturally occurring neurohypophysial hormones have so far been chemically identified (Table 1). The numerous investigations concerning the phyletic distribution of these hormones are extensively reviewed by Heller (1963). The following consideration is based on his review and on some later reports. The most primitive vertebrates are the Cyclostomata. They seem to synthesize only one active principle. On the basis of the close resemblance between the pharmacological

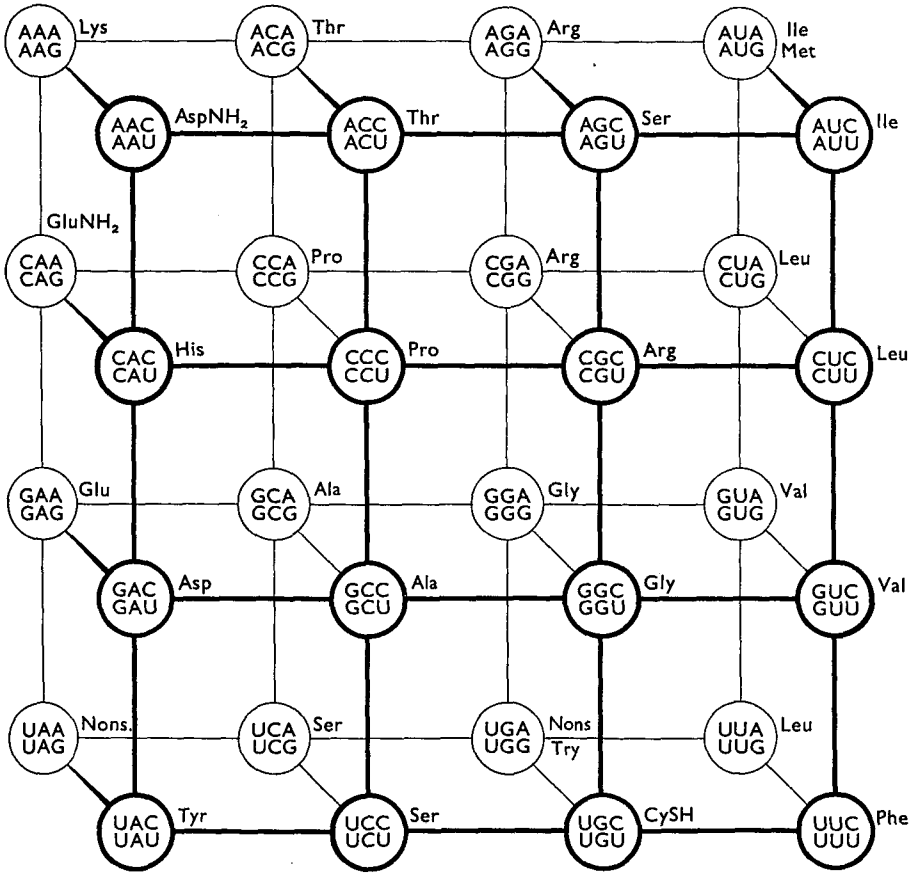

Fig. 1. Spatial diagram of the genetic code. The consequence of single base conversion in a codon, with respect to amino acid coding, can be read off on the three lines crossing the codon. AUA = isoleucine (Wahba *et al.* 1966), AUG = methionine (Nirenberg *et al.* 1965), UAA, UAG and UGA = nonsense (Brenner *et al.* 1965; Weigert & Garen, 1965; Brenner Barnett, Katz & Crick, 1967). For the remaining codons see: Brimacombe *et al.* (1965); Matthaei *et al.* (1965); Söll *et al.* (1965).

properties of pituitary extracts of *Petromyzon marinus* and of synthetic vasotocin, Sawyer, Munsick & Van Dyke (1961) suggested this hormone to be identical with vasotocin. A further examination (Sawyer, 1965) of this extract by chromatography on carboxymethylcellulose columns corroborated this opinion. Follett & Heller (1964*a*) investigated pituitary extracts of the cyclostomes *Lampetra fluviatilis* and

Myxine glutinosa by paper chromatographic and pharmacological methods. One clear spot was detected with the characteristics of vasotocin.

The class Chondrichthyes has separated from the main line of vertebrate evolution at an early stage. Representatives of the subclasses Elasmobranchii and Holocephali have been examined. Acher, Chauvet, Chauvet & Crepy (1965) have isolated two active principles from the ray *Raia clavata*. The hormone present in the most abundant concentration appeared to be 4-Ser,8-GluNH_2 oxytocin. The chromatographical and biological properties of the second hormone seemed to match those of vasotocin.

Table 1. *Chemical constitution of identified neurohypophysial hormones*

No.	Position 1 2 3 4 5 6 7 8 9
1	Cys-Tyr-Ile--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-Ile--GluNH_2-AspNH_2-Cys-Pro-Arg------ GlyNH_2
	vasotocin = 8-Arg oxytocin
5	Cys-Tyr-Ile--Ser-------AspNH_2-Cys-Pro-Ile--------GlyNH_2
	isotocin = ichthyotocin = 4-Ser,8-Ile oxytocin
6	Cys-Tyr-Ile--GluNH_2-AspNH_2-Cys-Pro-Ile--------GlyNH_2
	mesotocin = 8-Ile oxytocin
7	Cys-Tyr-Ile--Ser-------AspNH_2-Cys-Pro-GluNH_2-GlyNH_2
	glumitocin = 4-Ser,8-GluNH_2 oxytocin

These authors reported that the ratio oxytocic activity with Mg^{2+}:oxytocic activity without Mg^{2+} was 10 for 4-Ser,8-GluNH_2 oxytocin. In a further paper Chauvet, Chauvet, Beaupain & Acher (1965) the same ratio was obtained; they reported also that 4-Ser,8-GluNH_2 oxytocin has a higher R_F than their second principle in paper chromatography in the system *n*-butanol:acetic acid:water (4:1:5). Heller & Roy (1965*a*, *b*) too investigated extracts of the neuro-intermediate lobe of *Raia clavata* by paper chromatography in the same system. Two active fractions were obtained. The oxytocic ratio for the slower moving component was $6{\cdot}14 \pm 0{\cdot}94$ and for the fast moving $2{\cdot}07 \pm 0{\cdot}27$. Natriferic assays indicated that only the fast-moving component had such an activity. Thus the identity of the second hormone of *Raia clavata* is not quite clear. The hormones of *Scyliorhinus caniculus* (Heller & Roy, 1965*b*) and of *Raia batis* (Chauvet *et al.* 1965) seem to have a close resemblance to those of *Raia clavata*. Perks & Sawyer (1965) isolated an oxytocic fraction from pituitary extracts of *Raia ocellata*, which had biological properties clearly differing

from those of other elasmobranchs that have been studied. It had a higher specific oxytocic activity than 4-Ser,8-GluNH$_2$ oxytocin. However, its amino acid composition seemed to be identical with that of 4-Ser,8-GluNH$_2$ oxytocin. It is possible that the active component was heavily contaminated with weakly active 4-Ser,8-GluNH$_2$ oxytocin (W. H. Sawyer, personal communication). The slow-moving component of *Negaprion brevirostris* (lemon shark) may be different from that of *Raia clavata* (Heller & Roy, 1965*b*). Two active principles are present in *Squalus acanthias* (Heller & Pickering, 1961; Sawyer, 1964, 1965; Acher *et al.* 1965). The properties of one peptide matched almost exactly those of vasotocin (Sawyer, 1965). The identity of the other hormone is still unknown. It is clearly not 4-Ser,8-GluNH$_2$ oxytocin. The biological activities of purified pituitary extracts of the Holocephaleans *Hydrolagus collei* (Sawyer, 1964, 1965) and of *Chimaera monstrosa* (Heller & Roy, 1965*b*) indicate that in these animals probably other active peptides are present. Sawyer (1964) assumed that one principle might be identical to oxytocin. Thus it seems that a family of neurohypophysial hormones occurs in the cartilaginous fishes, to which 4-Ser,8-GluNH$_2$ oxytocin and possibly vasotocin and oxytocin belong.

The Actinopterygii can be divided into three main groups: the Teleostei, the Holostei and the Chondrostei. In teleostean species two active neurohypophysial hormones have been demonstrated: vasotocin and 4-Ser,8-Ile oxytocin (see Heller, 1963). Follett & Heller (1964*a*) compared the hormones of the three groups of the bony fishes. The hormones of the holosteans seemed to be also vasotocin and 4-Ser,8-Ile oxytocin. In the Chondrostei, however, only vasotocin was present. No or doubtful indications were found for the occurrence of a second hormone. Of great interest is the very primitive bony fish *Polypterus*, in which Sawyer (1964) showed the occurrence of vasotocin and of another oxytocic peptide, whose pharmacological characteristics resembled those of 8-Ile oxytocin.

The Dipnoi are closely related to the amphibians. Follett & Heller (1964*b*) examined the neurohypophysial hormones of the species *Protopterus aethiopicus* and *Neoceratodus forsteri*. The occurrence of vasotocin could be well established pharmacologically. The biological properties of the other oxytocic principle found seemed to be different from those of isotocin (4-Ser,8-Ile oxytocin) and of oxytocin. However, a close resemblance to the oxytocic hormone of the amphibians was observed, making not unlikely that the lungfishes investigated by Follett & Heller elaborated 8-Ile oxytocin. Sawyer (1964) too investigated the oxytocic peptide of *Protopterus*, but found it to be pharmacologically indistinguishable from oxytocin.

Acher, Chauvet, Chauvet & Crepy (1964*a*) chemically identified vasotocin and 8-Ile oxytocin in the amphibian *Rana esculenta*. Pharmacological support for the occurrence of these peptides in *Necturus maculosus*, *Triturus alpestris* and *Bufo bufo* was obtained by Follett & Heller (1964*b*), and in *Xenopus laevis* by Follett & Heller (1964*b*) and Acher, Beaupain, Chauvet, Chauvet & Crepy (1964*b*). Munsick (1966) purified the hormones of *Rana pipiens* chromatographically. On the basis of their pharmacological properties, the peptides seemed to be indistinguishable from vasotocin and oxytocin.

The hormones of the Reptilia have been little investigated. Heller & Pickering (1961) demonstrated the presence of two active principles. Munsick (1966) investigated the hormones of *Crotalus atrox* by chromatographical and pharmacological methods.

Two active peptides were again found, one seemed to be identical with vasotocin, the other with 8-Ile oxytocin.

The Aves elaborate vasotocin and oxytocin.

The neurohypophysial hormones of the Mammalia have been extensively investigated. All mammals investigated have oxytocin in common. With the exception of the pig-like animals, arginine vasopressin could be identified as the antidiuretic hormone (Ferguson & Heller, 1965). Lysine vasopressin was found in the Suiformes, sometimes together with arginine vasopressin. Single posterior pituitary lobes of the white lipped peccary, for example, contained either lysine or a mixture of arginine vasopressin and lysine vasopressin.

Discussion of the peptide evolution

The occurrence of vasotocin only in the Cyclostomata supports the suggestion that this hormone is the developmentally oldest peptide (Sawyer, 1964; Vliegenthart & Versteeg, 1965*a*). This, and the fact that in all other vertebrate classes two neurohypophysial hormones occur, suggests that two peptide series may have evolved after doubling of the vasotocin controlling gene. In the first series vasotocin remained unchanged until the appearance of the primitive Mammalia. The other series may consist of a variety of oxytocic principles derived from vasotocin by consecutive mutations.

(*a*) *The vasotocin series*

Up to and including the Aves, vasotocin appears to be present in every vertebrate class, with the possible exception of the Chondrichthyes.

In the primitive Mammalia vasotocin seems to have been converted to arginine vasopressin by the substitution of phenylalanine for isoleucine in position 3. This mutation can be explained by the single base change A → U in the triplets AUC or AUU coding for isoleucine, resulting in the triplets UUC or UUU for phenylalanine.

Lysine vasopressin is an aberrant hormone, specific for the Suina, which originated from arginine vasopressin by the replacement of arginine by lysine in position 8. The fact that this mutation could occur suggests that of the various codewords for arginine only AGA or AGG can be considered for arginine in arginine vasopressin since triplets coding for lysine cannot be obtained by a single base change in the other codons for arginine.

(*b*) *The oxytocin-like hormones*

The diversity in structures of the oxytocic principles makes it difficult to assign ancestors for each hormone, and to determine the most ancient derivative of vasotocin.

It seems that in the cartilaginous fishes, the Chondrichthyes, oxytocic hormones are present besides vasotocin for the first time. The variety of oxytocic peptides in this class suggests that a cluster of peptides has been evolved in the cartilaginous fishes independently from the main line of evolution. The structure of 4-Ser,8-GluNH_2 oxytocin, together with the indications for the presence of vasotocin and oxytocin, may suggest that 8-GluNH_2 oxytocin and 4-Ser,8-Arg oxytocin could also be members of this family. Thus glutamine and probably leucine may be found in position 8

instead of arginine. Codewords for these amino acids can be obtained by single base conversions in the codons CGA or CGG for arginine, yielding respectively CAA or CAG for glutamine and CUU or CUG for leucine. Here another codeword for arginine may have been involved than in the vasotocin series. The codewords CGA or CGG for arginine, however, can be converted to the arginine codons AGA or AGG by a point mutation (see Fig. 2).

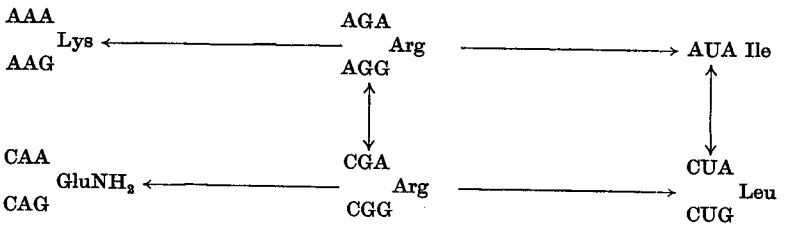

Fig. 2. Amino acid replacements in position 8, with the corresponding conversions in the codons.

For the derivation of 4-Ser,8-GluNH$_2$ oxytocin the substitution serine for glutamine in position 4 might have taken place either in 8-GluNH$_2$ oxytocin or in vasotocin. The alteration of CAA or CAG coding for glutamine in codewords for serine needs at least two steps. The most probable way may have been the conversion to the codons UCA or UCG for serine. CGA or CCG coding for proline or the nonsense codons UAA or UAG are possible intermediates. There are no indications in favour of either of these alternatives. Nonsense mutations in *E. coli* can be suppressed (see above), but for higher organisms there is no information available on this point.

The origin of 4-Ser,8-Ile oxytocin in the Holostei and Teleostei would be easy to explain from its phyletic distribution. 8-Ile oxytocin is probably present in the primitive Actinopterygian *Polypterus* and in the higher animals like the Dipnoi and the Amphibia. This suggests that 4-Ser,8-Ile oxytocin derives from 8-Ile oxytocin and that it represents a side-line specific for the higher bony fishes. As already pointed out, however, the substitution of glutamine by serine might have taken place by way of either proline or nonsense as an intermediate. The phylogenetic position of the Chondrostei in combination with the pharmacological properties of pituitary extracts of Acipenser and Polyodon species (Follett & Heller, 1964*a*) suggest that the gene controlling the intermediate between 8-Ile oxytocin and 4-Ser,8-Ile oxytocin may occur in these animals (see Fig. 3).

The origin of 8-Ile oxytocin is not easy to determine. Possibly vasotocin is its ancestor. A single base change in the codeword AGA for arginine can give rise to the codeword AUA for isoleucine. The same codon for arginine would be involved as in the pressor series for the replacement of arginine by lysine. This interpretation presumes that immediately after doubling of the vasotocin controlling gene a bifurcation took place, the first branch leading to the hormones of the Chondrichthyes, the other to those of the Actinopterygii. This view fits the facts known at the moment but it cannot be excluded that the evolutionary process has been more complicated, with perhaps one or more intermediates between vasotocin and 8-Ile oxytocin. It is even possible to consider oxytocin as an intermediate between these two hormones by the way of CGA as codon for arginine in vasotocin, since a single base conversion

can result in the codon CUA for leucine, which could be altered to AUA coding for isoleucine (see Fig. 2).

In the Dipnoi, the Amphibia and probably the Reptilia both oxytocin and 8-Ile oxytocin may be present. On the basis of the phyletic distribution it is not possible

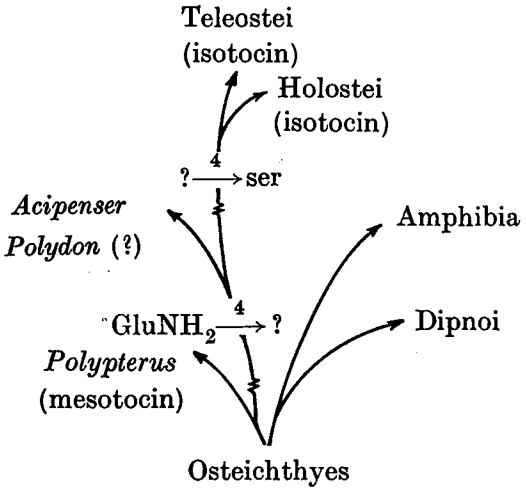

Fig. 3. Suggested derivation of 4-Ser,8-Ile oxytocin.

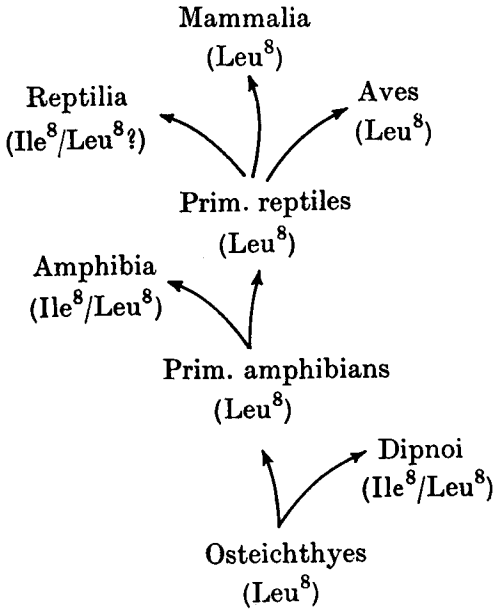

Fig. 4

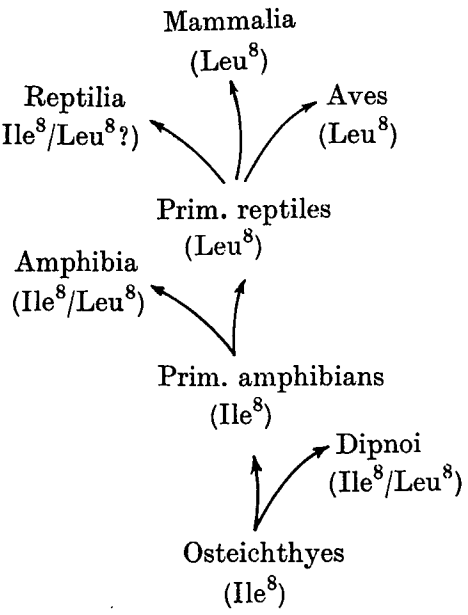

Fig. 5

Fig. 4. Suggested derivation of oxytocin, starting with the triplet CGA coding for arginine in vasotocin.

Fig. 5. An alternative for the suggested derivation of oxytocin starting with the triplet AGA coding for arginine in vasotocin.

to decide which of these peptides is the developmentally oldest (Sawyer, 1964, 1965; Munsick, 1966). The genetic code too, does not give much information on this point. There are two alternatives for the triplet coding for arginine in vasotocin in the main-line of evolution. Starting with the arginine codon CGA, oxytocin would appear in the main-line. 8-Ile oxytocin would always represent a side-line, which has in each case arisen *de novo* (Fig. 4).

The implication of the alternative arginine codon (AGA) would be that 8-Ile oxytocin represents the oxytocin-like principle of the main-line. The occurrence of oxytocin in the Dipnoi and the Amphibia would be the result of separate mutations. Since oxytocin is present in the Mammalia and the Aves it is reasonable that this peptide occurred in their common ancestors, the primitive reptiles (Fig. 5). As mentioned before, the two arginine codons CGA and AGA are related by a single base mutation. For their relation with leucine and isoleucine codons see Figs. 1 and 2.

Information about the neurohypophysial hormones of a great number of further representatives of all vertebrate groups, especially the Chondrichthyes, the Chondrostei, the Amphibia and the Reptilia, will be indispensable for a more definite answer to the evolutionary questions.

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