

Amino acid analysis of the purified fraction gave the following mole ratios: Asp<sub>1.1</sub>, Cys<sub>1.4</sub>, Glu<sub>1.0</sub>, Gly<sub>1.0</sub>, Ile<sub>1.4</sub>, Leu<sub>0.6</sub>, Tyr<sub>0.7</sub>, Pro<sub>0.9</sub> and small amounts (< 0.2 mole/mole Gly) of Ala, Ser, Thr and Val.

The amino acid analysis is compatible with an equimolar mixture of oxytocin and 8-Ile-oxytocin. The oxytocin activity of such a mixture would be potentiated 1.11 times by the presence of Mg<sup>2+</sup> and the ratio of chicken vasodepressor to oxytocic activity without Mg<sup>2+</sup> would be 1.23. Thus it appears that both these neurohypophysial hormones are present in cobra pituitaries.

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#### **Amino acid replacements in the neurohypophysial hormones during vertebrate evolution.** By D. H. G. VERSTEEG and J. F. G. VLIAGENTHART. *Afdeling Farmacologie and Afdeling Organische Chemie, R.U. Utrecht*

As pointed out previously (Vliegenthart & Versteeg, 1965) it is reasonable to propose that the neurohypophysial hormones are products of protein biosynthesis. The evolution of these nonapeptides must have taken place along the same lines as the evolution of the proteins. Recent advances in the study of the genetic code corroborated the idea of the universality of the code. The evolution of a protein with constant chain length can be conceived as a consequence of amino acid substitutions, which are the result of single base changes in the codons in messenger RNA.

The amino acid sequences of seven naturally occurring hormones have been reported: vasotocin, arginine vasopressin and lysine vasopressin (pressor series); oxytocin, 8-isoleucine oxytocin, 4-serine, 8-isoleucine, oxytocin and 4-serine, 8-glutamine oxytocin (oxytocic series).

The aim of the present report is to arrange these peptides according to their evolutionary appearance i.e. to trace the stages of amino acid replacements by means of the genetic code.

Vasotocin is considered as the developmentally oldest peptide, because of its occurrence as the single hormone in the Cyclostomata. The existence of two neurohypophysial hormones in representatives of the other vertebrate classes suggests that two peptide series may have evolved after doubling of the vasotocin controlling gene. In the pressor series two amino acid replacements are observed. In position 3, isoleucine has been substituted by phenylalanine, probably in the primitive mammals. This has been a point mutation: AUC or AUU → UUC or UUU. In the primitive *Suina* there has been the further mutation arginine to lysine in position 8. Only from the triplets AGA or AGG a codon for lysine (AAA or AAG) can be obtained.

In the oxytocic series the situation is more complicated by the great diversity in oxytocic principles. Besides that it is highly probable that other oxytocic peptides

exist. Furthermore, it is not necessarily so, that two peptides differing in one amino acid are directly related by a point mutation. The genetic code alone does not make it possible to determine the sequence of amino acid substitutions starting from vasotocin. Knowledge of the phyletic distribution of these peptides is quite indispensable in this respect.

Amino acid replacements are found in the positions 4 and 8. In two peptides, present in different side-lines, glutamine in position 4 has been substituted by serine. For this mutation at least 2 base changes have been necessary.

The amino acids occurring in position 8 are arginine, in the primeval peptide vasotocin, and glutamine, isoleucine and leucine in the evolutionary younger hormones. We suggest that the arginine codon pair CGA/CGG may have been involved in the substitution of arginine by glutamine (CAA/CAG) and possibly by leucine (CUA/CUG). The codon for isoleucine AUA can be obtained by a point mutation in the codeword AGA for arginine, or by a point mutation in the triplet CUA coding for leucine. Although we cannot discriminate between these alternatives, it is worthy of note that the codon AGA for arginine has also been involved in the replacement of arginine by lysine in the pressor series. The triplets AGA and CGA for arginine can be converted in each other by a one step mutation. The significance of 4-serine, 8-arginine oxytocin and 8-glutamine oxytocin as possible intermediates was considered.

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#### **Endocrine aspects of nutrition and excretion in the Colorado beetle.** By J. DE WILDE. *Laboratorium voor Entomologie, Landbouwhogeschool, Wageningen*

In the course of experiments on the neuro-endocrine control of reproduction in *Leptinotarsa*, we have made some observations on endocrine aspects of some vegetative functions.

It had been described in literature, that the female Colorado beetle, upon feeding with senescent leaves, soon ceases oviposition. This phenomenon has been related to nutritional deficiency, resulting in a lack of material for yolk production. We have found that implantation of active corpora allata in these beetles results in a marked increase in the rate of oviposition. This points to an endocrine rather than a nutritive deficiency.

Upon electrocoagulation of the medial neurosecretory cells, we have found that in about 30 % of the operated beetles a marked swelling occurs, leading to immobilization. This phenomenon, on closer examination, appeared to be due to retention of body water after feeding of potato leaves. Further experiments showed that retention of body water also occurs when the ventral nerve cord is severed at different levels. The possibility thus remains open that the brain lesions described above in some way interfere with the innervation of the cryptonephridial system which in *Leptinotarsa* mainly regulates water excretion.