## Position of the Methionine Residue in Bovine Neurophysin II[1]

In 1942 VAN DYKE et al.[2] described a protein fraction from bovine posterior pituitary lobes which exhibited oxytocic, pressor and antidiuretic activities. Subsequently, studies[3] have shown that this 'VAN DYKE protein' consists of neurohypophyseal hormones associated in non-covalent linkage with several closely related proteins, which CHAUVET et al.[4] designated as neurophysin. Evidence has accumulated which suggests that under a variety of conditions neurophysins reversibly form aggregates. For bovine neurophysins a monomeric molecular weight of approximately 10,000 has been reported[5]. One of the major components of the neurophysin fraction, neurophysin II, has been described by HOLLENBERG and HOPE[6]; a conspicuous feature of this protein is its single methionine residue. The successful scission of the amide chain at the carboxyl site of a methionine residue by the non-enzymatic cleaving reagent cyanogen bromide[7] has been demonstrated with many peptidyl substrates[8]. It was hoped that the cleavage of neurophysin II at the methionine position would result in 2 fragments of suitable size for a variety of further studies.

With this as background neurophysin II was purified by a modification of the procedure of HOLLENBERG and HOPE[6], and checked for the absence of neurohypophyseal hormones by avian vasodepressor[9] and rat pressor assay[10], and by the lack of precipitation with specific antibodies to oxytocin[11]; neurophysin II was tested for homogeneity by disc electrophoresis[12], isoelectric focusing[13] and amino acid analysis[14]. This material (10 mg), dissolved in 6 ml of 0.1 *N* HCl, was allowed to react with 60 mg of cyanogen bromide. The mixture, stirred until a homogenous solution resulted and allowed to stand at room temperature for 16 h, was diluted with 12 ml of glass distilled water and lyophilized. This powder was then taken up in a minimal amount of *N* formic acid and subjected to gel filtration on a Sephadex G-75 column. Elution with *N* formic acid resulted in two UV-absorbing peaks (254 nm); the first (CNBr-1) emerged about midway between the void- and bed-volume and the second (CNBr-2) at the bed-volume. The N-terminal of CNBr-1 was found to be serine. Amino acid analysis after acid hydrolysis with 6*N* HCl at 110 °C for 22 h showed that CNBr-2 contained 2 ninhydrin-active components, alanine and homoserine lactone, the latter resulting from methionine during the cyanogen bromide reaction. These results confirm that alanine is the N-terminal residue of bovine neurophysin II[5a] and show that methionine occupies the penultimate N-terminal position, and serine the third position, in this protein.

*Zusammenfassung.* Durch Spaltung mit CNBr wurde gezeigt, dass Neurophysin II aus Rinderhypophyse nur eine Methionin-Einheit enthält und dass die N-terminale Sequenz Ala-Met ist.

D. H. SCHLESINGER[15], J. D. CAPRA, I. L. SCHWARTZ and R. WALTER

*Departments of Physiology and Microbiology, Mount Sinai Medical and Graduate Schools, 100 Street and Fifth Avenue, New York (New York 10029, USA), 31 July 1970.*

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[15] Predoctoral fellow of the Department of Physiology.

## Identification of Teleost Gonadotrope Cells Using Methallibure (I.C.I. 33, 828) and Thiourea

In recent years increasing attention has been paid to the experimental identification of functional cell types in the teleost pituitary (see review by VAN OORDT[1]). Associated with this has been the realization that empirical staining techniques have, by themselves, only a morphological value, a fact emphasized in teleosts by the general failure of tinctorial methods to distinguish between thyrotrope and gonadotrope cells in the proximal pars distalis (PPD) of the pituitary gland[2].

In attempts to identify these cell types the most common approach has been to look for parallel changes in the pituitary following experimentally-induced changes in its target organs. In this way the identification of thyrotrope cells has been successfully accomplished in

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