ON THE BIOLOGICAL SIGNIFICANCE OF NEUROPHYSINS: PRESENCE OF A MAJOR NEUROPHYSIN IN THE SHEEP

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Received 3 March 1975

1. Introduction

Neurophysins [1] are proteins which are usually found associated with neurohypophysial hormones in the posterior pituitary gland. The components of the complex can be separated [2] and reassociated [3] to give a complex similar to the native one. Because of that, a role of neurophysins either in the biosynthesis of hormones or in their carriage from hypothalamus to the posterior pituitary lobe has often been postulated [4,5]. Two neurohypophysial hormones are generally present in the gland and it is assumed that a stoichiometric amount of a particular neurophysin is associated to each hormone. If such an assumption is correct, two neurophysins should be isolated in the same proportions as those found for the two hormones. Actually two major neurophysins have been characterized in ox (6–8) and pig [9,10], species in which oxytocin and vasopressin are present in approximately equal amount [11]. However in the sheep, despite the fact that oxytocin and arginine vasopressin exist in equal molar amounts in the posterior pituitary gland [12], we have found a single major neurophysin representing about 60–70% of the 'crude neurophysin'.

2. Materials and methods

Sheep neurophysin–neurohypophysial hormone complex is isolated under conditions previously described [3,11], from an acetone-desiccated posterior pituitary powder prepared with glands removed rapidly after death, The powder titrates at 0.8 U of oxytocic activity (1.6 nmol oxytocin) and 0.8 U of pressor activity (1.6 nmol of arginine vasopressin) mg^{-1} and the complex 18 U of both activities mg^{-1}. About 90% of both activities are recovered in the complex.

Purification of neurophysins is carried out first by dissociation of the complex by gel filtration on Sephadex G-25 in 0.2 M acetic acid which removes the hormones and gives 'crude neurophysin' (proteins with a mol. wt around 10 000 (fig.1.), then by ion exchange chromatography on Diethylaminoethyl-Sephadex A-50 which separates two different neurophysin fractions (fig.2.). A discontinuous gradient of 0.2–1 M pyridine acetate buffers pH 5.9 is used. From 50 mg of 'crude neurophysin', 43 mg are recovered: 30.5 mg are obtained in fraction A (0.2–0.4 M pyridine acetate pH 5.9) and 6.5 mg in fraction B (0.4–1 M pyridine acetate pH 5.9).

The material found in peak A appears homogeneous by electro-phoresis either on cellulose acetate (Cellogel) at pH 8.8 or on polyacrylamide gel at pH 9.5 [13]. On the other hand a single amino acid sequence is determined [14] by automated Edman degradation [15] in a Socosi model P 100 sequencer. From the sequence of the first 27 residues, it can be concluded that this neurophysin belongs to the MSEL-neurophysin group [14]. In contrast the material found in fraction B shows three components by electrophoresis on cellulose acetate. This fraction represents about 15% of the 'crude neurophysin'. By peptide mapping [16] of the tryptic peptides of the performic acid-oxidized fraction B, an octapeptide can be isolated which corresponds to the N-terminal sequence of the VLDV-neurophysins, the second type of neurophysins found in ox [8] and in pig [10]. Therefore a second neurophysin is present and it can be estimated, from the yield of

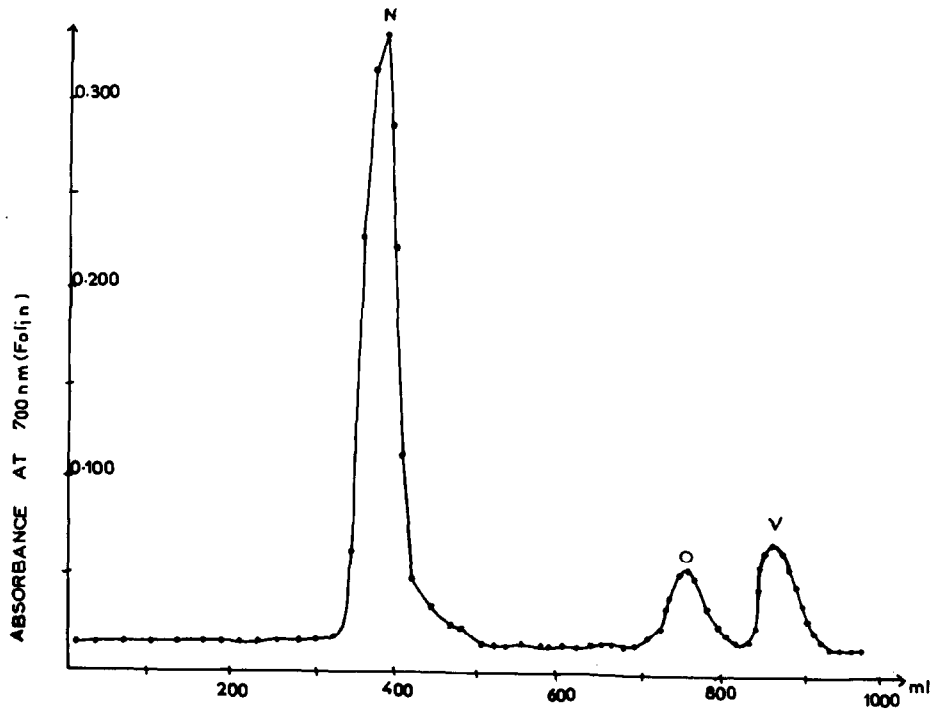

Fig.1. Dissociation of the sheep neurophysin–neurohypophysial hormone complex by gel filtration on Sephadex G-25 in 0.2 M acetic acid. N = 'crude neurophysin'. O = oxytocin. V = arginine vasopressin.

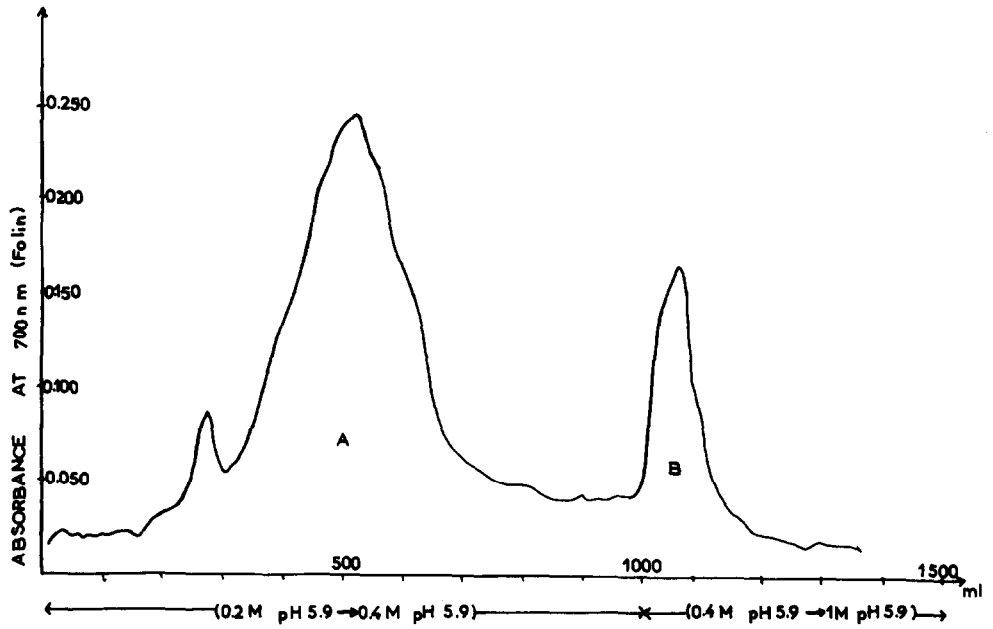

Fig.2. Fractionation of 'crude neurophysin' by ion chromatography on diethylaminoethyl-Sephadex A-50. Two ionic strength gradients were applied from 0.2 to 0.4 M and from 0.4 M to 1 M pyridine acetate (details on peaks A et B in the text).

the N-terminal peptide, that this neurophysin accounts roughly for 50% of fraction B, that means approx. 7% of the total neurophysins. The other components of fraction B are not yet identified but one could be a modified form of the major neurophysin. The major neurophysin would be at least seven or eight times more abundant than the minor neurophysin.

3. Discussion

The number of neurophysins in a given species is rather hard to determine because partially degraded forms of neurophysins are often present in the complex. In this respect the time elapsed between the death and the removal of gland seems of prime importance: proteolysis of neurophysins can occur, without concomitant loss of oxytocic or pressor activities, leading to modified neurophysins. When several components are observed by electrophoresis of the crude neurophysin, some of them can be slightly degraded forms of native neurophysins. Therefore the determination of the real number of molecular species requires chemical characterization of each component detected by electrophoresis.

The sheep major neurophysin isolated in the present work accounts for about 60–70% of the material regarded as 'crude neurophysin'. On the other hand, the minor second neurophysin is present in the complex in amount less than 10% of crude neurophysin. Therefore, there is no relationship between the relative amounts of the two hormones (molar ratio 1 : 1) and those of the two neurophysins (molar ratio 7 : 1).

Furthermore the absolute amount of the major neurophysin is superior to that of each hormone. From the specific activities of the complex, it can be calculated that 27 mg of the complex contain approx. 1 mg (1 μmol) of oxytocin, 1 mg (1 μmol) of arginine vasopressin and about 25 mg of neurophysin proteins with a mol. wt of 10 000. 2.5 μmol of neurophysin proteins are therefore associated with 2 μmol of peptide hormones. The major neurophysin accounts for 60–70% of neurophysin proteins and the molar proportions of the major neurophysin, oxytocin and arginine vasopressin in the gland are approx. 1.6 : 1 : 1. Apparently neurophysins are not merely produced by the cleavage of a protein precursor giving an active peptide and an inactive carrier protein but could be synthesized independently.

Acknowledgements

The authors wish to thank Miss Marie-Hélène Simon and Miss Monique Bourdin for their skilled technical assistance. This study was supported in part by grants from CNRS (ERA No.563) and INSERM (No.73–1–488–22).

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