Neurophysins of the Sheep

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Neurophysins are now recognized as a class of proteins of molecular weight approx. 10000 (Rauch *et al.*, 1969) associated with the polypeptide hormones oxytocin and vasopressin within the neurosecretory axons of the hypothalamo-neurohypophyseal system of the mammalian brain.

In both the ox (Rauch *et al.*, 1969) and the pig (Uttenthal & Hope, 1970) two major neurophysins, together with a minor component, have been purified and biochemically characterized. By subcellular fractionation of the bovine posterior pituitary lobes Dean *et al.* (1968a) have shown that oxytocin and neurophysin-I are associated within neurosecretory vesicles that are different from those containing vasopressin and neurophysin-II. It is still uncertain whether the minor neurophysin is associated with oxytocin or vasopressin or some other hormone such as that described by Hope & Watkins (1969).

To investigate whether the distribution of neurophysins found in the ox and the pig was common to other species, studies were undertaken to identify and purify the neurophysins of the sheep.

The pituitary glands of sheep were removed immediately after slaughter, and the posterior lobes were carefully dissected out and ground to a powder in cold (4°C) acetone. A sample (2g) of the powder was extracted overnight at 4°C with a mixture of 35 ml of 0.1 M-HCl and 8 ml of 1 M-HCl, and the insoluble material was removed by centrifugation. After re-extraction of the pellet for 12 h with a further 20 ml of 0.1 M-HCl, followed by centrifugation, the combined supernatants were adjusted to pH 7 with 2 M-NaOH and the insoluble material was removed by centrifugation. The pH of the combined supernatants was adjusted to 4 with 1 M-HCl, and NaCl was added to a final concentration of 10 g/100 ml. The precipitate that had formed after 18 h at 4°C was collected, dialysed against distilled water and then freeze-dried to give a protein-hormone complex as a white powder. Dissociation of the hormones from this protein complex was achieved by chromatography on a column (2 cm × 146 cm) of Sephadex G-25 with 0.1 M-formic acid as eluent. Oxytocin was eluted at 430 ml and [8-arginine]-vasopressin at 490 ml. The material that was eluted in the void volume of the column (220 ml) was collected, freeze-dried and further fractionated on a column (2 cm × 146 cm) of Sephadex G-75. On elution of this column

with 0.1 M-formic acid, three peaks emerged: peak A in the void volume of 175 ml, peak B at 210–280 ml and peak C at 330–430 ml. This last peak was eluted with a slow-rising leading edge followed by a rapidly falling trailing edge, a phenomenon that is characteristic of neurophysin under these conditions (Hollenberg & Hope, 1967; Uttenthal & Hope, 1970). The material from peaks A, B and C correspond respectively to 34, 12 and 55% of the total material eluted from the Sephadex G-75 column. The fact that a low E_{280}/E_{260} ratio is characteristic of ox and pig neurophysins further suggests that the material from peak C ($E_{280}/E_{260} = 0.70$) contains neurophysin. This was confirmed by establishing the ability of the material recovered from peak C to bind the hormones oxytocin and [8-arginine]-vasopressin during thin-film dialysis (Craig & Konigsberg, 1961) in sodium acetate buffer at pH 5.6 and 10.1.

On electrophoresis on the starch gel at pH 8.1, the protein from peak A gave a single band with a mobility identical with that of sheep serum albumin set against a diffuse background. Fraction B produced a diffuse smear on electrophoresis, and the proteins in the third peak were resolved into three components. Fraction C was fractionated into its three components by ion-exchange chromatography on a column of CM-Sephadex C-50 through a gradient of sodium acetate buffer, pH 4.40–5.0 and 10.1. These proteins all possessed the ability to bind both oxytocin and [8-arginine]-vasopressin in the above thin-film dialysis system and were designated neurophysins-I, -II and -III according to the order of their electrophoretic mobilities (Webb, 1964) (Fig. 1).

The sheep neurophysins were further characterized by comparison of their electrophoretic mobilities with those of the three pig and the three ox neurophysins. The most striking feature is that sheep neurophysin-III possesses an electrophoretic mobility almost identical with those of both pig neurophysin-III and ox neurophysin-II. On the other hand, the remaining two sheep neurophysins both migrate further on starch electrophoresis, with the mobility of sheep neurophysin-I close to that of pig neurophysin-II.

The yields of the sheep neurophysins-I, -II and -III isolated from peak C were approx. 15, 15 and 70% respectively. The percentage abundances of the sheep neurophysins are somewhat different from those of

Fig. 1. Diagrammatic representation of the starch-gel electrophoretograms of the soluble proteins extracted from ox, sheep and pig fresh posterior pituitary lobes

A sample of the lobes was homogenized in 25 μ l of 0.1 M-HCl and applied to wicks of Whatman no. 1 chromatography paper. The wicks were then inserted into slots in the starch, and electrophoresis was carried out by the method of Smithies (1955) with starch at a concentration of 27 g/200 ml of tris-borate buffer (Ferguson & Wallace, 1961), pH 8.1. After electrophoresis for 1½ h at a gradient at 28 V/cm, the gel was stained with Nigrosine (0.05%, w/v) in water-methanol-acetic acid (5:5:1, by vol.) for 18 h and then destained with several changes of water-methanol-acetic acid (5:5:1, by vol.). The positions of ox neurophysins-I and -II and minor component C (a) (Rauch *et al.*, 1969) and of pig neurophysins-I, -II and -III (c) (Uttenthal & Hope, 1970) are indicated for comparison against the sheep proteins (b). ● indicates the position of the serum albumin.

the neurophysins isolated from other species. In both the pig and the ox there are two major neurophysins in approximately equal amounts together

with a minor component corresponding to approx. 10% of the total neurophysins (Uttenthal & Hope, 1970; Rauch *et al.*, 1969).

Preliminary amino acid analyses showed that the three sheep neurophysins share some features with the ox and pig neurophysins, namely high contents of cystine and glutamic acid. Sheep neurophysins are also rich in proline and all contain one molecule of tyrosine/molecule of protein (based on an approximate molecular weight of 10000 as calculated from the amino acid analysis). Sheep neurophysins-I and -II each contain one molecule of histidine/molecule of protein but lack methionine, whereas sheep neurophysin-III contains no histidine but possesses one molecule of methionine/molecule of protein.

The results from this work provide a further example of a mammalian species possessing three neurophysins. However, unlike the ox and the pig, the sheep posterior pituitary contains one major and two minor neurophysins. Chauvet *et al.* (1960) have shown that there is a 1:1 ratio of oxytocin to [8-arginine]-vasopressin in the sheep neurohypophysis, whereas this present work indicates that sheep neurophysins-I, -II and -III are present in the approximate proportions 1:1:5. These results suggest that the stoichiometric relationship between molar amounts of the two polypeptide hormones and the two major neurophysins found by Dean *et al.* (1968b) in the ox is not general to mammalian species.

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