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AN ATTEMPT TO ISOLATE ARGININE VASOTOCIN FROM SHEEP AND BOVINE PINEAL BODY

BY

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Abstract

An attempt was made to isolate a peptide with pressor and oxytocic activities from sheep and bovine acetone desiccated pineal bodies by gelfiltration and column chromatography. A fraction with a small pressor effect was obtained. At no time oxytocic activity could be demonstrated in any of the fractions. The occurrence of arginine vasotocin in the pineal organ could not be confirmed.

MILCU *et al.* (1963) reported the occurrence of a polypeptide with pressor and oxytocic activities in the bovine pineal body. On the basis of pharmacological and paperchromatographical data of a partially purified product they concluded this polypeptide to be identical to or related to vasotocin. 1 g of this peptide fraction was obtained from 250 g bovine pineal body powder according to the procedure of KAMM *et al.* (1928) for the preparation of protopituitrin from posterior pituitary powder. The biological properties of their peptide fraction can be explained by a vasotocin content of 0.1 %.

We found it valuable trying to reproduce the work of MILCU *et al.* (1963) and, if possible, to isolate a pure polypeptide, the more so as it is peculiar that the bovine pineal body should contain a polypeptide which, up till now, has only been found in neurohypophyses from avians and lower vertebrates. We investigated bovine and sheep acetone dried powders of the pineal body by extraction and purification procedures, which are well established for the neurohypophyseal peptides (HELLER, 1963; WITTER *et al.*, 1964).

Materials and Methods

In the factory of A. Molendijk N.V. Import- en Exporthandel (Nieuwerkerk a/d IJssel, Holland) we collected in autumn and winter beef and sheep pineal bodies. 1 to 8 hours after death the heads of the animals were stored at 2° C. Within 4 to 20 hours after death the pineal bodies were extirpated and put into distilled acetone for 10 minutes. Then the organs were dried with filter paper and the adherent brain tissue was stripped off. The cleaned organs were stored in acetone at 4° C. The acetone

was refreshed daily for ten subsequent days. Thereafter the pineal bodies were dried in vacuo and ground to a powder.

Bioassays

Vasopressor activity was estimated on rats under urethane anesthesia and pretreated with phenoxybenzamine HCl (DEKANSKI 1952). The oxytocic activity was determined on the isolated rat uterus, which was suspended in a Mg^{++} free solution (HOLTON 1948).

Experiments and Results

I. Extraction with a 0.2 M pyridine–0.05 M acetic acid buffer.

I A. 20 g acetone desiccated sheep pineal powder were extracted with 0.2 M pyridine–0.05 M acetic acid buffer pH 5.9 at 2° C (LINDNER *et al.* 1959), as we described for pig posterior pituitary lobe powder previously in detail (WITTER *et al.* 1964). The lyophilized extract (4.80 g) was redissolved as completely as possible in the extraction buffer. This solution was filtered through a Sephadex G25 column (8 × 83 cm), which was equilibrated with the same buffer. The fractions were analyzed with a ninhydrin reagent before and after alkaline hydrolysis (WITTER *et al.* 1964). The fractions belonging to a peak were pooled and lyophilized. The results are summarized in Table I.

TABLE I

fraction		weight (mg)
lyophilized extract		4800
high mol. fraction	Sephadex	472
intermediate fraction	Sephadex	167
low mol. fraction	Sephadex	1912

Only in the "intermediate molecular fraction" a pressor effect was demonstrable, but the effect was too small to be compared with a vasopressin standard. All fractions were devoid of oxytocic activity up to a concentration of 5 mg/ml. Analogous to the neurohypophyseal hormones this pressor effect could be present as a protein complex. Therefore we treated samples of the "intermediate molecular" fraction with 0.2 M acetic acid at room temperature or with 1 M formic acid at 70° C (LINDNER *et al.* 1959) and filtered these solutions through Sephadex G25 columns equilibrated with the corresponding solvent. No real separation was demonstrated.

The pressor effect could be concentrated by chromatography of the "intermediate molecular" fraction dissolved in 0.25 % acetic acid over Amberlite CG 50 (VLIEGENTHART 1964). Elution with 0.25 % acetic acid

followed by 0.1 M ammonium acetate pH 5.0 yielded eluates containing 75 % of the applied material. The pressor activity was eluted by a gradient of increasing pH and salt concentration (LIGHT *et al.* 1957; ACHER *et al.* 1958). The elution pattern was not detectable with the Folin reagent (LOWRY *et al.* 1951). The pressor effect could only be found by direct assay of the collected fractions. The fractions containing activity were pooled; lyophilization yielded 1.5 % of the applied material. By paper chromatography in several solvent systems and by high voltage paper electrophoresis at pH 6.5 it appeared to be a rather complex mixture of ninhydrin positive components. The pressor effect, however, was completely destroyed during the lyophilization process.

II. Extraction with 0.25 % acetic acid.

II A. 20 g acetone desiccated sheep pineal powder were extracted thrice with 600 ml 0.25 % acetic acid by heating to 90° in 30 minutes under continuous stirring. The lyophilized extract (3.56 g) was redissolved as completely as possible in 0.25 % acetic acid and filtered through a Sephadex G 25 column (8 × 83 cm), which was equilibrated with 0.25 % acetic acid. The collected fractions were analyzed with a ninhydrin reagent before and after alkaline hydrolysis (WITTER *et al.* 1964). The fractions belonging to a peak were pooled and lyophilized. The results are summarized in Table II. Only in the intermediate molecular fraction we found a pressor effect. Oxytocic activity could not be demonstrated up to a concentration of 5 mg/ml.

TABLE II

fraction		weight (mg)
lyophilized extract		3557
high mol. fraction	Sephadex	285
intermediate fraction		665
low mol. fraction		1748

II B. 20 g acetone desiccated bovine pineal powder were extracted with 0.25 % acetic acid in the same way as we described for the sheep pineal powder. Thus the lyophilized extract (4.43 g) was redissolved and gelfiltrated. The results are summarized in Table III.

TABLE III

fraction		weight (mg)
lyophilized extract		4430
high mol. fraction	Sephadex	151
intermediate fraction		533
low mol. fraction		2478

In bovine material too a pressor effect was only present in the intermediate molecular fraction. Oxytocic activity did not occur in our fractions up to a concentration of 5 mg/ml.

Discussion

In this work we have shown the existence of a slight pressor activity in acetone dried sheep and bovine pineal powder. Because of its lability, the nature of this substance has not been further identified. In our fractions obtained by two different extraction procedures we did not observe any oxytocic effect. We have not been able to confirm the work of MILCU *et al.* (1963), who claim to have isolated a peptide with the properties of vasotocin from bovine pineal powder. However, from the extract we prepared with the pyridine acetate buffer from chicken posterior pituitary lobe powder it was easy to isolate vasotocin and oxytocin (VLIEGENTHART, unpublished results). These findings are in full agreement with those of MUNSICK (1964), who extracted chicken and turkey neural lobe powder for 10 minutes with 0.154 M NaCl adjusted to pH 3.0 with glacial acetic acid in a boiling water bath. We mention this experiment only to demonstrate the suitability of our methods.

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