IMMUNOCHEMICAL IDENTIFICATION OF GROWTH HORMONE AND PROLACTIN IN THE PITUITARY POLYPEPTIDE SPECTRUM OF CHICKENS

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The avian adenohypophysis is distinguished by marked zonality of the distribution of certain types of gland cells, hormones, and tissue-specific antigens. Tissue-specific adenohypophyseal antigen A-1, characteristic of the caudal lobe of this gland, is known to be present in chickens. The biochemical nature of this antigen is not yet known. Immunohistochemical investigations conducted on birds of various species have shown that antisera against pituitary hormones of mammals can react with avian pituitary and can be used to identify corresponding types of endocrine cells [6, 10, 11].

In the investigation described below immunoreactive somatotrophic hormone (STH) and prolactin were identified in chickens by the use of electrophoresis in polyacrylamide gel (PAG), electrophoretic blotting, and antisera against mammalian hormones.

EXPERIMENTAL METHOD

The adenohypophysis was removed from White Leghorn hens and divided into cephalic and caudal lobes. The tissues were homogenized in ice-cold distilled water, adjusted to neutral or weakly alkaline pH, and after centrifugation, the extracts were freeze-dried. The polypeptide composition of the tissues was studied by electrophoresis in PAG with sodium dodecylsulfate (SDS-PAGE) in Tris-HCl and a Tris-glycine buffer system [9]. Electrophoresis was carried out in plates 14 cm long, formed with the use of a linear acrylamide concentration gradient of 10-20%. The dose of the freeze-dried substance in the samples was 100 and 50 μl of application buffer. The gel after electrophoresis was stained with 0.04% Coomassie Bright Blue G-250 in a 3.5% aqueous solution of perchloric acid [8], photographed, and dried. The molecular weight of the polypeptides was determined by means of a low-molecular-weight kit (Pharmacia, Sweden), with a set of proteins with a range of molecular weights from 14 to 94 kilodaltons (kD).

Electrophoretic fractions of adrenohypophyseal polypeptides were transferred from the unstained plates to nitrocellulose filters (from Schleicher and Schuel, West Germany) by the electrophoretic blotting method in 0.025 M Tris-glycine buffer, pH 8.3 [14]. Control filters with transferred fractions were stained with a solution of Ponceau-S (Beckman, USA). For immunochemical detection of polypeptides, the blotted filters were treated with previously obtained rabbit antisera against human STH [2] and against bovine prolactin [3], and antiserum against extract of chicken pituitary gland, which revealed, in particular, tissue-specific antigen A-1 [1]. The reactions were developed with the aid of a peroxidase–antiperoxidase complex (PAP complex, from Dako, Denmark), by the method of unlabeled antibodies with the PAT complex [13] used in immunohistochemical investigations.

EXPERIMENTAL RESULTS

In the study of extracts prepared from whole adenohypophyses, and from the cephalic and caudal lobes, by the SDS-PAGE method, 48 fractions of polypeptides were discovered in the chicken adenohypophysis (Fig. 1). Approximately one-third of the electrophoretic spectrum of the adenohypophyseal polypeptides was composed of strong fractions (15 fractions), forming brightly stained bands in the gel. The remaining fractions stained

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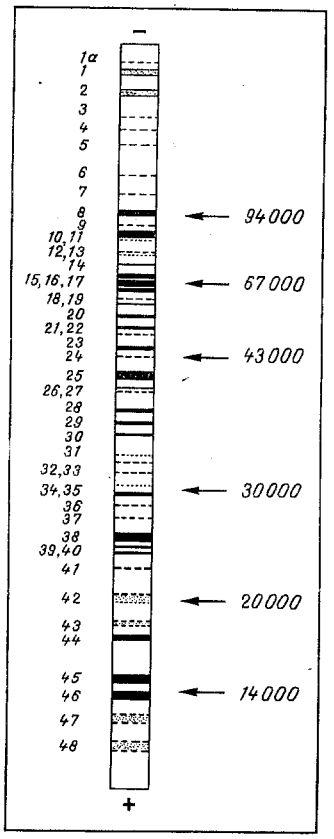

Fig. 1. Polypeptide composition of chicken adenohypophysis according to result of SDS-PAGE.

much less strongly, and they included 5 or 6 fractions which are rare. The chicken adenohypophysis contains 8 fractions of high-molecular-weight polypeptides with mol. wt. of over 95 kD, 39 fractions of polypeptides with mol. wt. of between 14 and 95 kD, and 2 fractions with mol. wt. of under 14 kD, migrating rapidly in SDS-PAGE. The cephalic and caudal lobes of the adenohypophysis, as shown by comparative analysis of their composition, have similar sets of polypeptides. At the same time, it was shown that one of the strong fractions, a fraction of polypeptides with mol. wt. of 26 kD, is characteristic of the caudal lobe and is not found in the cephalic lobe of the adenohypophysis. Meanwhile, fractions of polypeptides with mol. wt. of 27 and 25 kD and rapidly moving peptides with mol. wt. below 14 kD are seen more clearly in the cephalic lobe.

To detect polypeptides immunochemically similar to mammalian STH, electrophoretic blotting of fractions of extracts of the whole adenohypophysis and of extracts of the caudal lobe of the chicken adenohypophysis was used. On incubation with antiserum against human STH, in both versions of the reactions one band stained for peroxidase was developed, corresponding to a strong fraction of polypeptides with mol. wt. of 26 kD (Fig. 2).

To identify the tissue-specific antigen A-1 of the chicken adenohypophysis, a rabbit antiserum obtained against the cathodal fraction of chicken adenohypophysis, isolated by electrophoresis in agar gel [1], was neutralized with a freeze-dried extract of chicken liver, in a dose of 5 mg/ml. On development of the nitrocellulose filters with electrophoretic fractions of polypeptides from the whole adenohypophysis and from the caudal lobe of the chicken adenohypophysis, the antiserum reacted with polypeptide fractions with mol. wt. of 64, 37, and 26 kD (Fig. 2). Additional neutralization of the antiserum with freeze-dried extract of the cephalic lobe of the chicken adenohypophysis in doses of 1-5 mg/ml led to inhibition of the reaction with polypeptides with mol. wt. of 37 kD. On neutralization of the antiserum with a freeze-dried preparation of chicken blood serum in a dose of 10 mg/ml, marked weakening of the reaction with the fraction with mol. wt. of 64 kD took place, although under these circumstances complete "extinction" of the immunochemical reaction was not achieved. With an increase in the dose of the freeze-dried preparation from the cephalic lobe of the adenohypophysis to 10 mg/ml, the reaction with polypeptides of the fraction with mol. wt. of 26 kD also ceased, in agreement with data showing that the cephalic lobe of the chicken adenohypophysis contains only a small number of cells producing STH and of cells containing antigen A-1.

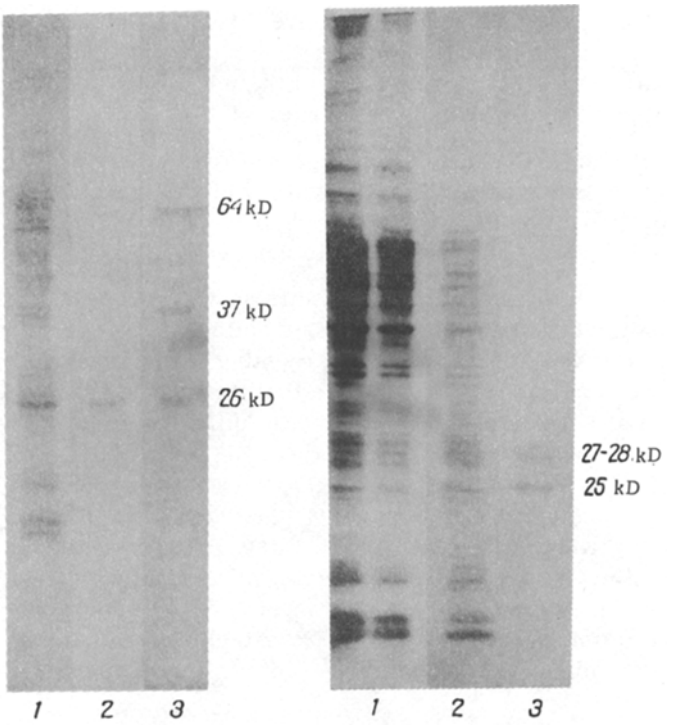

Fig. 2. Immunochemical identification of STH and of tissue-specific antigen A-1 of chicken adenohypophysis. 1) Electrophoretic blotting of fractions of extract of whole chicken adenohypophyses (control), stained with Ponceau-S solution; 2) blotting of parallel path in SDS-PAGE, the same extract. Development of antiserum against human STH; 3) electrophoretic blotting of parallel path in SDS-PAGE, the same extract. Development of antiserum against extract of chicken pituitary.

Fig. 3. Immunochemical identification of prolactin in chicken adenohypophysis. 1) Separation of extract of cephalic lobe of chicken adenohypophysis in SDS-PAGE, left path – 100 μg of freeze-dried preparation, right path – 50 μg. Stained with Coomassie Bright Blue G-250; 2) electrophoretic blotting of parallel path in SDS-PAGE, the same extract (electrical transfer control), stained with Ponceau-S solution; 3) blotting of parallel path in SDS-PAGE, the same extract. Development with antiserum against bovine prolactin.

Polypeptides immunochemically similar to mammalian prolactin were identified on filters after electrophoretic transfer of fractions of extracts of the whole chicken adenohypophysis and of its cephalic lobe. On development with antiserum against bovine prolactin, one distinct band corresponding to polypeptides with mol. wt. of 25 kD was found in extracts of the whole adenohypophysis. In reactions with extract of the cephalic lobe of the adenohypophysis, two other weakly stained polypeptide fractions with mol. wt. of 27 and 28 kD also were developed (Fig. 3).

It can be concluded from these results that in the polypeptide spectrum of the chicken adenohypophysis the fraction with mol. wt. of 26 kD, characteristic of the caudal lobe of the adenohypophysis, is immunoreactive STH. According to data in the literature, STH of the chicken pituitary gland, isolated by chromatography on DEAE-cellulose, has a mol. wt. of 23 kD, the same as human STH [7]. The cause of the discrepancy between these data and our own is not quite clear. As a control of accuracy of determination of mol. wt. of polypeptides in SDS-PAGE, using 10-20% gel, a preparation of human STH isolated from strains of Escherichia coli,

transformed by methods of genetic engineering [5], was investigated. Two polypeptide fractions were discovered in the sample of "bacterial" STH: the principal fraction with mol. wt. of 22.5 kD and a minor fraction with mol. wt. of 15 kD. The difference in determination of the molecular weight of the principal component from the value given in the literature of 22 kD [4, 5], is only 0.5 kD.

Data on immunochemical identification of polypeptides of the chicken adenohypophysis, obtained in experiments using antiserum against extract of chicken pituitary gland, are evidence that the tissue-specific A-1 antigen, characteristic of the caudal lobe of the adenohypophysis [1], is also a fraction of polypeptides with mol. wt. of 26 kD and, consequently, it is identical to chicken STH.

Immunoreactive prolactin, as the experimental results show, is represented in chicken adenohypophysis as a principal fraction with mol. wt. of 25 kD and 2 minor fractions of polypeptides with mol. wt. of 27 and 28 kD. The discovery of several fractions of polypeptides, reacting with antiserum against bovine prolactin, in the chicken adenohypophysis is in agreement with data in the literature on the polymorphism of this hormone in mammals and the existence of "isohormones" of prolactin, differing in their level of biological activity [12].

LITERATURE CITED

1. V. M. Barabanov and D. B. Nikolova-Kitova, Byull. Éksp. Biol. Med., No. 8, 209 (1978).
2. S. Yu. Kasumova, V. M. Barabanov, and R. Ya. Snigireva, Arkh. Patol., No. 5, 19 (1982).
3. S. Yu. Kasumova, R. Ya. Snigireva, and V. M. Barabanov, Prob. Éndokrinol., No. 2, 35 (1982).
4. L. Lasas and D. Lasene, Human Somatotrophin [in Russian], Vilnius (1981).
5. P. M. Rubtsov, A. Sh. Parsadanyan, P. S. Sverdlova, et al., Dokl. Akad. Nauk SSSR, 276, No. 3, 762 (1984).
6. M. P. Dubois, Bull. Assoc. Anat., 57, 63 (1973).
7. S. Harvey and C. G. Scanes, J. Endocrinol., 73, 321 (1977).
8. I. B. Holbrook and A. G. Leaver, Anal. Biochem., 75, 634 (1976).
9. U. K. Laemmli, Nature, 227, 680 (1970).
10. C.-R. Marchand, C. Bugnon, M. Herlant, et al., Bull. Assoc. Anat., 60, 603 (1976).
11. B. A. McKeown, Canad. J. Physiol. Pharmacol., 50, 1021 (1972).
12. C. S. Nicoll, Perspect. Biol. Med., 25, 369 (1982).
13. L. A. Sternberger, in : Immunocytochemistry, New York (1979), p. 104.
14. H. Towbin, T. Staehelin, and J. Gordon, Proc. Natl. Acad. Sci. USA, 76, 4350 (1979).