tassium holding current recorded at the end of the testing pulse, 60 msec in duration, remained unchanged.

The results of the voltage clamping experiments show that the decrease in amplitude of the action potential under the influence of adrenochrome is connected not only with membrane depolarization, but also with a change in the characteristics of the ionic channels of the electrically excitable membrane and, in particular, with a decrease in maximal conductance of the inward current system.

It can be stated on the basis of the Goldman—Hodgkin—Katz equation for a steady field [11, 12] that since the resting potential reached its initial values or was below them during the periodic transient shift of membrane potential, its dynamics under the influence of adrenochrome was not due to changes in ionic composition but to changes in membrane structures responsible for permeability to sodium and potassium ions. Changes in these structures may be connected with the direct action of adrenochrome on the neuron membrane or may take place through the intervention of cell metabolism.

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SYNTHESIS OF THE SPECIFIC LENS ANTIGEN (δ-CRYSTALLIN) IN THE ADENOHYPOPHYSEAL ANLAGE OF CHICK EMBRYOS

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KEY WORDS: adenohypophysis; chick embryos; crystallins.

The synthesis of crystallins is a specific biochemical sign of differentiation of the vertebrate lens. In their immunologic and physicochemical properties crystallins can be divided into several classes. For birds and, in particular, for hens there are three main classes of crystallins: α-, β-, and δ-crystallins. The first to appear during development of the lens in chick embryos are the δ-crystallins [10, 12], which account for most of the total quantity of lens proteins synthesized during embryonic development.

Recently δ-crystallins have been found immunochemically in cells of the adenohypophyseal anlage of chick embryos [2]. This a most interesting observation, for it may be evidence

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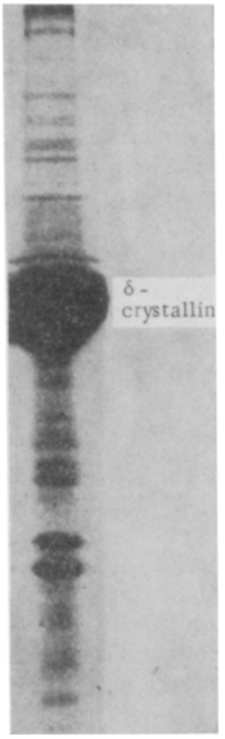

Fig. 1. Electrophoresis of lens extract of 8-day chick embryos in polyacrylamide gel gradient (7-15%) with 0.1% sodium dodecylsulfate in the presence of mercaptoethanol.

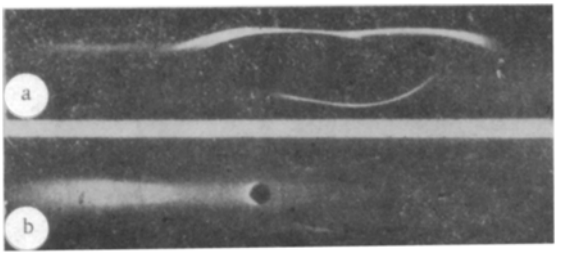

Fig. 2. Synthesis of δ-crystallins by cells of adenohypophyseal anlage of 4-day chick embryos: a) immunoelectrophoresis of hen lens extract with addition of extract of adenohypophyseal anlagen cultured in the presence of ^{14}C-leucine. Development with rabbit antiserum against δ-crystallins (below) and against hen lens β-crystallins (above); b) autoradiograph of the same preparation. Incorporation of ^{14}C-leucine into precipitation band of δ-crystallin.

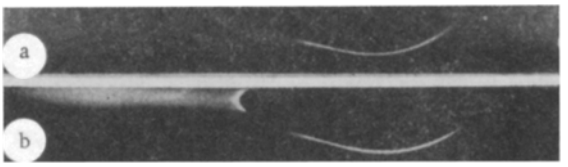

Fig. 3. Synthesis of δ-crystallins by lens of 4-day chick embryos. a) Immunoelectrophoresis of lens extracts of 4-day chick embryos cultured in the presence of ^{14}C-leucine. Development with antiserum against β-crystallins; b) autoradiograph of the same preparation. Incorporation of ^{14}C-leucine into precipitation band of δ-crystallins.

that the initial stages of biochemical differentiation of the embryonic anlagen of two different organs may be similar, if it could be shown that δ-crystallins are in fact synthesized in the adenohypophyseal anlage. The present investigation was devoted to a study of this problem.

EXPERIMENTAL METHODS

Synthesis of δ-crystallins in cells of the adenohypophyseal anlage of chick embryos was determined by immunoautoradiography using an unlabeled carrier [1], namely extract of hen lens. Fifty adenohypophyseal anlagen isolated from 4-day chick embryos were cultured in medium 199 containing 10% embryonic calf serum, antibiotics, and 2 μCi/ml of ^{14}C-leucine (from the Radiochemical Centre, Amersham, England). After culture for 15 h the anlagen were frozen with dry ice and homogenized in 20 μl of a solution of lens extract (protein concentration 20 mg/ml in 0.1 M Tris-buffer, pH 8.3). This mixture was used as antigen in the immunoelectrophoresis reaction. Immunoelectrophoresis in 1.25% agar in 0.1 M Tris-buffer, pH 8.3, was carried out by the method described previously [4]. Specific antiserum against δ-crystallins was obtained by immunizing rabbits with a preparation of δ-crystallins isolated by electrophoresis in a polyacrylamide gel gradient (7-15%) in the presence of sodium dodecylsulfate and mercaptoethanol in Tris-HCl — a Tris-glycine buffer system [9]. The δ-crystallins were isolated as a fraction of subunits with mol. wt. 45,000-50,000 daltons (Fig. 1) [11]. The purity of the isolated preparation and specificity of the resulting antiserum were verified by double immunodiffusion and immunoelectrophoresis in agar. Serum against β-crystallins was obtained previously.

Agar plates after immunoelectrophoresis were washed for 4 days in 0.01 M Tris-buffer, pH 8.3, fixed, dried, and exposed for 8 weeks on RF-3 film.

The controls were: 1) the reaction with antiserum against β-crystallins — for lens proteins not found in the adenohypophysis [2]; 2) addition of ^{14}C-leucine to lens extract — for binding of free label by the carrier [1]; 3) treatment of the agar plates after immunoelectrophoresis with a mixture of ethanol with chloroform and TCA — control for stability of incorporation of label into the protein antigens [1]; 4) the liver of the chick embryos, as an organ in which synthesis of crystallins is known not to take place, was cultured and processed under similar conditions; 5) the lenses of 4-day chick embryos, in which synthesis of δ-crystallins is well marked [5, 6], were cultured and processed under similar conditions.

EXPERIMENTAL RESULTS

Lens extract, added to adenohypophyseal anlagen, homogenized after culture, formed distinct precipitation bands of δ- and β-crystallins in the immunoelectrophoresis tests (Fig. 2a). Incorporation of ^{14}C-leucine was found on the autoradiograph of the immunoelectrophoresis reaction in the precipitation band of δ-crystallins and it was absent in the precipitation bands of β-crystallins (Fig. 2b). When the liver of the chick embryos was cultured, δ-crystallin was completely absent. As would be expected, δ-crystallin synthesis was much more distinctly found in the cultured lens of 4-day embryos (Fig. 3) than in the adenohypophyseal anlage.

On simple addition of ^{14}C-leucine to the carrier (extract of hen lens) no label was incorporated into the δ-crystallin precipitate.

Treatment of the precipitation arcs of δ-crystallins from the adenohypophyseal anlage and lens with a mixture of chloroform and methanol, and also with TCA did not affect discovery of the ^{14}C-leucine label in the autoradiographs, evidence of incorporation of the label into the protein component of the antigen, and not of absorption of nonprotein labeled impurities on the antigen or precipitate [1].

The results described above are evidence that significant synthesis of δ-crystallins takes place in the adenohypophyseal anlage of 4-day chick embryos. As has already been shown [3], in the earlier stages of development the oral region of the head ectoderm, including the presumptive anlage of the adenohypophysis, possesses lenticular determination. The ectoderm of the oral region, cultured *in vitro* togeter with the underlying mesenchyme, can form lentoids until the 19 somites stage, i.e., in the first stages of the formation of Rathke's pouch — the presumptive anlage of the adenohypophysis [3]. In the 25-30 somites stages (3 days of incubation) the isolated adenohypophyseal anlage develops in organ culture into the adenohypophysis [8, 10], i.e., it is determined for development in the histotypic direction. However, the lens-forming powers of the cells of the adenohypophyseal anlage are evidently preserved for some time longer. It has been shown, in particular, that during dissociation and subsequent culture of cells of the pituitary anlage of 4-day chick embryos, their ability to form lentoid bodies is manifested [7]. In the light of these findings, synthesis of δ-crystallins in the adenohypophyseal anlage of chick embryos can evidently be regarded as the residual form of manifestation of lens-forming ability of its cells, due to lenticular determination of the head ectoderm of chick embryos in the early stages of development.

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