DEVELOPMENTAL ASPECTS OF ACETYLCHOLINESTERASE ACTIVITY IN CHICK BRAIN

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1. Introduction

Acetylcholinesterase has been found to exist in a multiplicity of molecular forms in all organisms where its heterogeneity has been studied, (either by polyacrylamide gel electrophoresis or by zone sedimentation in sucrose gradients) [1]. In higher vertebrates, acetylcholinesterase molecular forms have been most extensively studied in the rat, where three main forms have been identified [2,3]; one of them, whose sedimentation coefficient was 16 S appeared to be restricted to peripheral nicotinic synapses, i.e., neuromuscular end plates [2,4,5] and sympathetic ganglia [6]. Two forms were found both in the periphery and in the brain, those of 4 S and 10 S, and their relative proportions in the brain were dramatically reversed during development [4]: the 4 S form was found to be predominant in the embryo (90% at 14 days of gestation) whereas both forms were equally represented at birth: finally, the 10 S form became the major component (90%) in the mature animal. From biosynthesis experiments performed with neuroblastoma cells in culture, the 4 S form appeared as a precursor of 10 S, [3,7] and, from solubilization criteria, seemed less firmly integrated into membrane structures [4]. Accumulation of the end product, that is the membrane-bound 10 S form, thus seemed to coincide with brain maturation.

In chickens, analogous molecular forms have been described [8]. In the chick brain, a heavy (11 S) form, and a light (6 S) form, together with a minor 4 S component, have been characterized, the 11 S form representing most of the acetylcholinesteratic activity in adult brain.

In this paper, we report an analysis of the light and heavy forms of acetylcholinesterase in chick brain before and after hatching.

2. Experimental

Eggs of the Vedette 419 strain were supplied by an industrial hatchery, after 11 days or 18 days of incubation. Incubation was continued in a Curfew incubator in the dark. After hatching, chicks were transferred into individual dark, thermostatically controlled cages. Groups of five birds were sacrified at various times, from 10 days before to 15 days after hatching. The brain was removed immediately after decapitation and dissected to separate the forebrain hemispheres and the optic tectum. The tissues were rapidly frozen in liquid nitrogen and then stored at −20°C until analysis of acetylcholinesterase activity was performed (no change in enzyme activity or in molecular forms pattern could be observed during the storage of frozen intact tissue).

The tissues were homogenized in four volumes of detergent-containing saline buffer (NaCl 1 M, $MgCl_2$ 0.05 M, Tris pH 7, 0.01 M, Triton X-100, 1%). After centrifugation (30 000 × *g* for 10 min), total acetylcholinesterase activity was measured in the supernatants, and 100 μl aliquots were layered on 4 ml sucrose

gradients (5–20% w/v) in Triton X-100/saline buffer. Centrifugation was carried out in an SW-60 Beckman rotor at 2°C (45 000 rev./min for 16.5 h) and fractions were collected and assayed by Ellman acetylthiocholine method [9,10]. Protein concentrations were determined as described by Lowry et al. [11].

3. Results

The variation of acetylcholinesterase activities found with age are shown in fig.1. A significant increase was apparent around hatching and was more pronounced in the optic tectum (OT) than in the forebrain hemispheres (FH).

During the period studied, acetylcholinesterase activity/gram tissue increased by a factor of 4.7 in the whole brain, 4.3 in the hemispheres and 7.5 in the optic tectum. Although the tectum represented only 20% of the brain weight at 15 days, it contained about 40% total enzyme.

We analyzed the participation of the different forms of the enzyme in both regions of the brain; in fig.2, the sedimentation patterns of acetylcholinesterase activity are shown at 10 days before hatching (A) and 2 weeks after hatching (B). In all cases, the main peaks corresponded to the 11 S and 6.5 S forms of acetylcholinesterase but in the forebrain hemisphere preparations a minor 4 S form was also apparent as a shoulder on the 6.5 S peak. The most conspicuous feature of these curves was, however, the dramatic change in the

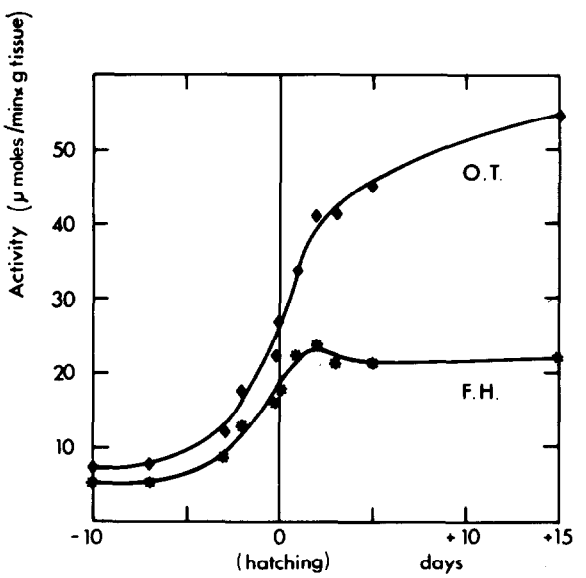

Fig.1. Increase in acetylcholinesterase activity during brain development. The tissues, stored at −20°C until use, were homogenized with a Potter homogenizer in 4 vol. Triton X-100/saline buffer (1 M: NaCl 0.06 M; $MgCl_2$ 0.01 M; Tris (pH 7); 1% Triton X-100). After centrifugation (30 000 × *g* 10 min) the supernatants were assayed for acetylcholinesterase activity according to the Ellman method [9]. One optical density unit variation (1 ml medium, 1 cm path-length) is assumed to represent an hydrolysis of 75 nmol acetylcholine [10]. Activity is expressed in μmol/g tissue/min. (F.H.) Forebrain hemispheres. (O. T.) Optic tectum.

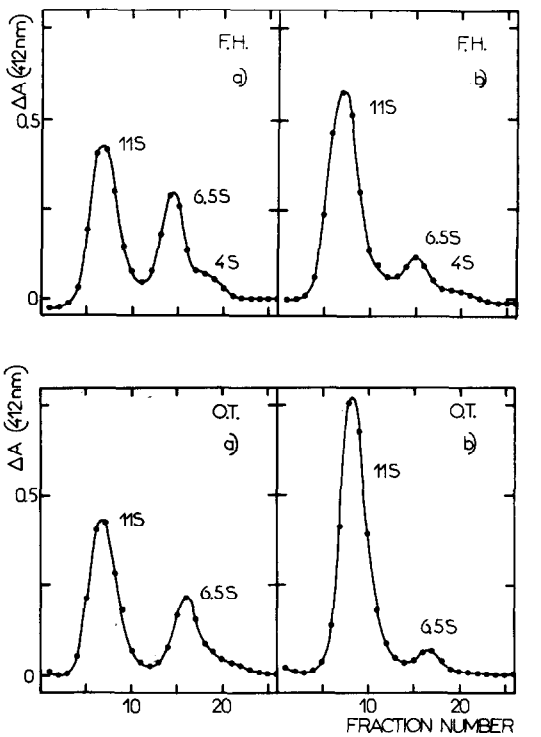

Fig.2. Sedimentation pattern of acetylcholinesterase. Tissue extracts prepared as described in fig.1 were diluted to equal activity (75 nmol ACh hydrolyzed/min/ml) and 100 μl aliquots were centrifuged in sucrose gradients. Centrifugation was performed on 5–20% w/v sucrose gradients in Triton X-100–saline buffer (SW 60 Beckman rotor, 2°C, 45 000 rev/min for 16.5 h). After collection, each fraction from the gradients was incubated with 1 ml Ellman's reagent, for 90 min at 20°C. Sedimentation constants were determined by including marker enzymes (not shown). (F. H.) Forebrain hemispheres. (O. T.) Optic tectum. (a) 10 days before hatching. (b) 2 weeks after hatching.

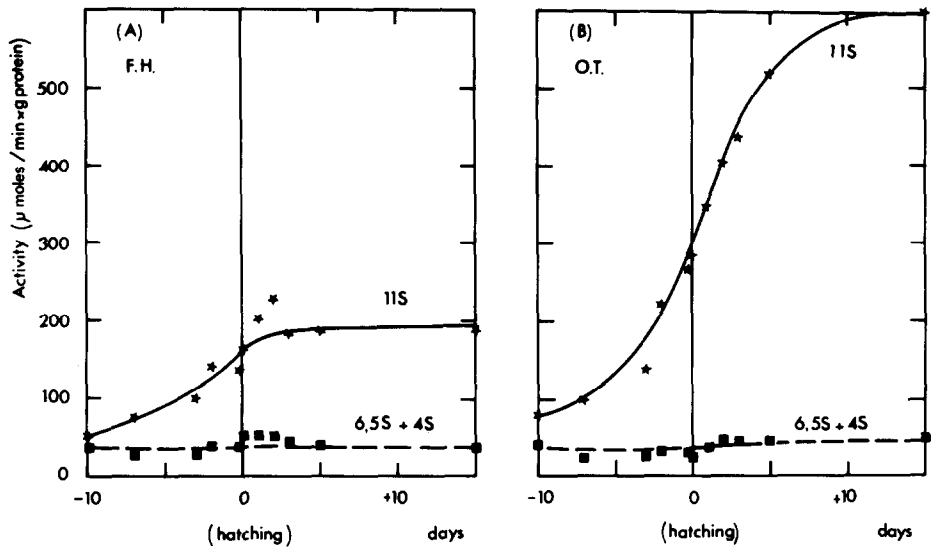

Fig.3. Variation of specific activities of the heavy (11 S) and light (6.5 S + 4 S) components of acetylcholinesterase during development. (A) forebrain hemispheres (B) optic tectum, the specific activities expressed as μmol acetylcholine hydrolyzed/min/g protein, was computed from the data shown in fig.1, the protein concentration in the extracts, and the ratios of heavy to light components obtained from the sedimentation patterns of acetylcholinesterase (fig.2).

relative proportions of heavy and light forms. In order to study the evolution of this phenomenon, we chose as a parameter the ratio of the activities of heavy to light forms (6.5 S and 4 S form being considered together). This ratio was calculated from the sum of activities found in the different fractions of each peak.

This ratio increased markedly with age, from a value of 1.43 and 1.88 (−10 days) to a value of 5.06 and 11.83 (+ 15 days) in the hemispheres and optic tectum respectively. From these data, we computed the specific activity of the heavy (11 S) and light (6.5 S + 4 S) components (fig.3). It is remarkable that the specific activity of the light component remained essentially constant and equal in both brain regions during the period examined, while that of the 11 S forms varied: its increase constituted the major part of the total activity increase, so that the 11 S curves in fig.3 closely parallel the curves of fig.1.

There are marked quantitative differences between the acetylcholinesterase activities of chicken and rat; comparing our results with those of Rieger and Vigny [4], we find that the activity/gram brain is about 10 times lower at birth in the rat, and in the adult, reaches only 40% of the value observed in the 15 day-old chick. Thus the increase in acetylcholinesterase is smaller and takes place later in the rat. However, in both animals the specific activity of the light forms appear unchanged from the perinatal to the adult stage, while an heavier molecular form accumulates.

In spite of differences in sedimentation coefficients, a certain homology between rat and chicken molecular forms of acetylcholinesterase is therefore likely. The 11 S form of chicken acetylcholinesterase thus appears as a maturated, probably membrane-bound, enzyme. The situation is more complex than in rat however, since the light component consists of two 4 S and 6.5 S forms. The minor 4 S was much more visible in the hemispheres than in the optic tectum although the specific activity of the light component was equal in the two brain regions.

Besides this qualitative difference, we observed that the 11 S form rose to a value three times higher in the optic tectum than in the hemispheres. Such a high concentration of acetylcholinesterase in the optic tectum suggest a high density of cholinergic synapses and might be related to the important role played by vision in birds.

Our results are thus qualitatively similar to those

reported for whole rat brain where accumulation of the 10 S form has been interpreted as the manifestation of a maturation process [4]. However, we find that the specific activity of acetylcholinesterase is markedly higher in chicken than it is in rat (1.4 nmol/min × g tissue at birth, to 9 μmol/min × g tissue in the adult). In addition, we observe both a quantitative and a qualitative difference in the two regions of the chicken which we have separately studied: the minor 4 S form was much more noticeable in the hemispheres than in the optic tectum, and although the specific activity of the light forms was essentially identical in both regions, the 11 S activity was increased to a value three times higher in the latter,

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