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THYROID HORMONE RECEPTORS OF DEVELOPING CHICK BRAIN ARE PREDOMINANTLY IN THE NEURONS

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The relative concentration of the triiodothyronine (T_3) receptors in the neuronal and glial nuclei of developing chick brain have been studied. Scatchard analysis indicate that the number of T_3 binding sites in the neuronal nuclei increases from 400 to 1600 sites/nucleus between 7-11 day of embryonic development without any concomitant change in the level of glial nuclear receptors (130 - 200 sites/nucleus). Both sites are of high affinity (K_a = 1-3 x 10^9 M^{-1}) at all ages examined. The abundance of the T_3-receptors in the neuronal nuclei and the close coincidence of the period of rise in the level of these receptors in these nuclei (7-11 day) with that of maximal neuronal growth and synaptogenesis (7-13 day) suggest that the neurons are the primary site of action of T_3 in the developing brain.

The essential role of thyroid hormones in the development and maturation of mammalian brain has been well documented (for review, see Ref 1). In hypothyroid rat brain, the major lesion is in the neuronal differentiation manifested by the formation of axons and dendrites (2-5). Available evidence(6) indicate that hypothyroidism retards the assembly of neurotubules - a process that is required for the growth of dendrites and axons (7-9).

Thyroid hormones mediate their effect by interaction with specific chromatin associated receptor proteins (10). Brain tissue is composed of a variety of cell types. Conceivably, the receptors for T_3 could be distributed randomly in different types of brain cells or localized selectively in certain target cells for the hormone. We report here the distribution of receptors in the nuclei of the two major cell types of brain, viz. - the neuron and the glial cells of the embryonic chick. Ease of availability

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and freedom from maternal hormonal influence prompted us to use the chick system. Since the embryonic age of 6-11 day of chick represent the period of rapid increase in the level of neurotubule protein (11) and that of 7-13 day the period of maximal growth of neuronal processes (12), we decided to study the ontogeny of T_3 receptors in the neuronal and glial nuclei of brains covering these embryonic ages.

MATERIALS AND METHODS

Fertile white Leghorn chick eggs were obtained from local poultry and incubated in the laboratory. L-[^{125}I] triiodothyronine (T_3), having specific activity 70-80 Ci/m mole, was purchased from Bhaba Atomic Research Centre, Bombay, India. Unlabeled T_3 was obtained from Sigma Chemical Co., USA.

Preparation of nuclei and their separation into neuronal and glial fractions were performed at 0-4°C using some modifications of the procedure described before (13-17) for rat brain. Briefly, whole brain tissues were homogenized in 10 parts (w/v) of 0.32 M sucrose containing MP buffer (3 mM $MgCl_2$ - 1 mM sodium phosphate, pH 6.5) and the nuclei pelleted at 700xg for 10 min. The pellets were suspended in 2.1(M) sucrose and centrifuged for 1 hr. at 58,500xg (for embryonic ages upto 13 day) or 86,500xg (for 19-day embryo and adult). These pellets of total nuclear population were then suspended in 9.0 ml 2.38 M (for adult) or 2.425 M (for embryonic ages) sucrose - MP buffer, overlayed with 25 ml of 2.1 M sucrose containing MP buffer and centrifuged at 58,500xg (embryonic ages up to 13-day) or at 89,900xg (for 19-day embryo and adult) for 1 hr. in a Sorvall ultracentrifuge using AH-627 rotor. Following centrifugation, the neuronal and glial nuclei appeared at the interphase and at the pellet respectively. These two fractions were collected and their identity was confirmed on the basis of differences in size, morphology and staining properties as described before (12-17); they were stained separately by mixing 1% aqueous toludine blue and 19 parts of nuclear suspension in 0.32 M sucrose - MP buffer. For microscopic examination and photography, a built-in camera attached to an American Optical inverted microscope was used. There was 5-10% neuronal contamination in the glial nuclei and 10-20% glial contamination in the neuronal nuclei and these cross-contaminations were taken into account in calculating the receptor sites per nucleus. Nuclear fractions were then suspended in 0.32 M sucrose - 0.25% triton-X-100 containing MP buffer, pelleted at 700xg, washed twice, once with the same buffer without triton-X-100 and then with 0.14 M NaCl - 3 mM $MgCl_2$ - and finally pelleted at 1000xg. Nuclei were usually preserved overnight at -20°C prior to extraction by vortexing at 0°C (18) with 0.4 M NaCl - 5 mM $MgCl_2$ made up in buffer A [20 mM Tris-HCl, pH 8.0, 2.0 mM EDTA, 5.0 mM mercaptoethanol and 10% (vol/vol) glycerol] using 1 ml buffer per 300 - 350 x 10^6 nuclei. After vortexing for 40 minutes, equal volume of buffer A was added to the suspension and it was clarified by centrifugation at 20,000xg for 20 minutes. Aliquots of the extract were incubated in duplicate with increasing concentrations of [^{125}I] T_3 (0.2-45 x 10^{-10}M) at 30°C for 40 min to allow dissociation of endogenously bound hormone followed by 17-18 hr. at 0°C as described by Schwartz and Oppen-

heimer (19). Parallel set of tubes were also incubated in presence of 1000 fold excess of unlabeled T_3 to correct for non-specific binding. Bound and free hormones were separated by passing through 2.5 ml columns of Sephadex G-25 and the data analyzed by the method of Scatchard (20).

RESULTS

Fig.1 shows the representative photographs of neuronal and glial nuclear preparations used in the present study. Morphological and size differences between the two types of nuclei are similar to those reported for rat brain (13).

In all age-groups studied (7, 9, 11, 13, 19-day embryo and adult), analysis of the T_3-binding data of nuclear receptors were performed by Scatchard plots. The binding affinities (equilibrium association constants) and the binding capacities (expressed as the number of receptors per nucleus) were determined from these plots. Fig.2 shows the typical Scatchard plots for nuclei from brains of 9 day and 13 day embryos. A single class of binding sites, characterized by a single slope was observed in all cases. The equilibrium association constants of the neuronal and glial nuclear receptors for T_3 ranged between 1.3-3 x 10^9 M^{-1} and

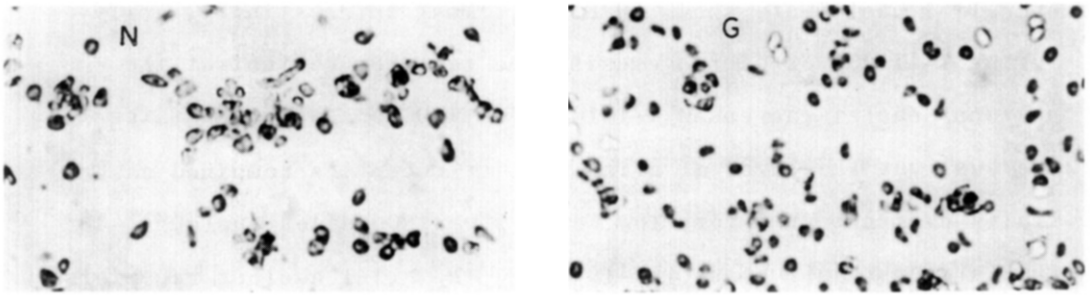

Fig.1 Representative photographs of neuronal(N) and glial(G) nuclei from 13 day embryonic chick brain. Both types of nuclei were stained with toluidine blue and photographed under identical conditions using a built-in camera attached to an American Optical Inverted Microscope. Note that neuronal nuclei appeared relatively large and pale with one or two well defined nucleoli. The size difference between the neuronal and glial nuclei increased progressively with age.

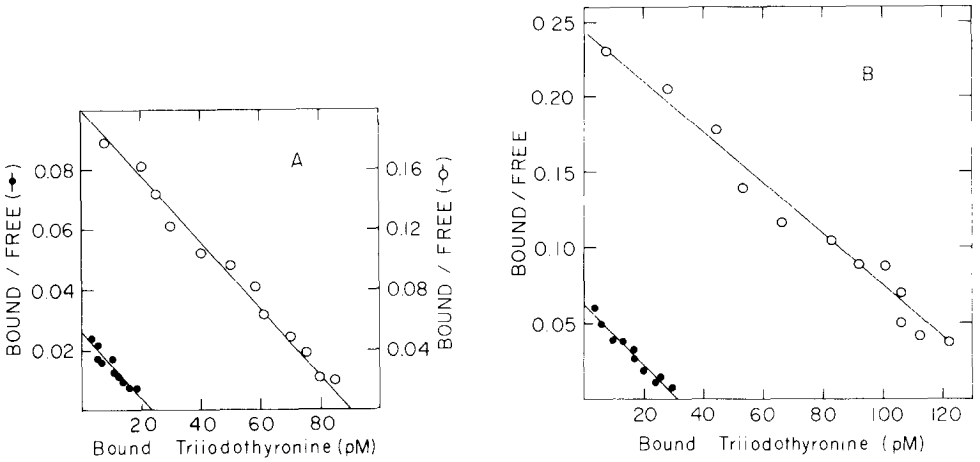

Fig.2 Scatchard analysis of T_3 binding to neuronal (○——○) and glial (●——●) nuclear receptors of 9 day (A) and 13 day (B) embryonic chick brain. Aliquots of nuclear extracts corresponding to 70 and 60 x 10^6 nuclei for 9 and 13 day brain respectively were incubated with ^{125}I-T_3 in duplicate in a volume of 1 ml in the presence and absence of 1000-fold unlabeled T_3. Bound and free hormone were determined as described in "Methods".

0.77-2.8 x 10^9 M^{-1} respectively at various ages. These affinity constants are comparable to those reported for embryonic chick liver (21).

In contrast to rather constant binding affinity, the binding capacity of the neuronal nuclei increased progressively with age. Developmental changes in the T_3-binding capacity of the neuronal and glial nuclei are shown in Fig.3. Most interestingly, early during 7-11 day of embryogenesis, the receptor content of the neuronal nuclei increased 4-fold (from 400 to about 1600 sites/nucleus) but the level of glial nuclear receptors remained essentially constant at a very low level (130 - 200 sites/nucleus). In the late embryonic phase (11-19 day), the glial nuclear receptors increased slightly from 200 to 440 sites/nucleus; correspondingly the receptors in the neuronal nuclei increased from 1600 to 2000 sites/nucleus. Finally in the adult brain too, the level of T_3 receptors in the neuronal nuclei (8000 sites/nucleus) is far greater than that in the glial nuclei (1000 sites/nucleus).

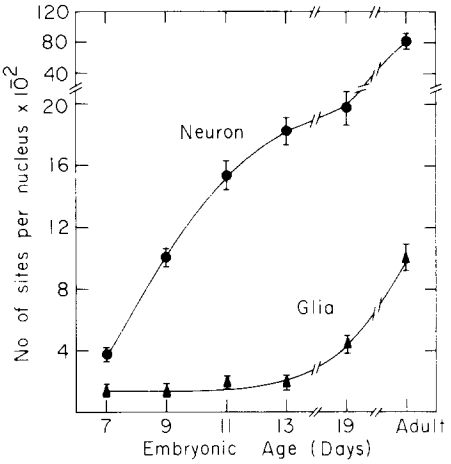

Fig.3 Age-related changes in the level of T_3-receptors (expressed as number of T_3 binding sites/nucleus) in the neuronal (●——●) and glial (▲——▲) nuclei. Binding data for all the indicated ages were subjected to Scatchard analysis and the number of sites/nucleus was calculated as described in "Methods". Bar represents the range of variation observed between replicate experiments.

DISCUSSION

The most important outcome of the experiments presented here is the finding that in both embryonic as well as in adult brain, the receptors for T_3 are localized predominantly in the neuronal nuclei. Fig.3 indicate that at embryonic ages of day 7 and 9, the ratio of T_3-receptors in the neuronal : glial nucleus is about 2 : 1 and 4 : 1 respectively. At all other ages examined, the level of the receptor in the neuronal nucleus is 5-8 fold greater than that in the glial nucleus. Our own microscopic observation of total nuclear population indicate that early during embryogenesis (9-11 day) the ratio of the two types of nuclei remains nearly identical and thereafter the proportion of glial cell increases reaching about 70-80% of the total population at adulthood. This is in accordance with the reports (22,23) that mitosis of chick neuroblasts occur rapidly between 7-11 day and ends by day 12. Even if these developmental alterations in the neuronal and glial cell population are taken into consideration,

the majority of the T_3 receptors in the embryonic chick brain appears to be in the neuronal cells.

Studies on the ontogeny of T_3 receptors using total nuclear population of rat brain (11) indicate that the level of these receptors is very high in the first two weeks after birth which corresponds to the period of rapid neuronal differentiation. Our studies on the level of T_3 receptors in the total nuclei of chick brain (unpublished data) also indicate a similar correspondence.

The relative abundance of the T_3 receptors in the neuronal nuclei, the low level of these receptors in the glial nuclei, and the temporal coincidence of the period of increase of the receptors in the neuronal nuclei with that of maximum neuronal processes and synaptogenesis (12) suggest that the major role of thyroid hormone in the developing brain lies in the differentiation and maturation of neurons.

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