TRIIODOTHYRONINE BINDING TO BRAIN CYTOSOL RECEPTORS DURING MATURATION

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1. Introduction

Thyroid hormones play a unique role in the development of the central nervous system. Indeed, thyroid hormone deficiency at birth results in irreversible brain damage, if not recognised at an early age. The observation that late treatment is ineffective indicates that during development the brain becomes for a limited period of time a specific target for thyroid hormones. In vivo [1] and in vitro [2] experiments have established the presence of nuclear binding sites in adult brain. Some of the properties of nuclear binding sites have been described in developing rat brain [3]. Except for a report on rat cerebellum [4], no other data are available on cytosolic brain receptors during development. We therefore studied the properties of cytosolic receptors for triiodothyronine (T_3) in brain and liver in rats aging from 3–50 days.

2. Materials and methods

Brain and liver were removed from young rats killed by decapitation. The tissues were kept on ice and rinsed twice in phosphate saline buffer (0.01 M, pH 7.5, NaCl 0.15 M). The assays were always performed on fresh preparations. All subsequent steps were carried out at 0–4°C. The tissues were homogenised with a motor-driven Teflon pestle in 5 vol. 0.25 M sucrose, 20 mM Tris–HCl, 1 mM $MgCl_2$, 1 mM dithiothreitol and 5% glycerol, at pH 7.6. The homogenate was centrifuged at 3000 × *g* for 12 min, the supernatant collected and further centrifuged at 105 000 × *g* for 90 minutes at 4°C. Protein concentration was determined by the Lowry method.

The binding of 3′,3,5-triiodothyronine (T_3) to cytosolic receptors was studied by incubating cytosol with labelled hormone, and increasing amounts of unlabelled T_3 (detailed in fig.1). The amount of cytosol proteins was in the range where the binding was proportional to the concentration of protein. The binding increased with incubation time up to 30 min, leading to the binding of 15% of the tracer hormone. The plateau was maintained for at least another 30 min. The reversibility of the reaction was ascertained: an excess amount of unlabelled T_3 (10^{-6} M) added when maximum fixation of the tracer was attained, completely displaced the tracer after 75 min. As in [4], we found that it was important to maintain the temperature close to 0°C. Indeed, no binding was observed at different temperatures tested: 8, 15, 20 and 37°C, whereas at 4°C the binding was slightly decreased (12% versus 15% at 0°C). The bound hormone was separated from the free hormone by adsorption of the free hormone on dextran-coated charcoal (0.2 ml; 5% charcoal, 1% dextran in 20 mM Tris–HCl, pH 7.8). The tubes were vortexed, kept on ice for 10 min and centrifuged at 3000 × *g* for 8 min. An aliquot of the supernatant (0.2 ml) was taken for measuring the radioactivity.

Affinity constants and maximum binding capacity were derived from the Scatchard analysis of the data.

3. Results and discussion

A marked difference was observed in the behaviour of brain and liver receptors during maturation. Liver receptors show a decrease in the affinity constant and

an increase in the maximum binding capacity, whereas in brain the affinity constant of the T_3 for the binding sites seems to peak between days 12 and 15, with only small changes in the number of binding sites. The maximal binding capacity and the number of sites per cell seems to be the lowest, when the K_a values reach their highest value (table 1, fig.1).

The observed differences between the K_a values of brain cytosol receptor in the three groups (group I, 3, 5 and 7 days; group II, 12 and 15 days; group III, 25 and older) were statistically significant ($p < 0.001$).

Our results on liver receptors in development are in agreement with recent data also showing an increase in the number of binding sites with a small decrease in the K_a [4]. The data on brain tissue are at variance with the observations of the same author on rat cerebellum showing a marked and progressive decline in the affinity constant with age, at the stages studied (10, 20 and 50 days) whereas we observe a surge in the K_a during a limited period around days 12–15. Interestingly enough the peak in K_a occurs precisely when cytodifferentiation in the brain is maximal [5].

The physiological role of cytosolic receptors is still debated. It has been established that 10% of the intracellular T_3 is nuclear bound and that the access of thyroid hormone to the nuclear receptor does not require a prior binding of the hormone to cytosol proteins as this is the case for steroid hormones. The presence of specific extranuclear receptors for thyroid

Table 1
Association constants (K_a), maximal binding capacity (R_0) and no. receptors/cell (N) of brain and liver during maturation

	Age (days)	K_a (10^8 M^{-1})	R_0 (10^{10} M)	N
Brain				
	3	1.73	5.55	–
	5	1.37	5.90	9800
	7	1.45	6.15	–
	12	2.69	5.10	5614
	15	2.94	4.72	5930
	25	0.58	6.57	–
	Adult	0.63	5.55	7995
Liver				
	5	3.94	1.7	–
	15	1.92	4.3	–
	25	2.07	8.45	–

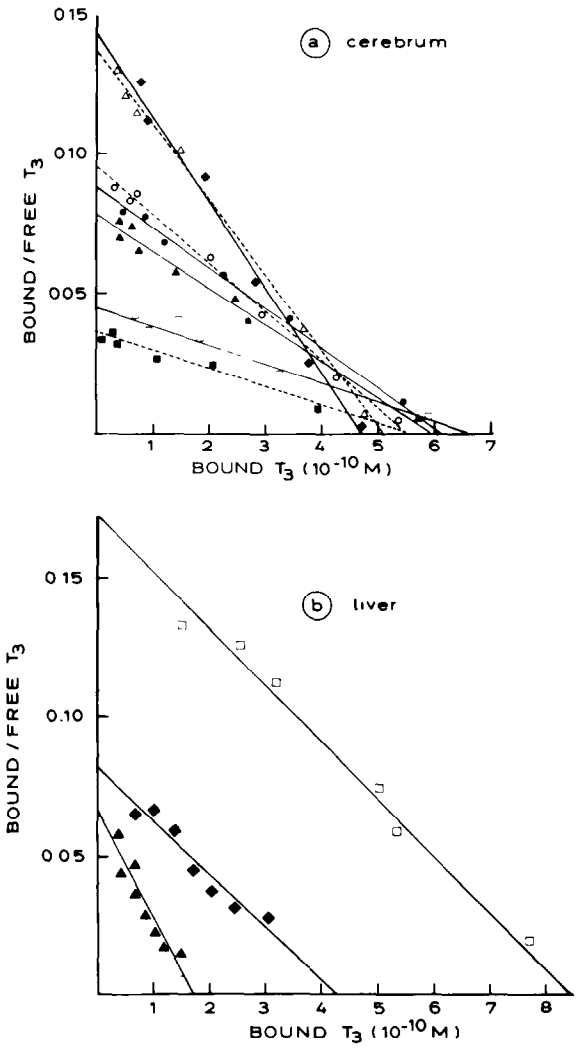

Fig.1. Scatchard plots of the binding of T_3 to cytosol proteins prepared from rat cerebral tissue (a), and liver (b). Cytosol (120 μg protein) was incubated with labelled hormone ([^{125}I]-T_3, spec. act. 573 mCi/mg, final conc. 2×10^{-10} M) and increasing amounts of T_3, ranging from 10^{-10}–10^{-6} M. Binding of [^{125}I]T_3 at 10^{-6} M T_3 was considered as non-specific, and subtracted from the radioactivity observed at lower concentration. Incubations were performed in polystyrene tubes in final vol. 0.5 ml. After 45 min at 0°C, the bound hormone was separated from the free hormone by the addition of 0.2 ml dextran-coated charcoal (5% charcoal, 1% dextran in 10 mM Tris–HCl, pH 7.8). The tubes were vortexed, kept on ice for 10 min and centrifuged at $3000 \times g$ for 8 min. An aliquot of the supernatant (0.2 ml) was taken for measuring the radioactivity. Following signs were used to identify the different days: (○) 3, (▲) 5, (●) 7, (△) 12, (♦) 15, (□) 25, (■) 50.

hormone has been documented for several subcellular fractions (membrane [6,7], cytosol [8–10], mitochondria [11]) leading to the concept that the interaction of thyroid hormone with the nucleus would not be the sole stimulus for hormonal expression [12]. Indications are indeed available that membrane transport [7], and the activity of enzymes as diphosphoglycerate mutase and adenylate cyclase are directly influenced by thyroid hormones [13]. The number of cytosolic binding sites has been reported to decrease [14] or to remain unaltered [12] in the liver of hypothyroid rats, without significant changes in the affinity constant. How thyroid hormones could act on their receptors, and how this effect could be different in various organs has yet to be defined. The same is true for the nuclear binding sites where seemingly discrepant results are available. A decrease in T_3 serum levels increases the number of nuclear binding sites in the brain [3], in cultured pituitary cells [15], and either depresses the number of sites in the liver [6], or does not affect it [4].

Since no direct involvement of the cytosolic binding proteins has been clearly identified up to now, it would only be safe to conclude that these proteins would act as buffer system and/or storage of thyroid hormones for extranuclear hormonal effects. In this regard a higher K_a during development could provide the brain cell with a regulatory mechanism for increasing its hormonal supply at a critical period of maturation.

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