

Thyroxine preferentially stimulates transcription by isolated neuronal nuclei in the developing rat brain cortex

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The administration of thyroxine to neonatal rats stimulates RNA synthesis by neuronal nuclei isolated from the developing rat brain cortex. Glial nuclei are relatively resistant to thyroxine treatment. The activity of neuronal RNA polymerase II is particularly stimulated by the hormone. Thyroxine also affects neuronal chromatin structure as shown by changes in the relative proportion of different subnuclear fractions obtained by gentle micrococcal nuclease digestion of nuclei from hormone-treated rats.

Thyroid hormone	Development	Rat brain cortex	Brain cell nucleus	RNA polymerase
		Micrococcal nuclease		

1. INTRODUCTION

The mechanism by which thyroid hormones promote growth and maturation of the mammalian brain is not fully understood [1]. Available data suggest that thyroid hormones stimulate RNA and protein syntheses in target tissue by interacting with specific chromatin-bound receptors [2]. Thus it has been shown that triiodothyronine enhances the synthesis of poly(A)-containing RNA in liver [3] and increases the level of growth hormone mRNA in rat pituitary cells in culture [4,5]. Previous studies have also shown that triiodothyronine administration increases the activities of RNA polymerases in rat liver [6] and brain tissue [7].

Recently it was shown that neuronal RNA and protein syntheses are influenced by the thyroid status of neonatal rats [8]. This study was undertaken to investigate the effect of neonatal thyroxine treatment on RNA metabolism in isolated nuclear fractions enriched in neuronal and glial cell nuclei. It was also examined whether thyroxine administration affects brain cell chromatin structure as studied by gentle micrococcal nuclease digestion of isolated nuclei [9,10].

2. MATERIALS AND METHODS

[³H]UTP and [³H]poly(U) were purchased from the Radiochemical Centre (Amersham) and α -amanitin was obtained from Boehringer-Mannheim. Micrococcal nuclease was obtained from Worthington Biochemicals. The source of the rest of the materials has been described in [8].

Neonatal rats of the Wistar strain received saline or sodium L-thyroxine (10 μ g/animal) every third day as in [11]. The animals were killed 24 h following the last hormone injection and the brains were rapidly removed. Cortical tissues were homogenized in 20–25 vols ice-cold 1.6 M sucrose in TM buffer (3 mM MgCl₂–10 mM Tris–HCl, pH 7.5, at 20°C) and the homogenate fraction was centrifuged over 2.0 M sucrose in TM buffer to prepare nuclei. The crude nuclear pellet obtained was subsequently resuspended in 2.38 M sucrose in TM buffer and neuronal and glial cell enriched nuclei were prepared by discontinuous sucrose density gradient centrifugation as in [12–14]. The purity of nuclear fractions was studied by phase-contrast microscopy and different nuclei were identified by the criteria described in [14,15]. Nuclei were resuspended in 25% (v/v) glycerol and 1 mM

MgCl₂–10 mM Tris–HCl (pH 7.9) and were used immediately for the study of RNA synthesis in vitro.

The standard nuclear reaction mixture was essentially as in [16]. It contained in a final volume of 0.2 ml, nuclei (40–60 µg DNA), 40 mM Tris–HCl (pH 7.9), 2 mM MgCl₂, 1.5 mM MnCl₂, 0.5 mM dithiothreitol, 0.3 mM each of ATP, CTP, GTP, 0.05 mM unlabelled UTP, 1 µCi [³H]UTP (spec. act. 45 Ci/mmol) and either 25 or 250 mM (NH₄)₂SO₄ (low- and high-salt conditions, respectively). Where indicated, α-amanitin (1 µg/ml) was added to the reaction mixture to inhibit specifically the activity of RNA polymerase II [17]. Following incubation at 25°C the reaction was terminated by the addition of 0.5 ml ice-cold 10% (w/v) trichloroacetic acid and 0.5 mg bovine serum albumin. The samples were washed with 5% trichloroacetic acid and the acid-insoluble radioactivity was determined as in [8].

The poly(U)-degrading activity of brain nuclei was measured under identical conditions to those described above except that the nucleoside triphosphates were omitted from the reaction and 50 nCi [³H]poly(U) was used as the substrate. Following incubation for 60 min at 25°C the reaction was terminated as described above. The activity was expressed as the amount of radioactivity liberated from poly(U) into the supernatant frac-

tion during the reaction. Blank values obtained with samples kept on ice for the same period of time were subtracted from all data points.

Micrococcal nuclease treatment of brain nuclei, washed with 0.1% (v/v) Triton X-100, was performed essentially as in [9]. Digestion was carried out for 2 min at 25°C with 0.5 units nuclease per A₂₆₀ unit nuclei and the reaction was stopped by the addition of 2 mM EGTA. Subnuclear fractions were subsequently prepared as in [9] and the recovery of DNA in different fractions was determined as in [8].

3. RESULTS AND DISCUSSION

The results presented in table 1 show that thyroxine treatment of neonatal rats significantly (36%) stimulates the RNA synthetic activity of neuronal nuclei in both age groups studied. The activity of glial nuclei is relatively more resistant to the hormone administration (table 1). These results are in keeping with that of a recent study showing that thyroid hormone receptors in developing chick brain reside predominantly in the neuronal nuclei [18].

Table 1 also shows that the stimulation of neuronal transcription by thyroxine is diminished in the presence of a low concentration of α-amanitin to inhibit the activity of RNA polymerase

Table 1
The effect of thyroxine treatment on the synthesis of RNA by isolated brain cell nuclei

Age (days)	α-Amanitin	[³ H]UMP incorporated (× 10 ⁻³) (cpm/mg DNA)			
		Neuronal nuclei		Glial nuclei	
		Control	+ thyroxine	Control	+ thyroxine
9 (9)	–	13.2 ± 0.4	17.9 ± 1.00 ^b	14.6 ± 1.1	15 ± 0.9
	+	7.0 ± 0.6	7.8 ± 0.7 ^a	12.2 ± 0.7	12.9 ± 0.8
18–21 (8)	–	9.9 ± 1.2	13.9 ± 1.1 ^b	6.6 ± 0.6	6.8 ± 1.0
	+	4.8 ± 0.5	5.9 ± 0.6 ^a	3.3 ± 0.2	3.4 ± 0.5

^a *p* < 0.05

^b *p* < 0.01 as determined by Student's *t*-test

Nuclear fractions enriched in neuronal and glial cell nuclei were prepared from the developing brain cortex of control and thyroxine-treated rats (see section 2). Isolated nuclei were incubated in vitro for 10 min at 25°C under low-salt conditions in the absence and presence of α-amanitin (1 µg/ml) as described in section 2. The results are given as means ± SD of the number of different preparations of nuclei (in parentheses)

II. The addition of a higher concentration of α -amanitin (100 μ g/ml) to the assays, to inhibit RNA polymerase III activity [17], resulted in a 10% further decrease in the activity of different brain nuclei (not shown). Thus the results in table 1 indicate that thyroxine treatment significantly enhances the activity of neuronal RNA polymerase II and has a minor effect on that of RNA polymerase I. This finding agrees with the results of a previous study showing no substantial effect of repeated thyroxine injections on the activity of RNA polymerase I in isolated nuclei from hypothyroid rat cerebrum [7]. However, previous studies have shown that triiodothyronine treatment markedly enhances the activity of RNA polymerase I in rat liver nuclei [6] and in hindlimb nuclei of *Rana catesbeiana* tadpoles [19].

The discrepancies in the results obtained could be due to the different rates of ribosomal RNA synthesis in rat liver and brain tissue [20]. It is also possible that thyroxine activates the two forms of neuronal RNA polymerases at different times following the hormone treatment as has been shown for the corresponding enzymes in rat liver [6,21]. Here, animals were decapitated 24 h after the last hormone injection.

The time course of [3 H]UTP incorporation into nascent RNA by isolated brain nuclei is shown in fig.1. RNA synthesis by different brain nuclei in vitro is linear for about 10 and 30 min under low- and high-salt conditions, respectively. More than 80% of the activity measured in the presence of

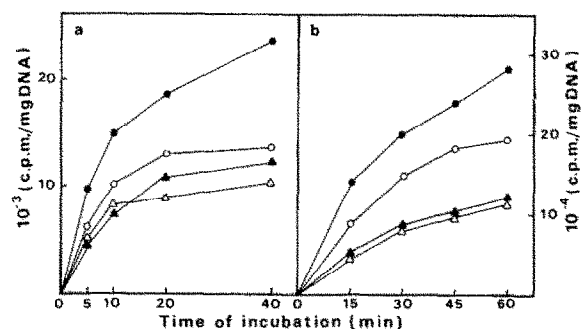


Fig.1. Time course of [3 H]UTP incorporation into RNA by isolated brain nuclei from 18-day-old rats. Nuclei were incubated in vitro for different time intervals at 25°C under low-salt (a) and high-salt (b) conditions. Neuronal nuclei: (○—○) control rats; (●—●) hormone-treated rats. Glial nuclei: (Δ—Δ) control rats; (▲—▲) hormone-treated rats. The ordinates are different in (a) and (b).

high-salt was inhibited by low concentrations of α -amanitin and thus represents transcription due to RNA polymerase II (cf. [14]). Thyroxine increases the activity of neuronal nuclei assayed at different ionic strength without affecting that of glial nuclei (fig.1).

To determine whether thyroxine influences the degradation of nascent RNA by isolated brain nuclei, incubation was performed in the presence of radioactive poly(U). The results presented in table 2 show that neuronal nuclei from euthyroid and thyroxine-treated rats exhibit the same

Table 2

The effect of thyroxine treatment on poly(U)-degrading activity in brain nuclei

Source of nuclei	Nuclear fraction	Poly(U)-degrading activity (cpm/mg DNA)	
		– ATA	+ ATA
Control rats	Neurone	37.9 ± 1.2	6.9 ± 0.8
	Glia	26.7 ± 0.6	4.2 ± 0.5
Thyroxine-treated rats	Neurone	39.1 ± 1.9	6.2 ± 0.7
	Glia	25.8 ± 1.2	4.6 ± 0.8

Brain nuclei obtained from 9-day-old rats were incubated for 60 min at 25°C in the presence of radioactive poly(U) (see section 2). Aurin tricarboxylic acid (0.1 mM) was added to the assays to inhibit ribonuclease activity. The results are means ± SD of 5 different experiments, and represent the amount of radioactivity liberated from the [3 H]poly(U) substrate during incubation

poly(U)-degrading activity. It is also shown that the antibiotic aurin tricarboxylic acid, which is a potent inhibitor of ribonuclease [22], decreases the endogenous nuclease activity of control and hormone-treated nuclei to the same extent (table 2). Pulse-chase experiments employing unlabelled UTP also revealed that nascent RNA chains synthesized by neuronal nuclei from control and hormone-treated rats have the same stability *in vitro* (not shown).

The results of a number of studies indicate that the activation of transcription is accompanied by changes in chromatin structure [23]. Micrococcal nuclease has been shown to excise preferentially genes being actively transcribed by attacking the linker region between nucleosomes [24,25]. To investigate whether thyroxine induces a change in the structure of neuronal chromatin, nuclei from control and hormone-treated rats were gently digested with micrococcal nuclease. This procedure has been shown to resolve the chromatin into two fractions with different transcriptional capacities [9,26]. The results in table 3 show that thyroxine treatment increases the relative proportion of the subnuclear fraction P₂, which sediments at high speed and which has been shown to be enriched in active transcribing chromatin [9,26]. Thus DNA in this neuronal fraction comprises about 10% of total DNA in controls, whereas the corresponding figure in thyroxine-treated rats is increased to 14% (table 3). It has also been shown that the susceptibility of rat liver chromatin to digestion with micrococcal nuclease is enhanced following triiodothyronine treatment of thyroidectomized rats [27]. The structural basis for the increased nuclease sensitivity of nuclei from hormone-treated rats remains unknown.

Thyroid hormones have been shown to exert multiple effects on cellular metabolism in target tissue [2]. Our results demonstrate that thyroxine preferentially stimulates RNA synthesis by isolated neuronal nuclei in the developing rat brain cortex. The relative resistance of RNA metabolism in glial nuclei to thyroxine treatment is consonant with the preponderance of thyroid hormone receptors in neuronal nuclei [18]. The results of previous studies indicate that the outgrowth of neuronal processes and the differentiation of the nerve cell are markedly retarded in the hypothyroid brains of neonatal rats [1,8]. However, the process of

Table 3

Distribution of DNA in different subnuclear fractions obtained by mild micrococcal nuclease treatment of neuronal nuclei from control and hormone-treated rats

Source of nuclei	Subnuclear fraction	DNA recovered (% of total)
Control rats (<i>n</i> = 6)	P ₁ pellet	88.3 ± 0.4
	P ₂ pellet	9.9 ± 0.2
	Supernatant	1.8 ± 0.3
Thyroxine-treated rats (<i>n</i> = 10)	P ₁ pellet	84.9 ± 0.3 ^a
	P ₂ pellet	14.0 ± 0.3 ^a
	Supernatant	1.1 ± 0.2

^a *p* < 0.01

Neuronal nuclei were digested with micrococcal nuclease and the different subnuclear fractions were obtained by differential centrifugation as in section 2

myelination is also impaired in these animals with a possible permanent deficit in the total amount of myelin deposited [28]. It remains to be elucidated whether the derangement of glial cell metabolism in hypothyroidism is secondary to alterations in neuronal RNA and protein syntheses or whether thyroid hormones directly influence myelination as has been suggested [29].

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