ences concern hormones involved in the regulation of stress reactions and in the redistribution of energy resources in stressed and hypoxic animals. The LR and HR groups did not differ significantly in blood levels of testosterone.

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Regulation of RNA Polymerase Activity in Liver and Brain Cell Nuclei by a Cytoplasmic Thyroxine Modulator in Rats of Various Ages

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Hormones play important roles in the regulation of the morphological and functional differentiation which cells undergo during ontogeny. The role of the thyroid hormones in this differentiation consists essentially in the activation of protein synthesis in cell nuclei and ribosomes via the nuclear hormone-receptor complex [4,12]. However, regulation of metabolic processes by the thyroid also occurs at the level of numerous membrane structures of the cell, as is indicated by the presence of highly specific binding sites for thyroid hormones in mitochondria [10], cytosol [6], and on plasma membranes [8]. Most of the published information on receptor structures of thyroid hormones has been obtained in studies of mature organisms. The number of investigations examining thyroid hormone receptors in the course of ontogeny is small. The best studied are nuclear receptors of developing tissues [5], and there is some scant information about receptors localized in the cytosol and other cell organelles [1].

Previously, we identified a thyroxine-binding protein designated thyroxine modulator, or T_4M, that mediates certain effects of thyroxine in the nucleus and mitochondria [2,3]. The present study was undertaken to compare the effects of this modulator on RNA polymerase activity in liver and brain cell nuclei of rats during ontogeny.

MATERIALS AND METHODS

For the experiments, 20-day hyperthyroid embryos and 7-, 20-, 45-, and 90-day-old rats of the

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TABLE 1. Effect of Thyroxine Modulator (T_4M) on RNA Polymerase Activity of Liver Cell Nuclei from Rats of Various Ages (the Values are Means±SEM; $n = 10$)

	Treatment	^{3}H-UMP incorporation	
		cpm	%
20-day embryos	T_4 alone	1701.5±56	100
	$T_4 + T_4M$	2211.3±93	130
7-day-old rats	T_4 alone	1983.0±41	100
	$T_4 + T_4M$	2636.1±69	138
20-day-old rats	T_4 alone	2763.3±143	100
	$T_4 + T_4M$	3936.0±178	143
45-day-old rats	T_4 alone	3556.1±193	100
	$T_4 + T_4M$	9680.0±267	264
90-day-old rats	T_4 alone	3778.2±224	100
	$T_4 + T_4M$	11711.1±300	310

Wistar strain were used. The cytoplasmic thyroxine modulator (T_4M) was isolated from their liver and brain nuclei as described earlier [2]. In the embryos and 7-day-old rats, hyperthyroidism was induced by injecting the mother rat with L-thyroxine (T_4) at 0.5 μg/g body weight for 7 days starting with day 13 of pregnancy. In the rats aged 20, 45, and 90 days, it was produced by injection of the hormone at 1 μg/g over the same period starting with day 7 before sacrifice.

Cell nuclei from the liver and brain were isolated by the standard procedure [11], homogenizing their tissues in 10 volumes of 0.25 mol/liter sucrose and 10 mmol/liter Tris-HCl (pH 7.5) and then applying the nuclear sediment onto 2.2 mol/liter sucrose. Total RNA polymerase activity [11] was measured using a medium containing, in a final volume of 250 μl, 6 mmol/liter TrisHCl, pH 7.6; 1 mmol/liter $MgCl_2$; 5 mmol/liter $MnCl_2$; 1 mmol/liter DDT, 200 mmol/liter $(NH_4)_2SO_4$, 0.4 mmol/liter ATP, 0.4 mmol/liter GTP, 0.4 mmol/liter CTP, and 0.03 mmol/liter ^{3}H-UTP (0.05 μCi). The reaction, carried out at 37°C, was initiated by adding 25 μg DNA to each nuclear sample and stopped after 30 min by adding an equal volume of cool 10% trichloroacetic acid (TCA). The precipitates were transferred to Millipore filters (Synpor, Czechoslovakia) after washing the filters with 5% TCA and 85% ethanol, and radioactivity was measured in a Rackbeta 1217 scintillation counter (LKB, Sweden).

RESULTS

In the first (control) series of tests, thyroxine in a physiological concentration (10^{-8} mol/liter) was found to have little or no effect on RNA polymerase activity of isolated liver or brain nuclei from rats of various ages. In the second series, where the cytoplasmic thyroxine modulator (T_4M) was used, RNA polymerase activity was 130% relative to the control value in liver nuclei from rat embryos and progressively higher in those isolated from rats of increasing age (Table 1). In this series, liver cell nuclei were also incubated with T_4M isolated from brains of hyperthyroid rats and found to have virtually the same levels of RNA polymerase activity as did the control samples.

In the third series, in which T_4M was tested for its impact on RNA polymerase activity of brain cell nuclei, the modulator increased this activity by 67% in brain cell nuclei from embryos, by 100%

TABLE 2. Effect of Thyroxine Modulator (T_4M) on RNA Polymerase Activity of Brain Cell Nuclei from Rats of Various Ages

	Treatment	^{3}H-UMP incorporation	
		cpm	%
20-day embryos	T_4 alone	1696±85	100
	$T_4 + T_4M$	2832.6±140	167
7-day-old rats	T_4 alone	1985.0±97	100
	$T_4 + T_4M$	3989.0±275	201
20-day-old rats	T_4 alone	2102.1±43	100
	$T_4 + T_4M$	4394.0±189	208
45-day-old rats	T_4 alone	2414.0±103	100
	$T_4 + T_4M$	2776.1±133	115
90-day-old rats	T_4 alone	2892.0±154	100
	$T_4 + T_4M$	3023.0±131	108

in nuclei from 7-day-old rats, and by 108% or more in nuclei from 20-day-old rats; in the T_4M-treated brain cell nuclei from older rats, however, RNA polymerase activity was almost at the same level as in the control (Table 2). In additional tests, where the modulator isolated from liver cell nuclei was incubated with brain cell nuclei, no substantial increase in RNA polymerase activity of these nuclei was noted at any time of ontogenetic development.

The results of this study indicate that the influence of T_4M from the liver on RNA polymerase activity in this organ differs from that of T_4M in the brain. In the liver, which has been traditionally regarded as an organ responsive to thyroxine, the modulator stimulated the polymerase increasingly as the animal grew. In brain cells of embryos and young rats under 1 month of age it stimulated this enzyme but was virtually nonstimulatory in cells of older rats. These findings are consistent with the reported [7,9] maximal binding of thyroxine to its receptors in the cytoplasm of brain cells during fetal and early postnatal life; thus, the entry of thyroid hormones into the brain in sufficient amounts appears to be assured in the period when they are needed most for the developing brain. In adult life, the brain is less sensitive to these hormones, probably as a result of decreases in the amount or activity of the modulator. The present results may help in understanding the differential sensitivity of tissues to thyroid hormones at different ages.

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