The Early Enhancement of Rat Liver Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase II Activity by Tri-iodothyronine

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(Received 14 April 1975)

It is shown that tri-iodothyronine injected intravenously into thyroidectomized rats induces an early and transient activation of rat liver RNA polymerase II which could be demonstrated to occur 40–80 min after hormonal treatment. There was a simultaneous increase in the concentration of acidic proteins bound to chromatin.

The mode of action of tri-iodothyronine on a target organ (rat liver) involves stimulation of the transcription processes, the earliest of which appeared to be the incorporation of orotic acid into heterogeneous nuclear RNA detectable 3 h after hormonal treatment, followed by an activation of the Mg^{2+} -stimulated RNA polymerase after 10 h and then the Mn^{2+} -stimulated RNA polymerase after 45 h (Tata & Widnell, 1966). Further studies performed 12 h or more after tri-iodothyronine administration showed that the RNA species synthesized in the nucleus under the influence of the hormone behaved as rRNA (Wyatt & Tata, 1968) and that the stimulated RNA polymerase was α -amanitin-insensitive (Smuckler & Tata, 1971). Subsequently, it has been shown that binding of tri-iodothyronine to the nucleus is maximal 30 min after injection of the hormone (Oppenheimer *et al.*, 1974).

In the present study we attempted to demonstrate changes in RNA polymerase II activity (known to be involved in the synthesis of DNA-like RNA) and in the chromatin-associated proteins, shortly after the administration of tri-iodothyronine.

Materials and methods

Sprague–Dawley rats weighed approx. 75 g at the time of thyroidectomy. The drinking water of the surgically manipulated animals was supplemented with 0.85% calcium gluconate for the first week after surgery. The animals were treated intravenously on the thirtieth day after surgery with tri-iodothyronine (0.1 μ g/100 g body wt. dissolved in 1% bovine serum albumin); control animals were injected with an identical volume of 0.15 M-NaCl and the animals were fed until they were killed.

Livers were minced and homogenized in 2 vol. of ice-cold 0.25 M-sucrose in TKM buffer (25 mM-KCl–10 mM-MgCl₂–50 mM-Tris–HCl buffer, pH 7.6 at 20°C). The homogenization and preparation of

nuclei were performed as described by Blobel & Potter (1966) except that treatment of the nuclear pellet with Triton X-100 was omitted. The purity of nuclei was assessed by electron and phase-contrast microscopy and chemical analysis (the average RNA/DNA ratio was 0.132). Nuclei were kept at –15°C in a solution containing 25% (v/v) glycerol in 1 mM-MgCl₂–50 mM-Tris–HCl buffer, pH 7.9 at 20°C, and used in subsequent steps within 24 h.

RNA polymerase I and II activities were assayed in the presence and in the absence of α -amanitin respectively as described by Glasser *et al.* (1972). Briefly, 25 μ l of nuclei (50 μ g of DNA), solubilized in the glycerol–Tris–MgCl₂ buffer (pH 7.9), was incubated in the reaction mixture (250 μ mol final volume), which contained: 10 μ mol of Tris–HCl buffer, pH 7.9, 0.4 μ mol of 2-mercaptoethanol, 0.16 μ mol each of GTP, CTP and ATP, 0.05 μ mol of ³H-labelled UTP (49 Ci/mol), 2 μ mol of KCl, 0.4 μ mol of MnCl₂ and 0.5 μ mol of MgCl₂. The reactions were started by the addition of nuclei, and incubations were for 10 min at 15°C. Assays in low-salt (0.01 M-KCl) conditions contained 0.2 μ g of α -amanitin, whereas those in high-salt [0.25 M-(NH₄)₂SO₄] conditions contained no α -amanitin. The activity was expressed in terms of pmol of UMP incorporated/10 min per mg of DNA.

Histones and acidic (non-histone) proteins were extracted from the chromatin as described by Spelsberg *et al.* (1971).

Protein, DNA and RNA were determined by the methods of Lowry *et al.* (1951), Burton (1956) and Munro & Fleck (1966) respectively. [³H]UTP was obtained from Amersham/Searle Co., Oakville, Ont., Canada; α -amanitin was purchased from Boehringer, Mannheim, Germany.

Results and discussion

Effect of α -amanitin on RNA polymerase I activity.
When RNA polymerase I is assayed at a low ionic

strength a portion of the total incorporation observed can be attributed to polymerase II activity. This activity can, however, be inhibited by the addition of α -amanitin (Jacob *et al.*, 1970). In our study, when RNA polymerase I was assayed in the presence of α -amanitin (0.2 μ g/250 μ l) a 38% inhibition of the total polymerase activity was found in both the tri-iodothyronine- and saline-treated animals examined up to 180 min after injection. On the other hand, in animals examined between 20 and 48 h after injection the inhibition was significantly greater in those injected with tri-iodothyronine than in the control group (63% versus 38%), a finding that was also noticed by Smuckler & Tata (1971) at 22 h after hormone administration. Since it was found that polymerase I activity remained constant after the first 180 min after tri-iodothyronine administration (results given below), and since the proportion of this activity that can be inhibited by α -amanitin remained constant over this period, it followed that if any change occurred in the activity of RNA polymerase II it would not appear as ' α -amanitin-sensitive polymerase I'.

Effect of ionic strength. RNA polymerase II activity was assayed at two different concentrations of $(\text{NH}_4)_2\text{SO}_4$ (0.05 and 0.25 M). The highest concentration of $(\text{NH}_4)_2\text{SO}_4$ was associated with an 84% increase in UMP incorporation, and there was no significant difference in the effect of ionic strength between paired groups of tri-iodothyronine and control animals over a period of 0–180 min and 20–48 h after injection, suggesting that tri-iodothyronine stimulated a homogeneous class of RNA

polymerase II. Thus, in the data shown in Fig. 1, RNA polymerase II and I activities were assayed in the presence and in the absence of 0.25 M- $(\text{NH}_4)_2\text{SO}_4$ respectively, the latter enzyme being studied in the presence of α -amanitin.

Effect of tri-iodothyronine on RNA polymerase I and II activities. Intravenous injection of tri-iodothyronine into thyroidectomized rats resulted in rapid enhancement of polymerase II activity of isolated liver nuclei assayed *in vitro* (Fig. 1). The activity of polymerase II reached a peak at 40 min, remained significantly increased at 60 and 80 min and returned to control values at 120 min. No significant stimulation of polymerase I activity occurred between 0 and 180 min. Additional studies at 20, 36 and 48 h indicated a secondary stimulation of both forms of polymerase; this late increase confirms the previous report by Tata & Widnell (1966). An effect due to the stress of intravenous administration was reflected by an increase in RNA polymerase II activity apparent at 80 min after saline injection.

The early and transient increase in the activity of RNA polymerase II shown in Fig. 1 follows immediately the maximum binding of tri-iodothyronine to rat liver nuclei, which occurs at 30 min (Oppenheimer *et al.*, 1974), and precedes the earliest significant increase in the synthesis of heterogeneous nuclear RNA (Tata & Widnell, 1966). However, the dependence of RNA polymerase II activation on the nuclear binding cannot be established at this stage. The significance of the transient activation of the form II enzyme followed by a secondary stimulation of both forms is not clear. There might be a qualitative

Fig. 1. Time-course of activities of RNA polymerases I and II after the administration of tri-iodothyronine

(a) Animals were dosed with 0.1 μ g of tri-iodothyronine/100 g body wt. or saline as described in the text under 'Materials and methods'. RNA polymerase assays were performed as described by Glasser *et al.* (1972) for form I polymerase of tri-iodothyronine-treated ($\circ$ — $\circ$) and control ($\circ$ --- $\circ$) animals and for form II polymerase of tri-iodothyronine-treated ($\bullet$ — $\bullet$) and control ($\bullet$ --- $\bullet$) animals. Each point represents the average of four separate experiments (three animals per point per experiment) $\pm$ S.E.M. $\star P < 0.05$ as performed by Student's *t* test on paired samples. (b) Ratios of form I ($\circ$ — $\circ$) and of form II ($\bullet$ — $\bullet$) polymerase activity from tri-iodothyronine-treated animals to those from non-treated controls. Each point is derived from the data of (a).

Table 1. *Effect of tri-iodothyronine on the protein/DNA ratio of chromatin proteins*

Tri-iodothyronine or saline was administered intravenously 40min before the rats were killed. Histones and non-histone proteins were prepared from chromatin as described by Spelsberg *et al.* (1971). The results are given as means $\pm$ s.e.m.; numbers in parentheses refer to the numbers of experiments. * $P < 0.05$ as performed by Student's *t* test with respect to the group of thyroidectomized rats not injected or injected with the saline solution.

Treatment	Histones/DNA	Non-histones/DNA
Thyroidectomized, no injection	1.048 $\pm$ 0.076 (6)	1.240 $\pm$ 0.094 (6)
Thyroidectomized, 40min saline	1.054 $\pm$ 0.094 (7)	1.243 $\pm$ 0.105 (7)
Thyroidectomized, 40min tri-iodothyronine	1.066 $\pm$ 0.102 (6)	1.749 $\pm$ 0.174 (6)*
Normal, 40min saline	1.037 $\pm$ 0.084 (4)	1.724 $\pm$ 0.173 (4)*

difference between the products of the two phases of activation of RNA polymerase II, the former being responsible for the synthesis of mRNA coding for a key induced protein, which would subsequently act, via a cascade phenomenon, on the transcription of rRNA and other species of mRNA(s). This model has been proposed by Baulieu *et al.* (1972) for the early events associated with oestradiol action in the uterus.

Failure to detect an early activation of RNA polymerase II by Tata & Widnell (1966) may be due to one of the following factors: (1) subcutaneous rather than intravenous injection of tri-iodothyronine used in our assay; (2) different dose of tri-iodothyronine; (3) the action of ribonuclease at 37°C, which becomes negligible when the assay is performed at 15°C (Chambon *et al.*, 1968); (4) the transient nature of the increase, which may not be apparent with long-interval time-course experiments.

Effect of tri-iodothyronine on chromatin proteins. The protein composition of chromatin was investigated 40min after tri-iodothyronine administration, since this time corresponds to the peak of early increase in RNA polymerase II activity. Table 1 shows that at this time the non-histone protein concentrations in tri-iodothyronine-treated thyroidectomized rats were significantly higher than those in thyroidectomized and saline-injected thyroidectomized rats. This finding, together with the observed increase of RNA synthesis *in vitro*, is consistent with the observation that non-histone proteins activate gene transcription (Dingman & Sporn, 1964; Kamiyama & Wang, 1971).

In conclusion, an early and transient (40–80min) increase of RNA polymerase II activity has been observed after tri-iodothyronine injection. This phenomenon precedes a second phase of activation of both forms of the enzyme. Such an early activation preceding the physiological effects of tri-iodothyronine has been observed in two other hormonal systems: oestradiol-17 β in rat uterus (Glasser *et al.*, 1972) and 1 α ,25-dihydroxycholecalciferol in chick intestine (Zerwekh *et al.*, 1974). The observed

simultaneous increase in the amount of nuclear acidic proteins associated with the DNA, known to activate gene transcription, may lead to the appearance of newly synthesized species of regulatory proteins, which could, in turn, activate cistrons more closely related to the physiological effects of tri-iodothyronine.

This work was supported by a grant from the Medical Research Council of Canada (M.R.C.). S. J. is a Fellow and H. S. a Scholar of the M.R.C.

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