Early Stimulation of Rat Liver Microsomal Protein Synthesis after Tri-iodothyronine Injection *in vivo*

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In an effort to determine the physiological significance of previous studies showing stimulation of microsomal protein synthesis by thyroxine added *in vitro*, an early effect of tri-iodothyronine injected *in vivo* was sought. Tri-iodothyronine (25 µg/100 g) administered to euthyroid rats stimulated microsomal protein synthesis *in vitro* within 3–6 h. This effect occurred much earlier than the 26 h lag previously reported after tri-iodothyronine administration to hypothyroid rats. This early effect of tri-iodothyronine on protein synthesis is prevented by α -amanitin, suggesting that it is dependent on RNA synthesis. The failure to find a direct effect *in vivo* of tri-iodothyronine on translation casts doubt on the physiological significance of previous studies that have shown a direct stimulation of translation by thyroxine added *in vitro*.

Tata *et al.* (1963) reported that tri-iodothyronine stimulation of liver microsomal protein synthesis did not begin until 26 h after injection of the drug into hypothyroid rats. Furthermore, these investigators found that tri-iodothyronine stimulated nuclear and microsomal RNA synthesis before any effect on microsomal protein synthesis (Tata & Widnell, 1966). On the other hand, previous studies from our laboratory have shown that thyroxine added *in vitro* directly stimulates the translational steps of peptide synthesis (Carter *et al.*, 1975), specifically the reactions of peptide-chain elongation (Carter *et al.*, 1976). We have attempted to determine whether tri-iodothyronine, administered in a more physiological manner by injection *in vivo*, may also stimulate translation independently of transcription.

These studies show that tri-iodothyronine injected into euthyroid rats stimulates liver microsomal protein synthesis *in vitro* within 3–6 h, which is much earlier than the 26 h delay previously reported. Nevertheless, this early effect of tri-iodothyronine on protein synthesis is abolished by α -amanitin, suggesting that it, too, is dependent on RNA synthesis.

Materials and Methods

The disodium salt of phosphocreatine, creatine kinase, AMP, L-tri-iodothyronine and α -amanitin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. L-[U- 14 C]Valine (specific radioactivity 238 µCi/µmol) was purchased from New England Nuclear Corp., Boston, MA, U.S.A. Sprague–Dawley rats, weighing 125–200 g, were obtained from Charles

River Laboratories, Wilmington, MA, U.S.A., and fed on Purina chow *ad lib*. Animals were starved for 18 h before death.

At the designated time before death, experimental animals were given the tri-iodothyronine dosage intraperitoneally dissolved in 0.1–0.2 ml of 5 mM-NaOH in 0.9% NaCl. Control animals were given an equivalent volume of diluent. Animals were killed by decapitation, livers rapidly removed and homogenized in 4 ml of ice-cold 0.25 M-sucrose/g in a motor-driven Teflon/glass homogenizer. The homogenate was centrifuged at 12000g for 20 min. The 12000g post-mitochondrial supernatant was then centrifuged at 100000g for 60 min. The resulting microsomal fraction was resuspended in a volume of 0.25 M-sucrose equal to half the original homogenate volume. The active tissue fraction was reconstituted by adding 1 part of microsomal fraction, 1 part of 100000g supernatant and 1 part of 0.25 M-sucrose. Reaction mixtures and conditions are described in the Tables. Samples (0.1 ml) of reaction mixtures obtained before and after incubation were placed on 23 mm-diameter Whatman filter-paper discs, dried in hot air for 20 s, and placed in ice-cold 10% (w/v) trichloroacetic acid. The discs were washed in hot 5% trichloroacetic acid, ethanol/ether (1:1, v/v) and then ether. Hot-acid-insoluble radioactivity remaining on the discs was counted in a Packard scintillation counter at 65% efficiency. For counting, each paper disc was placed in a vial containing 5 ml of scintillation mixture consisting of 4 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre of toluene. RNA was determined by the method of Munro & Fleck (1966).

Table 1. *Effect of tri-iodothyronine (25 µg/100 g) injected in vivo on microsomal protein synthesis in vitro*

Reaction mixtures contained the following components (mm): sucrose, 100; K₂HPO₄ buffer, pH 7.0, 20; AMP, 3.0; MgCl₂, 6.0; and phosphocreatine, 18. The total volume of reaction mixtures was 0.5 ml. Each reaction mixture also contained 0.75 mg of 100000g-supernatant protein, approx. 100 µg of microsomal RNA, and 50 µg of creatine kinase (Sigma). The amount and specific radioactivity of the L-[U-¹⁴C]valine precursor added to reaction mixtures is indicated in the Table. Reaction mixtures were incubated in air at 37°C for 30 min. Samples (0.1 ml) of reaction mixtures were taken before and after incubation for measurement of hot-trichloroacetic acid-insoluble radioactivity. Values presented in the Table (c.p.m. of [¹⁴C]valine incorporated/mg of microsomal RNA) are means of the numbers of experiments indicated. Each experiment in turn represents the mean of two control and two tri-iodothyronine-treated animals studied simultaneously. ΔT_3 values are the means $\pm$ s.e.m. of the percentage tri-iodothyronine effect calculated for each paired experiment. *P* values were calculated by using the paired Student's *t* test: **P* < 0.05; ***P* < 0.02; ****P* < 0.01.

Tri-iodothyronine injection	(a) [¹⁴ C]Valine (2.1 nmol/ml; 238 µCi/µmol)				(b) [¹⁴ C]Valine (100 nmol/ml; 9.1 µCi/µmol)			
	No. of expts.	Control	+ Tri-iodothyronine	ΔT_3 (%)	No. of expts.	Control	+ Tri-iodothyronine	ΔT_3 (%)
3 h before death	10	32 500	38 200	+19.7 $\pm$ 8.5*	—	—	—	—
6 h before death	6	46 500	56 200	+20.8 $\pm$ 2.5***	6	6400	7210	+13.0 $\pm$ 2.4***
Daily for 3 days before death	5	33 300	43 600	+31.2 $\pm$ 7.6**	5	4950	6640	+34.8 $\pm$ 8.4**

Table 2. *Effect of tri-iodothyronine (100 µg/100 g) injected in vivo on microsomal protein synthesis in vitro*

Reaction mixtures, incubation conditions and data presentation are as described in Table 1. Where indicated, α -amanitin (Sigma) plus tri-iodothyronine or tri-iodothyronine diluent were administered by separate intraperitoneal injection 6 h before death of the animals. **P* < 0.05; ****P* < 0.01, by the paired *t* test.

Tri-iodothyronine injection	(a) [¹⁴ C]Valine (2.1 nmol/ml; 238 µCi/µmol)				(b) [¹⁴ C]Valine (100 nmol/ml; 9.1 µCi/µmol)			
	No. of expts.	Control	Tri-iodothyronine	ΔT_3 (%)	No. of expts.	Control	Tri-iodothyronine	ΔT_3 (%)
6 h before death	13	33 200	40 400	+26.1 $\pm$ 6.5***	13	4980	5720	+17.4 $\pm$ 4.4***
12 h before death	5	41 700	57 800	+42.7 $\pm$ 13.1*	5	6770	8980	+34.3 $\pm$ 10.3*
Plus α -amanitin (50 µg/100 g) injected 6 h before death	7	31 300	29 900	-1.7 $\pm$ 10	7	4740	4590	-2.9 $\pm$ 5.5

Results

In searching for a direct effect of tri-iodothyronine on translation, the earliest time interval at which the drug injected *in vivo* stimulates protein synthesis *in vitro* was sought. Table 1 shows the effect of 25 μ g of tri-iodothyronine/100g administered at various time intervals before death. Stimulation by tri-iodothyronine occurred whether reaction mixtures contained small amounts (2.1 nmol/ml) of high-specific-radioactivity [14 C]valine precursor (238 μ Ci/ μ mol) (Table 1a) or much larger amounts (100 nmol/ml) of low-specific-radioactivity [14 C]valine (9.1 μ Ci/ μ mol) (Table 1b). The high-specific-radioactivity precursor was initially used for convenience in obtaining greater radioactivity incorporation. The low-specific-radioactivity precursor was included to ensure that a saturating amount of [14 C]valine was added and that variation in endogenous free valine content of reaction mixtures would not influence valine specific radioactivity. Tri-iodothyronine injected 3h before death caused a 19.7% increase in liver microsomal protein synthesis in reactions containing the high-specific-radioactivity precursor. Tri-iodothyronine administered 6h before death caused a 20.8% stimulation in reactions containing the high-specific-radioactivity precursor and a 13% stimulation in reactions containing the low-specific-radioactivity precursor. The stimulation observed at 3-6h after tri-iodothyronine injection represents an appreciable fraction of the maximum 31.2 and 34.8% effects of tri-iodothyronine observed after three daily injections of the drug.

Table 2 illustrates the effect of 100 μ g of tri-iodothyronine/100g on microsomal protein synthesis in reaction mixtures containing high- and low-specific-radioactivity valine precursors. This dose of the drug injected 6h before death caused a 26.1% stimulation in reactions containing the high-specific-radioactivity precursor and a 17.4% stimulation in reactions with the low-specific-radioactivity precursor. This stimulation is slightly greater than that observed 6h after injection of 25 μ g of tri-iodothyronine/100g. When administered 12h before death, 100 μ g of tri-iodothyronine/100g caused a 42.7 and 34.3% stimulation of liver microsomal protein synthesis, as shown in Table 2. The lower portion of Table 2 shows the effect of 100 μ g of tri-iodothyronine/100g plus 50 μ g of α -amanitin/100g injected 6h before death. Control animals received the same dose of α -amanitin. As shown, α -amanitin completely prevented stimulation by tri-iodothyronine of liver microsomal protein synthesis in reactions containing both high- and low-specific-radioactivity valine precursors. α -Amanitin is an effective inhibitor of mammalian mRNA synthesis, and has been shown to prevent tri-iodothyronine induction of hepatic α -glycerophosphate dehydrogenase and 'malic' enzyme when administered in the present dose (Dillman *et al.*, 1976). The

α -amanitin inhibition strongly suggests that this early tri-iodothyronine effect on microsomal protein synthesis is nevertheless dependent on prior stimulation of RNA synthesis.

Discussion

Tata *et al.* (1963) observed an initial 13% increase in liver microsomal protein synthesis 26h after administration of 17-21 μ g of tri-iodothyronine/100g to hypothyroid rats. In the present study, a comparable or larger initial increase in protein synthesis was seen 3-6h after administration of 25 μ g of tri-iodothyronine/100g to euthyroid rats. The earlier effect observed in the present study may be due to the use of euthyroid rather than hypothyroid rats and to intraperitoneal injection of tri-iodothyronine rather than subcutaneous injection.

Using euthyroid rats, Sokoloff *et al.* (1968) also observed an early 18% stimulation of microsomal protein synthesis *in vitro* 2h after administration of 38-60 μ g of tri-iodothyronine/100g *in vivo*. However, these investigators found the early tri-iodothyronine effect to be dependent on the presence of mitochondria in the reaction. They concluded that tri-iodothyronine stimulation of microsomal protein synthesis resulted from an initial interaction of mitochondria and thyroid hormone. In contrast, the present studies show an early effect of tri-iodothyronine in reactions that did not contain mitochondria. Therefore tri-iodothyronine appears to have an early effect on microsomal protein synthesis that is not dependent on mitochondria.

This study was carried out to determine whether thyroid hormone administered *in vivo* would stimulate translation independent of transcription. Such an effect was suggested by previous studies *in vitro* from this laboratory that have shown a direct stimulation of translation by thyroxine (Carter *et al.*, 1975, 1976). Although the present study shows an earlier effect of tri-iodothyronine *in vivo* on liver microsomal protein synthesis than had been previously reported, this effect nevertheless appears to be dependent on RNA synthesis. Since a direct effect of tri-iodothyronine *in vivo* on translation has not been found, the physiological significance of the previously demonstrated effects of thyroxine *in vitro* is uncertain.

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