HIGH SENSITIVITY OF A RAT LIVER NUCLEOPLASMIC PROTEIN TO TRIIODOTHYRONINE

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Received 23 February 1982

1. Introduction

Most of the actions of T3 appear to be exerted at the nuclear level [1]. The hormone binds to a chromatin receptor and increases RNA and protein synthesis. However, little is known of how T3 modifies nuclear functions. Most known effects of T3 on the nucleus are non-specific and reflect pleiotypic actions of the hormone. For example, T3 increases the activity of RNA polymerases, the number of polymerase initiation sites, the synthesis of total and poly(A)-containing RNA and the turnover of nuclear proteins (reviews [1–3]).

The general increment in RNA synthesis brought about by thyroid hormone may be due to the control of nuclear proteins involved in the regulation of nuclear function. In [4], 102 different protein subunits of rat liver nuclei were shown to be dependent on thyroid hormones. This is consistent with our findings of changes in the rate of nuclear protein turnover or in the electrophoretic patterns of nuclear proteins after thyroidectomy and thyroid hormone treatment [5,6]. We found that thyroid hormones exert an opposite control on two nuclear proteins [7]; one, of 120 000 M_r, is increased by thyroid hormones; the other, of 81 000 M_r is decreased. These polypeptides were found in the nucleoplasmic fraction. However, their physiological roles are unknown. Here, we show that the 81 000 M_r polypeptide is very sensitive to small amounts of T3 administered to thyroidectomized rats.

2. Materials and methods

Wistar rats were thyroidectomized surgically at ~100 g body wt. One week after thyroidectomy 100 μCi ^{131}I was administered to each rat to ensure complete removal of thyroid remnants. One month after thyroidectomy a blood sample was withdrawn from the retrobulbar venous plexus with a micropipette to determine plasma T4 and T3. Only animals with undetectable thyroid hormone levels (T4 $\leqslant$ 0.33 μg/100 ml, T3 $\leqslant$ 15 ng/100 ml) were subsequently used. Four months after thyroidectomy, 3 groups of animals were prepared at random. Two of the groups received T3 in the drinking water. The calculated doses per rat were 0.1 and 0.5 μg T3/day, respectively. Tracer amounts of [^{125}I]T3 were also added to the drinking water (450 000 cpm/ml). T3 was administered for 3 days and the animals were sacrificed in the morning of the fourth day.

The animals were sacrificed by cervical dislocation. Nuclei were obtained by ultracentrifugation of the crude nuclear fraction through 2.2 M sucrose [5–7]. All buffers contained 1 mM phenylmethanesulphonylfluoride. The nuclear extracts were obtained by resuspending the nuclei (0.5 ml/g tissue) in 0.15 M NaCl/1 mM $MgCl_2$/10 mM Tris–HCl (pH 8.0). The suspension was incubated for 15 min at 0°C, and centrifuged at 6000 rev./min in a Sorvall HS-4 rotor. Polyacrylamide gel electrophoresis of the nuclear extracts was performed in 9 × 0.5 cm gels according to [8]. Staining was done overnight in 0.1% Coomassie blue R-250. Plasma T4, T3 pituitary GH were measured by specific radioimmunoassays [9,10]. Nuclear T3 was calculated from the counting rates of aliquots of the nuclear suspensions and the specific activity of the administered T3 + [^{125}I]T3 mixture. Liver malic enzyme and α-glycerophosphate dehydrogenase activities were measured in aliquots of the cytosol and mitochondrial fractions as in [11] and [12], respectively. Protein was measured as in [13].

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

Table 1
Effect of low doses of T_3 on body weight and thyroid hormone concentration

	No.	Body wt (g)	Serum		Nucleus
			T_3 ng/100 ml	T_4 μg/100 ml	T_3 fmol/100 μg DNA
T	8	123 ± 10	⩽15	⩽0.33	–
+ 0.1 μg T_3	6	126 ± 11	21.3 ± 6.3	⩽0.33	2.6 ± 0.4
+ 0.5 μg T_3	6	126 ± 9	26.6 ± 9	⩽0.33	10.9 ± 1.3

The amount of the 81 000 M_r polypeptide in the stained gels was measured after scanning the gels in a 659 ISCO gel scanner with UA-5 absorbance monitor. To increase the sensitivity, the 81 000 M_r zone was scanned at a full scale of 0.2 and chart speed 300 cm/h. The same amount of protein was loaded on each gel, and care was taken that the time of staining and destaining was the same in all samples. The recorder response was proportional to the amount of protein present in the gels. This was shown by measuring the amount of 120 000 and 81 000 M_r in samples that contained mixtures of normal and thyroidectomized rat extracts in different proportions. The amounts of 120 000 and 81 000 M_r polypeptides obtained were proportional to the amount of normal or thyroidectomized rat extract present in the sample.

3. Results

The effects of low doses of T3 are shown in tables 1 and 2. Table 1 shows data on body weight and hormone concentrations in the animals used. T3 was undetectable in the plasma of thyroidectomized animals. Administration of 0.1 and 0.5 μg T3 daily for 3 days led to small increases to 21.3 and 26.6 ng/100 ml. Plasma T4 was undetectable in all animals. Nuclear T3 concentrations were 2.6 and 10.9 fmol/100 μg DNA. These values would represent nuclear receptor occupancies of 4% and 15%, respectively [14].

The responses of several end effects of thyroid hormone action is shown in table 2. As indexes of T3 action we measured pituitary GH content, liver malic enzyme and α-glycerophosphate dehydrogenase activities and the 120 000 and 81 000 M_r nuclear polypeptides. The 120 000 M_r polypeptide was not included since it was almost undetectable in all groups. Treatment with 0.5 μg T3 caused a small increase, but the electrophoretic band was still too faint to be quantitated. The 81 000 M_r polypeptide was present as a prominent band in thyroidectomized animals. Treatment with 0.1 μg T3 led to a 55% decrease, and 0.5 μg to a further small decrease. The smallest dose of T3 did not have any effect on α-glycerophosphate dehydrogenase or malic enzyme activities. It caused a small effect on pituitary GH. T3 at (0.5 μg) caused a further increase of pituitary GH and a clear effect on liver enzymes.

4. Discussion

Here, we provide evidence for a differential sensitivity to thyroid hormones of 2 nuclear polypeptides, shown to be under thyroid hormone control

Table 2
Sensitivity to T_3 of several end points of thyroid hormone action (mean ± SD)

	No.	GH (μg/gland)	ME (×10^5) (U/ml protein)	α-GDP(×10^4) (U/mg protein)	Nuclear protein (81 000 M_r)
T	8	0.11 ± 0.03	42 ± 10	27 ± 10	103 ± 13
+ 0.1 μg T_3	6	1.5 ± 0.6	57 ± 27	32 ± 15	47 ± 5
+ 0.5 μg T_3	6	35.5 ± 7	135 ± 29	70 ± 7	36 ± 7

[5–7]. In particular, the 81 000 M_r polypeptide, which is negatively regulated by thyroid hormones, appears to be extremely sensitive to T3. Very low doses of this hormone, having no effect on the 120 000 M_r polypeptide or liver enzymes caused a clear decrease of the 81 000 M_r polypeptide. GH responded to 0.1 μg T3, but the magnitude of the response was only 7% of that attained with the 0.5 μg dose. In contrast, the response of 81 000 M_r polypeptide to the lowest dose of T3 was 83% of that with the higher dose. The high sensitivity of 81 000 M_r polypeptide to thyroid hormones explains that in thyroidectomized animals with moderate degree of hypothyroidism (i.e., low but detectable levels of T3) we find a decrease of the 120 000 M_r but no changes of 81 000 M_r polypeptide (unpublished). Here, the animals were selected on the basis of their plasma T3 and only those with undetectable levels of the hormone were used.

The reason for the high sensitivity of the 81 000 M_r polypeptide to T3, as compared to other responses, is unknown. The thyroid hormone control of protein concentrations in the cell is currently explained by effects at the level of mRNA formation [1–3]. Both positive and negative control of hepatic gene expression by thyroid hormone has been shown [15]. It is possible that the response of 81 000 M_r to T3 represents a primary effect of the hormone, in contrast with the response of other proteins and enzymes which have been found to be modulated by both thyroid hormones and other factors [16,17].

References

[1] Oppenheimer, J. H. (1979) Science 203, 971–979.
[2] Bernal, J. and DeGroot, L. J. (1980) in: The Thyroid Gland (DeVischer, M. ed) pp. 123–143, Raven, New York.
[3] Eberhard, N. L., Apriletti, J. W. and Baxter, J. D. (1980) in: Biochemical Actions of Hormones (Litwack, G. ed) vol. 7, pp. 311–394, Academic Press, New York.
[4] Nikodem, U. M., Trus, B. L. and Rall, J. E. (1981) Proc. Natl. Acad. Sci. USA 78, 4411–4415.
[5] Bernal, J., Coleoni, A. H. and DeGroot, L. J. (1978) Endocrinology 102, 452–459.
[6] Barsano, C. P., Coleoni, A. H. and DeGroot, L. J. (1980) Endocrinology 106, 1475–1488.
[7] Rodriguez-Pena, A. and Bernal, J. (1982) Acta Endocrinol. in press.
[8] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[9] Obregon, M. J., Pascual, A., Morreale de Escobar, G. and Escobar del Rey, F. (1979) Endocrinology 104, 1467–1473.
[10] Hervas, F. and Morreale de Escobar, G. (1974) Horm. Metabol. Res. 6, 300–303.
[11] Hsu, R. Y. and Lardy, H. A. (1969) Methods Enzymol. 13, 230–235.
[12] Lee, Y.-P. and Lardy, H. A. (1965) J. Biol. Chem. 240, 1427–1436.
[13] Lowry, D. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. S. (1951) J. Biol. Chem. 193, 269–275.
[14] Bernal, J., Coleoni, A. H. and DeGroot, L. J. (1978) Endocrinology 103, 403–413.
[15] Seelig, S., Liaw, C., Towle, H. C. and Oppenheimer, J. (1981) Proc. Natl. Acad. Sci. USA 78, 4733–4737.
[16] Mariash, C. N., McSwigan, C. R., Towle, H. C., Schwartz, H. L. and Oppenheimer, J. H. (1981) J. Clin. Invest. 68, 1485–1490.
[17] Wilson, E. J. and McMurray, W. C. (1981) J. Biol. Chem. 11657–11662.