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Thyroid hormone regulates protein expression in C6 glioma cells

A.G. Trentin[1] and M. Alvarez-Silva[2]

Departamentos de [1]Biologia Celular, Embriologia e Genética, and [2]Microbiologia e Parasitologia, Divisão de Imunologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil

Abstract

Thyroid hormone (T_3) is essential to normal brain development. Previously, we have shown that T_3 induces cerebellar astrocyte proliferation. This effect is accompanied by alteration in glial fibrillary acidic protein (GFAP) and fibronectin organization. In the present study, we report that the C6 glioma cell line, which expresses GFAP and is classified as an undifferentiated astrocytic cell type, is a target for T_3 action. The C6 monolayers were treated with 50 nM T_3 for 3 days, after which the cells were maintained for 2 days without medium changes. In C6 cells, T_3 induced the expression of proteins of 107, 73 and 62 kDa. The hormone also up-regulated protein bands of 100 (+50%), 37 (+50%) and 25.5 kDa (+50%) and down-regulated proteins of 94 (-100%), 86.5 (-100%), 68 (-100%), 60 (-100%), 54 (-33%), 51 (-33%) and 43.5 kDa (-33%). We suggest, on the basis of molecular mass, that the 54-, 51- and 43.5-kDa proteins could be the cytoskeletal proteins vimentin, GFAP and actin, respectively. The down-regulation of these proteins may be involved in the effects of thyroid hormone on C6 differentiation.

Correspondence
A.G. Trentin
Departamento de Biologia Celular, Embriologia e Genética
Centro de Ciências Biológicas, UFSC
88040-900 Florianópolis, SC
Brasil
Fax: +55-48-331-9672
E-mail: atrentin@ccb.ufsc.br

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- Vimentin
- Differentiation

The importance of thyroid hormone (T_3) for normal development of the central nervous system is well documented. T_3 deficiency during the early stages of brain development produces severe mental retardation in both animals and humans (1). It is known that during rat brain development, astrocytes are target cells for thyroid hormones (2,3). Thus, neonatal hypothyroidism results in an increased number of astrocytes in rat cerebellum and hyperthyroidism produces the opposite effect (2). Thyroid hormone regulates the transition between radial glia and mature astrocytes in the basal forebrain and hippocampus (3). *In vitro*, thyroid hormone induces the phosphorylation of specific proteins in astrocytes (4). The presence of thyroid hormone receptor isoforms has been demonstrated in cultured type 1 and type 2 astrocytes (5). We have previously demonstrated that T_3 induces the synthesis and release of growth factors by astrocytes. The growth factors secreted by cerebral hemisphere astrocytes promote morphological differentiation in cells derived from cerebral hemispheres of normal and hypothyroid newborn rats. In contrast, the growth factors secreted by cerebellar astrocytes induce proliferation in astrocytes from normal neonatal cerebellum, accompanied by reorganization of glial fibrillary acidic protein (GFAP) filaments without changes in total GFAP amount (6-8).

The rat glioma cell line C6, which was

cloned from a chemically induced rat brain tumor, was classified as an undifferentiated astrocytic cell type (9). Ortiz-Caro and colleagues (10) demonstrated the presence of nuclear T_3 binding capacity in C6 glioma cells and it has been reported that human glioma cell lines transport thyroid hormone through the plasma membrane (11). The C6 glioma cell line has been used as an *in vitro* model for the study of glial cell properties. Thus, C6 cells provide means of studying the effects of thyroid hormone on tumors of glial origin.

Alterations in the response of specific brain proteins to thyroid hormone are well documented (12). However, the precise mechanisms by which T_3 regulates the expression of target genes involved in brain development remain uncertain. The alteration in cellular proteins could be important for the effects of thyroid hormone on cellular differentiation. The objective of the present study was to determine the regulation of cell protein expression in rat C6 glioma cells by thyroid hormone.

The C6 cell line was grown in the presence of Dulbecco's modified Eagle's medium (DMEM, Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal calf serum (FCS). The cultures were incubated at 37°C in a humidified 5% CO_2 and 95% air atmosphere. After confluence, the cells were treated with 50 nM 3-3'-5 triiodo-L-thyronine (T_3, Sigma) for 3 days, with the T_3 medium changed after days 1 and 2. The T_3 was dissolved in FCS-free DMEM. Control cultures were maintained in DMEM without FCS during treatment. These cultures were then maintained for 2 days without medium changes. The cells were harvested, treated with trypsin, and lysed by successive freezing and thawing. The total proteins extracted from the cells were analyzed by SDS-PAGE 10% acrylamide gels according to Laemmli (13). Fifty micrograms of protein per sample was loaded in each lane and stained with Coomassie blue.

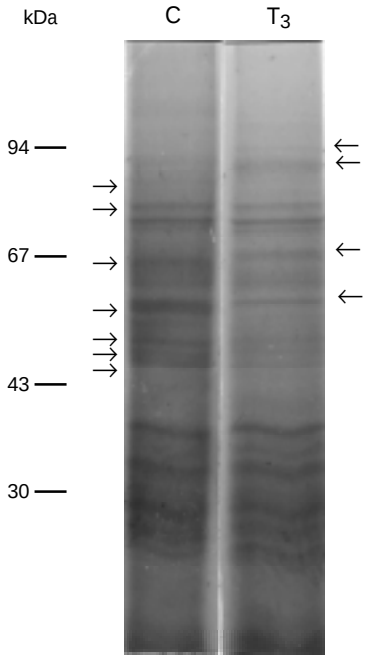

Figure 1 - Effects of T_3 on protein expression in C6 cells. Confluent cells were maintained in culture medium without serum (control cells) or treated with 50 nM T_3 for 3 days without serum. The monolayers were then harvested with trypsin and total protein extracted from the cells was analyzed by SDS-PAGE as described in the text. Arrows indicate protein bands of different intensities in control and T_3-treated extracts.

The effect of thyroid hormone on protein expression in C6 cells was demonstrated experimentally (Figure 1 and Table 1). T_3 induced the expression of protein bands with molecular weights of 107, 73 and 62 kDa, that were absent in untreated C6 cultures. The expression of proteins of 73 and 62 kDa was more evident on the basis of the amount of stained protein. We also observed a slight increase in protein bands with molecular weights of 37 and 25.5 kDa and a strong enhancement in a protein of 100 kDa after the hormone treatment (Figure 1 and Table 1).

However, after T_3 treatment the intensity of protein bands of 94, 86.5, 68, 60, 54, 51 and 43.5 kDa was reduced. The most significant effect was observed with the 68- and 60-kDa proteins (Figure 1 and Table 1). The 51- and 54-kDa proteins may be intermediate filament proteins GFAP and vimentin based on their relative molecular masses (14,15). The 43.5-kDa protein could be the cytoskeletal protein, actin (16).

The rat glioma cell line C6 was shown to express the intermediate filament proteins GFAP and vimentin (15). The expression of GFAP was the principal criterion used to

classify this cell line as an undifferentiated astrocytic cell type. However, the expression of GFAP depends on specific culture conditions, indicating that this cell line represents a heterogeneous population (15,17). In addition, vimentin expression was reported to be present in the majority of C6 cells (15). In our experiments, the 51- and 54-kDa proteins, which were increased in control C6 cultures, may correspond to GFAP and vimentin subunits, respectively.

Thyroid deficiency in early life reduces both the density and process length of the GFAP-labeled astrocytes in the rat parietal cortex and hippocampal formation (18). On the other hand, neonatal thyroid hormone treatment results in a premature disappearance of vimentin-immunoreactive radial glia and a premature appearance of GFAP-immunoreactive astrocytes in the basal forebrain and hippocampus (3). Previously, we confirmed reports that astrocytes cultured from neonatal rat cerebellum proliferate after thyroid hormone treatment. This effect was accompanied by reorganization of GFAP filaments that were spread in the cytoplasm of astrocytes and became organized around the cell nucleus after T_3 treatment (7). In those experiments, we observed that total GFAP expression was not changed (8). Thus, thyroid hormone seems to control many aspects of astrocyte differentiation. We suggest that T_3 may regulate C6 glioma metabolism similarly to cerebellar astrocytes. T_3 may induce C6 cells to proliferate and the regulation in vimentin and GFAP expression could be involved in this event. This hypothesis is currently under investigation in our laboratory.

Recent reports have shown that thyroid hormones modulate the biogenesis and assembly of actin (16,19,20). Faivre-Sarrailh and colleagues (19) showed an increase in actin mRNA content in the hypothyroid brain relative to that of normal brain. Paul and colleagues (20) demonstrated an increase in total actin in neuronal cultures from hypothyroid brain as compared to normal brain. These findings may possibly account for the enhancement of total actin in C6 cultures before thyroid hormone treatment observed here.

Table 1 - Molecular masses of protein bands of different intensities in control and T_3-treated extracts of C6 cells.

The molecular mass of the protein bands was calculated by comparison with standard proteins and the intensities are indicated by +.

Mass (kDa)	Control	T_3-treated
107	-	+
100	++	++++
94	+	-
86.5	+	-
73	-	+++
68	+++	-
62	-	+++
60	+++++	-
54	+++	+
51	+++	+
43.5	+++	+
37	+	++
25.5	+	++

Taken together, these data indicate that the C6 glioma cell line was responsive to thyroid hormone. In our experimental system, T_3 controlled the expression of C6 proteins. However, the hormone regulated these proteins differently. We observed that T_3-treated C6 cells induced the expression of 107-, 73- and 62-kDa proteins. T_3-treated C6 cells up-regulated the proteins of 100, 37 and 25.5 kDa and down-regulated the proteins of 94, 86.5, 68, 60, 54, 51 and 43.5 kDa. We suggest that the 54-, 51- and 43.5-kDa proteins could be vimentin, GFAP and actin, respectively. The down-regulation of these proteins may be involved in the effects of thyroid hormone on C6 differentiation.

References

1. Porterfield SP & Hendrich CE (1993). The role of thyroid hormones in prenatal and neonatal neurological development - current perspectives. *Endocrinology Reviews*, 14: 94-106.
2. Clos J & Legrand J (1973). Effects of thyroid deficiency on the different cell populations of the cerebellum in the young rat. *Brain Research*, 63: 450-455.
3. Gould EG, Frankfurt M, Westlind-Danielsson A & McEwen BS (1990). Developing forebrain astrocytes are sensitive to thyroid hormone. *Glia*, 3: 283-292.
4. Ruel J, Gavaret JM, Luo M & Dussault JH (1986). Regulation of protein phosphorylation by triiodothyronine (T_3) in neural cell cultures. Part I: astrocytes. *Molecular and Cellular Endocrinology*, 45: 223-232.
5. Carlson DJ, Strait KA, Schwartz HL & Oppenheimer JH (1996). Thyroid hormone receptor isoform content in cultured type 1 and type 2 astrocytes. *Endocrinology*, 137: 911-917.
6. Trentin AG, Rosenthal D & Moura Neto V (1995). Thyroid hormone and conditioned medium effects on astroglial cells from hypothyroid and normal rat brain: factor secretion, cell differentiation and proliferation. *Journal of Neuroscience Research*, 41: 409-417.
7. Trentin AG & Moura Neto V (1995). T_3 affects cerebellar astrocyte proliferation, GFAP and fibronectin organization. *NeuroReport*, 6: 293-296.
8. Lima FRS, Trentin AG, Rosenthal D, Chagas C & Moura Neto V (1997). Thyroid hormone induces protein secretion and morphological changes on astroglial cells with increase in GFAP expression. *Journal of Neuroendocrinology*, 154: 167-175.
9. Benda P, Lightbody J, Sato G, Levine L & Sweet W (1969). Differentiated rat glial cell strain in tissue culture. *Science*, 161: 370-371.
10. Ortiz-Caro X, Yusta B, Montiel F, Villa A, Aranda A & Pascual A (1986). Identification and characterization of triiodothyronine receptors in cells of glial and neuronal origin. *Endocrinology*, 119: 2163-2167.
11. Gonçalves E, Lakshmanan M, Pontecorvi A & Robbins J (1990). Thyroid hormone transport in a human glioma cell line. *Molecular and Cellular Endocrinology*, 69: 157-165.
12. Bernal J & Nunez J (1995). Thyroid hormones and brain development. *European Journal of Endocrinology*, 133: 390-398.
13. Laemmli U (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.
14. Eng LF (1985). Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. *Journal of Neuroimmunology*, 8: 203-214.
15. Rosser K, Bohn W, Giese G & Mannweiler K (1991). Subclones of C6 rat glioma cells differing in intermediate filament protein expression. *Experimental Cell Research*, 197: 200-206.
16. Siegrist-Kaiser CA, Juge-Aubry C, Tranter MP, Ekenbarger DM & Leonard JL (1990). Thyroxine-dependent modulation of actin polymerization in cultured astrocytes. *Journal of Biological Chemistry*, 265: 5296-5302.
17. Coyle DE (1995). Adaptation of C6 glioma cells to serum-free conditions leads to the expression of a mixed astrocyte-oligodendrocyte phenotype and increased production of neurite-promoting activity. *Journal of Neuroscience Research*, 41: 374-385.
18. Rami A & Rabie A (1988). Effect of thyroid deficiency on the development of glia in the hippocampal formation of the rat: an immunocytochemical study. *Glia*, 1: 337-345.
19. Faivre-Sarrailh C, Ferraz C, Liautard JP & Rabie A (1990). Effect of thyroid deficiency on actin mRNA content in the developing rat cerebellum. *International Journal of Developmental Neuroscience*, 8: 99-106.
20. Paul S, Das S & Sarkar PK (1992). Effect of hypothyroidism on different forms of actin in rat cerebral neuronal cultures studied by an improved DNase I inhibition assay. *Journal of Neurochemistry*, 59: 701-707.