Published in final edited form as:

Exp Clin Endocrinol Diabetes. 2011 February ; 119(2): 81–85. doi:10.1055/s-0030-1262860.

Stanniocalcin 1 Induction by Thyroid Hormone Depends on Thyroid Hormone Receptor β and Phosphatidylinositol 3-kinase Activation

L. C. Moeller¹, N. E. Haselhorst¹, A. M. Dumitrescu², X. Cao⁴, H. Seo⁴, S. Refetoff^{2,3}, K. Mann¹, and O. E. Janssen^{1,5}

¹Department of Endocrinology and Division of Laboratory Research, University of Duisburg-Essen, Essen, Germany

²Department of Medicine, The University of Chicago, Chicago, Illinois, USA

³Department of Pediatrics and Committees on Genetics and Molecular Medicine, The University of Chicago, Chicago, Illinois, USA

⁴Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

Abstract

Context—Thyroid hormone (TH) mediated changes in gene expression were thought to be primarily initiated by the nuclear TH receptor (TR) binding to a thyroid hormone response element in the promoter of target genes. A recently described extranuclear mechanism of TH action consists of the association of TH-liganded TR β with phosphatidylinositol 3-kinase (PI3K) in the cytosol and subsequent activation of the PI3K pathway.

Objective—The aim of this study was to examine the effect of TH, TR β and PI3K on stanniocalcin 1 (STC1) expression in human cells.

Design—We treated human skin fibroblasts with triiodothyronine (T₃) in the absence or presence of the PI3K inhibitor LY294002, a dominant negative PI3K subunit, p85 α , and the protein synthesis inhibitor cycloheximide (CHX). The role of the TR β was studied in cells from patients with resistance to thyroid hormone (RTH). STC-1 mRNA expression was measured by real-time PCR.

Results—We found an induction of STC1 by T₃ in normal cells, but less in cells from subjects with RTH (2.7 ± 0.2 vs. 1.6 ± 0.04 , $P < 0.01$). The effect of T₃ was completely abrogated by blocking PI3K with LY294002 (3.9 ± 0.5 vs. 0.85 ± 0.5 ; $P < 0.05$) and greatly reduced after transfection of a dominant negative PI3K subunit, demonstrating dependency on the PI3K pathway.

Conclusion—These results establish STC1 as a TH target gene in humans. Furthermore, we show that STC1 induction by TH depends on both TR β and PI3K activation.

Correspondence. L. C. Moeller, Department of Endocrinology, University of Duisburg-Essen, Hufelandstraße 55, 45122 Essen, Germany, Tel.: + 49/201/723 2821, Fax: + 49/201/723 5187, lars.moeller@uni-due.de.

⁵Present Address: Endokrinologikum, Hamburg 22767, Germany

Conflict of Interest: None.

Keywords

nongenomic action; hypoxia inducible factor 1; HIF-1

Introduction

Thyroid hormones (TH) are essential for normal development, growth and metabolism (Yen, 2001). Their action is principally mediated through triiodothyronine (T_3), acting as a ligand for the TH receptors (TRs) α and β (Cheng et al., 2010, Yen, 2001). In the classical mode of TH action, the TRs are bound to TH response elements (TREs) in the promoter of target genes as homodimers or heterodimers with the retinoid X receptor (RXR). In the case of positively regulated genes, T_3 binding then leads preferentially to a heterodimer formation with RXR, exchange of corepressors for coactivators, recruitment of basal transcription factors such as the RNA polymerase II and, subsequently, increased transcription of the TH target gene.

In addition to this classical mode of TH action, TH effects mediated by TRs but independent of TREs have recently been recognized (Moeller et al., 2006). Cao and others described a new mechanism of TH action in which the liganded TR β interacts with the regulatory subunit of phosphatidylinositol 3-kinase (PI3K), p85 α (Cao et al., 2005, Lei et al., 2004). This leads to activation of the PI3K signal transduction pathway with subsequent phosphorylation and activation of protein kinase B (PKB) and mammalian target of rapamycin (mTOR). It could be demonstrated that this initially non-genomic action of TH ultimately has genomic consequences. PI3K activation leads to induction of ZAKI4 α , known to be strongly induced by TH (Cao et al., 2005). Furthermore, this mechanism of T_3 /TR β action leads to induction of hypoxia-inducible factor (HIF-1) α , the tightly regulated subunit of the transcription factor HIF-1, and the HIF-1 target genes glucose transporter 1 (GLUT1) and phosphofructokinase (PFKP) (Moeller et al., 2005a, Moeller et al., 2005b). We also found an induction of the lactate transporter MCT4, which has recently been described as a HIF-1 target gene as well (Ullah et al., 2006).

A microarray study found stanniocalcin (STC)1 to be positively regulated by TH (Moeller et al., 2005b). STC1 is a polypeptide hormone that was originally identified as a regulator of calcium/phosphate homeostasis in fish (Wagner and Dimattia, 2006). During evolution from fish to mammals, STC1 maintained a function in calcium homeostasis, regulating mammalian intestinal calcium and phosphate transport (Madsen et al., 1998), but in evolutionary advanced animals, its role seems to have broadened to be involved in cellular metabolism, muscle and bone development and endothelial gene expression (Chakraborty et al., 2006, Filvaroff et al., 2002, McCudden et al., 2002). In humans, STC1 is expressed in heart, kidney, liver, ovary, prostate, thyroid c-cells and neurons (Chang et al., 2003, Sheikh-Hamad et al., 2003). Its physiological roles in humans as well as its regulation are not fully understood yet. STC1 has recently gained interest due to its involvement in cancer biology. Human STC1 was originally cloned in a screen for cancer-related genes and enhanced STC1 expression has been found in hepatocellular, colorectal and breast carcinoma as well as in medullary thyroid cancer (Chang et al., 1995, Chang et al., 2003).

In this communication we show that TH stimulate STC1 mRNA expression dependent on TR β and PI3K activation.

Material and Methods

Cell culture and treatments

Human skin fibroblasts were obtained by punch biopsy from 2 normal individuals and 2 patients with RTH due to TR β gene mutations, one heterozygous for a dominant negative TR β mutation A317T (TR β_{mut}) and the other homozygous for a TR β gene deletion (TR β_0). Their use was approved by the Institutional Review Board of the University of Chicago. Primary cultures of fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS) as previously described in detail (Murata et al., 1987). At confluency, the medium was replaced with one containing thyroid hormone depleted bovine serum (T $\times$ BS), produced by treatment of FBS with anion exchange resin (Samuels et al., 1979). 48 h later, T₃ was added to a final concentration of 0.1, 0.5 or 2 nM. Based on the free T₃ concentration in the medium containing 10% FBS, measured by equilibrium dialysis, these 3 T₃ doses correspond to a third to half, 1.5–2 times and 3–5 times physiological serum levels in humans. The inhibitors LY294002 (50 μ M, Sigma-Aldrich GmbH, Taufkirchen, Germany) and cycloheximide, CHX (10 μ g/ml, Sigma-Aldrich) were added to the medium 1 h before T₃ treatment. Infections with recombinant adenovirus either expressing green fluorescent protein (Ad-GFP) or a dominant negative form of the p85 α -regulatory subunit of PI3K (Ad-p85 α) prior to T₃ treatment were carried out as described previously (Cao et al., 2005). The experiments were terminated 24 h after T₃ treatment by RNA extraction for real-time PCR. All experiments were carried out at early cell passages (< 10 passages).

Isolation and reverse transcription of RNA

The medium was removed and the dish was washed twice with ice cold Hank's Buffered Saline Solution (HBSS, Invitrogen, Karlsruhe, Germany). Total RNA was extracted using phenol/guanidine isothiocyanate (TRIZOL, Invitrogen). RNA was reverse transcribed with the SuperscriptTM III RNase H Reverse Transcriptase Kit (Invitrogen) using 2 μ g of total RNA and 100 ng of random hexamers.

Quantification of mRNA

Quantification of mRNAs was performed in an ABI Prism 7 700 Sequence Detection System (Applied Biosystems, Foster City, CA), using SYBR[®] Green I as detector dye. The reaction mixtures contained 25 μ l QuantiTectTM SYBR[®] Green PCR Kit (QIAGEN, Hilden, Germany), 0.3 μ Mol/L of each primer, 10 ng template cDNA and nuclease-free water to a final volume of 50 μ l. The oligonucleotide primers were designed to cross introns, based on a Human BLAST Search result (<http://genome.ucsc.edu>). STC1 (NCBI Entrez Nucleotide mRNA access number NM_003155): forward primer 5'-TGTGAGCCCCAGGAAATCC-3' (exon 1), reverse primer 5'-TTCCTGCACCTCAGCAATCA-3' (exon 3); BTEB1 (NM_001206): forward primer 5'-CTC CCA TCT CAA AGC CCA TTA C-3' (exon 2), reverse primer 5'-TGA GCG GGA GAA CTT TTT AAG G-3' (exon 3). The primers were purchased from Operon Biotechnologies (Cologne, Germany). The reaction conditions were

95 C for 2 min followed by 40 cycles of 95 C for 15 s and 60 C for 45 s. To ensure specificity of the amplification, the PCR products were run on a gel and the single bands were of the expected size. STC1 expression was calculated relative to that in untreated cells and normalized for the housekeeping gene ubiquitin-conjugating enzyme E2D 2 (UBE2D2), using the 2^{-CT} method (Livak and Schmittgen, 2001). Quantification of TH dependent genes by microarray was carried out as described previously (Moeller et al., 2005b).

Data analysis

Real-time PCR results are expressed as mean $\pm$ SE and statistical analysis was done by ANOVA.

Results

STC1 expression is induced by T_3 via the TR β

STC1 was represented on the microarray chips that were used to study TH dependent gene expression in human fibroblasts as described previously (Moeller et al., 2005b). Primary cultures of human skin fibroblasts were treated for 24 h with increasing doses of T_3 , ranging from 0.1 to 2 nM following 48 h of TH-depleted medium to test for a dose response. In addition to cells from 2 normal individuals, fibroblasts from 2 patients with resistance to thyroid hormones were used. One patient has a heterozygous mutation (A317T, TR β_{mut}) and the other a homozygous deletion of the TR β gene (TR β_0). STC1 mRNA abundance after T_3 treatment is expressed relative to that in fibroblasts cultured for 72 h in TH depleted medium. A dose dependent increase in STC1 mRNA was observed in cells from 2 normal individuals: 1.4- and 1.3-fold increase after 0.1 nM T_3 and 1.8- and 2.1-fold increase after 0.5 nM T_3 , and 2.7 and 3.1-fold increase after 2 nM T_3 (Fig. 1a). In the fibroblasts from subjects with RTH, no such effect of TH was observed, as 1.3- and 1.0-fold changes after 0.1 nM T_3 , 1.4- and 0.9-fold changes after 0.5 nM T_3 and 1.3- and 0.7-fold changes after 2 nM T_3 were found for the TR β_{mut} and TR β_0 fibroblasts, respectively (Fig. 1a). These results demonstrate that STC1 is induced by TH. This effect is dose dependent and requires an intact TR β .

These results were confirmed by real-time PCR in an independent series of experiments. A similar dose dependent increase in STC1 mRNA was observed in normal fibroblasts 24 h after addition of 0.5 and 2 nM T_3 compared to untreated fibroblasts and expressed as fold-change (2.0 ± 0.2 ; $P < 0.01$ after 0.5 nM T_3 and 2.7 ± 0.2 ; $P < 0.005$ after 2 nM T_3) (Fig. 1b). This increase was greatly reduced in the RTH fibroblasts (1.3 ± 0.3 ; n.s. after 0.5 nM T_3 and 1.6 ± 0.4 ; $P < 0.005$ after 2 nM T_3) (Fig. 1b). The difference between the STC1 mRNA levels in normal and RTH fibroblasts was significant for the 2 nM T_3 treatment (2.7 ± 0.2 vs. 1.6 ± 0.4 ; $P < 0.01$). These results confirm both the stimulatory effect of TH on STC1 expression as well as the crucial role of the TR β .

T_3 -induced increase in STC1 mRNA requires de novo protein synthesis

Next, we investigated whether STC1 induction is direct or indirect, requiring de novo protein synthesis. Fibroblasts from a normal individual were cultured in T_3 -depleted medium for 48 h, followed by treatment with 2 nM T_3 for 24 h in the absence or presence of the translation

inhibitor CHX (10 µg/ml), added 1 h before T₃. Again, an increase in STC1 mRNA after T₃ treatment was observed. This increase was significantly diminished by pretreatment with CHX (2.7 ± 0.3 vs. 1.3 ± 0.1 , $P < 0.0005$) (Fig. 2). These results indicate that STC1 is indirectly induced by TH.

T₃-induced increase in STC1 mRNA is dependent on PI3K activation

To test whether the PI3K pathway is involved in mediating the T₃ effect on STC1 expression, we treated normal fibroblasts with T₃ in the absence and presence of the PI3K inhibitor LY294002. STC1 mRNA levels, measured by real-time PCR and expressed as fold-change relative to those in cells grown in T₃-depleted serum, are shown in Fig. 3. Consistent with the previous results shown in Fig. 1, T₃ treatment increased STC1 mRNA levels (3.9 ± 0.5 ; $P < 0.005$). This effect of T₃ was completely abrogated by LY294002 (0.85 ± 0.5 ; n. s.). These results demonstrate that the stimulatory effect of TH on STC1 mRNA requires PI3K activation.

This was confirmed by a different approach. PI3K consist of the catalytic p110 subunit and the regulatory p85α subunit. Transfection of a dominant negative p85α mutant, p85α, can inhibit PI3K. Fibroblasts cultured in T₃-depleted medium were incubated with T₃ for 12 h after infection with either a green fluorescent protein expressing adenovirus (Ad-GFP) or an adenovirus expressing p85α (Ad- p85α). As shown in Fig. 4a, the infection with p85α reduced T₃-induced STC1 expression to half compared to cells transfected with Ad-GFP, further demonstrating the involvement of PI3K. Basic transcription element binding protein 1 (BTEB1) mRNA induction by T₃ in human cells on the other hand is independent of PI3K (Moeller et al., 2005a) and could therefore serve as control. BTEB1 expression was not affected by infection with Ad- p85α (Fig. 4b), suggesting that reduction of STC1 expression is a specific effect of PI3K inhibition.

Discussion

A wide range of TH effects are mediated by its influence on gene expression. In recent years, several new mechanisms of TH effects on gene expression were described in addition to the classic nuclear mechanism of a TR/RXR heterodimer bound to a TRE in the promoter of a target gene. For example, TH can induce genes independent of TRs, as shown for basic fibroblast growth factor 2 (FGF2). TH binds to a membrane bound integrin, α_vβ₃, which subsequently leads to activation of the MAPK pathway and FGF2 mRNA increase (Davis et al., 2004).

Another mechanism of TH action leading to increased gene expression is the TR-mediated activation of the PI3K pathway by protein-protein interaction of the liganded TRβ with the p85α subunit of PI3K. The definition of the TRβ therefore has broadened beyond being merely a ligand-dependent transcription factor, acting on a TRE in the promoter of target genes. It can also induce gene expression through TRE-independent activation of signal transduction pathways such as the PI3K pathway (Cheng et al., 2010, Moeller et al., 2006).

We show here that STC1 is a TH inducible gene with a dose dependent mRNA response for physiological TH concentrations. The action of TH is TRβ mediated, because cells from

patients with RTH showed a significantly reduced STC1 response to TH compared to cells from normal individuals.

In addition to TR β , STC1 induction by TH is dependent on PI3K activation, since inhibiting PI3K with either LY294002 or p85 α reduced the TH effect on STC mRNA expression. We observed the same pattern of TR β - and PI3K-dependent induction for HIF-1 α and subsequently its then known target genes PFKF and GLUT1 as well as for MCT4, which was later found to be a HIF-1 target gene, too (Moeller et al., 2005a, Ullah et al., 2006). Because STC1 was recently also reported to be a target gene of HIF-1 (Law et al., 2010, Yeung et al., 2005), it is reasonable to assume that TH exerts its effect on STC1 through the same TR β /PI3K mediated mechanism.

There was no effect of T₃ on STC1 expression in cells with homozygous deletion of the TR β . As the resistance to TH in the case of heterozygous TR β mutations is partial, a minimal response of STC-1 expression to T₃ could be observed in these cells. Due to the variable degree of resistance among different mutations, there may be differences in the degree of STC1 expression, dependent on the mutation. In contrast, higher STC1 levels seem possible in the case of the TR β PV mutant, because in TR β ^{PV/PV} knock in mice, the TR β PV mutant bound significantly more to the PI3K-regulatory subunit p85 α , resulting in a greater increase in the kinase activity than did TR β 1 in wild-type mice (Furuya et al., 2006).

To which physiological or pathophysiological effects could this mechanism of TR β /PI3K mediated TH action contribute? A potential role of thyroid hormone in tumor progression was recently supported by the findings of Davis *et al.* They could demonstrate in glioblastoma cells that TH induced cell proliferation, which was mediated by the integrin α v β 3 and the MAPK pathway (Davis et al., 2006). Interestingly, STC1 was linked to angiogenesis in a glioblastoma cell line (Lal et al., 2001) and STC1 expression was enhanced in several types of cancer (colon, breast, hepatocellular and medullary thyroid cancer) (Chang et al., 2003). Further studies are needed to determine whether PI3K activation and STC1 induction by TH contribute to tumor progression.

Acknowledgments

This work was supported in part by grants DK 15070 from the National Institutes of Health and RR 00055 from the US public Health Service (to S. R.); Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (Monbukagakusho) of Japan (nos. 13470217 and 16390269) (to H.S.); by the 21st Century COE program "Integrated Molecular Medicine for Neuronal and Neoplastic Disorders" of Monbukagakusho (to H.S. and X.C.), the Howard Hughes Medical Institute (Predoctoral Fellowship to A.M.D.) and the Deutsche Forschungsgemeinschaft DFG (Mo 1018/1-1 to L.C.M.).

References

1. Cao X, Kambe F, Moeller LC, et al. Thyroid hormone induces rapid activation of Akt/protein kinase B-mammalian target of rapamycin-p70S6K cascade through phosphatidylinositol 3-kinase in human fibroblasts. *Mol Endocrinol.* 2005; 19:102–112. [PubMed: 15388791]
2. Chakraborty A, Brooks HL, Zhang P, et al. Stanniocalcin-1 regulates endothelial gene expression and modulates trans-endothelial migration of leukocytes. *Am J Physiol Renal Physiol.* 2007; 292:895–904.
3. Chang AC, Janosi J, Hulsbeek M, et al. A novel human cDNA highly homologous to the fish hormone stanniocalcin. *Mol Cell Endocrinol.* 1995; 112:241–247. [PubMed: 7489828]

4. Chang AC, Jellinek DA, Reddel RR. Mammalian stanniocalcins and cancer. *Endocr Relat Cancer*. 2003; 10:359–373. [PubMed: 14503913]
5. Cheng SY, Leonard JL, Davis PJ. Molecular Aspects of Thyroid Hormone Actions. *Endocr Rev*. 2010; 31:139–170. [PubMed: 20051527]
6. Davis FB, Mousa SA, O'Connor L, et al. Proangiogenic action of thyroid hormone is fibroblast growth factor-dependent and is initiated at the cell surface. *Circ Res*. 2004; 94:1500–1506. [PubMed: 15117822]
7. Davis FB, Tang HY, Shih A, et al. Acting via a cell surface receptor, thyroid hormone is a growth factor for glioma cells. *Cancer Res*. 2006; 66:7270–7275. [PubMed: 16849576]
8. Filvaroff EH, Guillet S, Zlot C, et al. Stanniocalcin 1 alters muscle and bone structure and function in transgenic mice. *Endocrinology*. 2002; 143:3681–3690. [PubMed: 12193584]
9. Furuya F, Hanover JA, Cheng SY. Activation of phosphatidylinositol 3-kinase signaling by a mutant thyroid hormone beta receptor. *Proc Natl Acad Sci USA*. 2006; 103:1780–1785. [PubMed: 16446424]
10. Lal A, Peters H, St Croix B, et al. Transcriptional response to hypoxia in human tumors. *J Natl Cancer Inst*. 2001; 93:1337–1343. [PubMed: 11535709]
11. Law AY, Ching LY, Lai KP, et al. Identification and characterization of the hypoxia-responsive element in human stanniocalcin-1 gene. *Mol Cell Endocrinol*. 2010; 314:118–127. [PubMed: 19628018]
12. Lei J, Mariash CN, Ingbar DH. 3,3',5-Triiodo-L-thyronine up-regulation of Na, K-ATPase activity and cell surface expression in alveolar epithelial cells is Src kinase- and phosphoinositide 3-kinase-dependent. *J Biol Chem*. 2004; 279:47589–47600. [PubMed: 15342623]
13. Livak KJS, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{−(ΔΔC_T)} Method. *Methods*. 2001; 25:402–408. [PubMed: 11846609]
14. Madsen KL, Tavernini MM, Yachimec C, et al. Stanniocalcin: a novel protein regulating calcium and phosphate transport across mammalian intestine. *Am J Physiol*. 1998; 274:G96–G102. [PubMed: 9458778]
15. McCudden CR, James KA, Hasilo C, et al. Characterization of mammalian stanniocalcin receptors. Mitochondrial targeting of ligand and receptor for regulation of cellular metabolism. *J Biol Chem*. 2002; 277:45249–45258. [PubMed: 12223480]
16. Moeller LC, Cao X, Dumitrescu AM, et al. Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor beta through the phosphatidylinositol 3-kinase pathway. *Nucl Recept Signal*. 2006; 4:e020. [PubMed: 16862226]
17. Moeller LC, Dumitrescu AM, Refetoff S. Cytosolic action of thyroid hormone leads to induction of hypoxia-inducible factor-1alpha and glycolytic genes. *Mol Endocrinol*. 2005a; 19:2955–2963. [PubMed: 16051672]
18. Moeller LC, Dumitrescu AM, Walker RL, et al. Thyroid hormone responsive genes in cultured human fibroblasts. *J Clin Endocrinol Metab*. 2005b; 90:936–943. [PubMed: 15507505]
19. Murata Y, Ceccarelli P, Refetoff S, et al. Thyroid hormone inhibits fibronectin synthesis by cultured human skin fibroblasts. *J Clin Endocrinol Metab*. 1987; 64:334–339. [PubMed: 3793852]
20. Samuels HH, Stanley F, Casanova J. Depletion of L-3,5,3'-triiodothyronine and L-thyroxine in euthyroid calf serum for use in cell culture studies of the action of thyroid hormone. *Endocrinology*. 1979; 105:80–85. [PubMed: 446419]
21. Sheikh-Hamad D, Bick R, Wu GY, et al. Stanniocalcin-1 is a naturally occurring L-channel inhibitor in cardiomyocytes: relevance to human heart failure. *Am J Physiol Heart Circ Physiol*. 2003; 285:H442–H448. [PubMed: 12663264]
22. Ullah MS, Davies AJ, Halestrap AP. The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. *J Biol Chem*. 2006; 281:9030–9037. [PubMed: 16452478]
23. Wagner GFD, Dimattia GE. The stanniocalcin family of proteins. *J Exp Zool A Comp Exp Biol*. 2006; 305:769–780.
24. Yen PM. Physiological and molecular basis of thyroid hormone action. *Physiol Rev*. 2001; 81:1097–1142. [PubMed: 11427693]

25. Yeung HY, Lai KP, Chan HY, et al. Hypoxia-inducible factor-1-mediated activation of stanniocalcin-1 in human cancer cells. *Endocrinology*. 2005; 146:4951–4960. [PubMed: 16109785]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Induction of STC1 mRNA expression by T_3 in cultured human fibroblasts. Human skin fibroblasts were cultured in TH-depleted medium (TxBS) for 48 h and then treated with 3 different doses of T_3 for 24 h prior to submission to microarray analysis. **a**, T_3 -dose dependent response of STC1 occurred in fibroblasts from normal individuals (left panels), but not in fibroblasts from 2 patients with RTH due to the dominant negative $TR\beta$ gene mutation, A317T ($TR\beta_{mut}$) and homozygous deletion ($TR\beta_0$) (right panels). **b**, The effect of 0.5 and 2 nM T_3 on STC1 mRNA expression was measured by real time PCR in fibroblasts

from a normal individual and a patient with RTH (TR β mutation A317T). Shown is the mean $\pm$ SE (n = 3 for each treatment).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2.

Effect of T₃ on STC1 mRNA in the absence and presence of the protein synthesis inhibitor CHX. Normal fibroblasts were cultured in DMEM with 10% TH-depleted bovine serum for 48 h and then treated with 2 nM T₃ without or with pretreatment with CHX (10 µg/ml). Cells were harvested 24 h after T₃ treatment. The relative amount of STC1 mRNA, as measured by real-time PCR and compared to fibroblasts not treated with T₃, is plotted on the ordinate as fold increase (mean ± SE, n = 8).

Fig. 3.

Effect of T₃ and the PI3K inhibitor LY294002 on STC1 mRNA levels. Normal fibroblasts were cultured for 48 h in DMEM supplemented with 10% TH-depleted bovine serum (TxBS) and then treated for 24 h with 2 nM T₃ in the absence or presence of LY294002 (LY, 50 μM), added 1 h before the addition of T₃. The relative amount of mRNAs, as measured by real-time PCR, and compared to that in fibroblasts cultured in TH-depleted medium is plotted on the ordinates as fold increase. Data are expressed as mean ± SE (n = 3 for each treatment).

Fig. 4.

Inhibition of T₃-dependent STC1 expression by p85 α . Adenovirus expressing either green fluorescent protein (Ad-GFP) or p85 α (Ad- p85 α) was infected at a M.O.I of 200 for 1 h. Fibroblasts were then incubated in DMEM with 5% T₃-depleted FBS for 48 h and treated with T₃ (10 nM) for 12 h. The relative amount of **a**, STC1 and **b**, BTEB1 mRNAs, measured by real-time PCR and compared to that in fibroblasts cultured in TH-depleted medium, is plotted on the ordinates as fold increase (mean of 2 experiments).