Thyroid hormone responsiveness of the L1210 murine leukemia cell line

J Brtko, P Filipčík, J Knopp, V Sedláková, L’ Rauová

Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava; Research Institute of Rheumatic Diseases, Piestany, Czechoslovakia


The presence of saturable and high affinity 3,5,3’-triiodothyronine (T3) binding sites was demonstrated in L1210 murine leukemia cell nuclei. Scatchard analysis revealed one class of receptors for T3 with Kd = 2.187 × 10⁹ l/mol and a maximum binding capacity (Bmax) of 3.96 fmol/10⁶ cells. The effects of T3 on protein phosphorylation and growth rate of L1210 cells were investigated in a medium containing T3-depleted fetal calf serum. T3 was observed to be effective in enhancing protein phosphorylation (153.06% ± 5.99%) compared to cells grown in the absence of T3 (81.49% ± 13.50%). Moreover, in the presence of high T3 concentration (11.15 nmol/l) T3 was found to significantly increase the cell growth rate. In addition, the T3 receptor-associated alterations during the cell cycle, as measured by flow cytometry, suggest that the presence of T3 receptors becomes evident during the late G1 phase of the cell cycle, and T3 receptor numbers increase during the S phase. These results suggest that in vitro conditions representing high T3 concentration, the number of L1210 leukemia cells may be increased by T3 via nuclear receptors. The L1210 leukemia cell line may serve as a convenient tool for in vitro studies of nuclear receptors and/or mechanism of action of T3. The binding affinity of T3 receptors is similar to that found in rat hepatocytes or human lymphocytes.

Július Brtko, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárská 3, 833 06 Bratislava, Czechoslovakia

Studies on thyroid hormone–nuclear receptor interaction and of thyroid hormone responsive genes have demonstrated multiple levels of control. Specific examples include both positive and negative regulation of genes by thyroid hormone (1). In rat pituitary tumour GC cells 3,5,3’-triiodothyronine (T3) regulates cellular growth rate, growth hormone production and levels of nuclear thyroid hormone receptor via distinct dose–response ranges (2). The thyroid hormone nuclear receptors are high-affinity low-capacity binding sites with an in vitro or in vivo apparent association constant (Kd) in the order of 2.0 × 10⁹ l/mol (3). The T3 receptors are considered to be intrinsic chromosomal acidic non-histone proteins having a molecular mass of approximately 50000 Da, whose localization is not dependent on the presence of thyroid hormone (4). Treatment of chromatin by micrococcal nuclease releases a predominant 6.5 S form from GH1 cell nuclei and its entity seems to be composed of the 3.5 S receptor and additional proteins which are associated with 35–40 base fragment of DNA (5). It has been shown independently by Weinberger et al. (6) and Sap et al. (7) that the cellular counterpart (c-erbA) of the viral oncogene v-erbA encodes the thyroid hormone receptor. In mammals at least two distinct but closely related genes encode thyroid hormone receptors. These genes, which are localized on chromosomes 17 and 3, have been termed c-erbA-α and c-erbA-β genes (8).

The aim of the present study was to examine L1210 murine leukemia cell nuclei for T3 receptors and to evaluate their T3 receptor binding characteristics: L1210 cells are widely used for testing a variety of new anti-cancer drugs. In addition, the effects of T3 on protein phosphorylation and cell growth rate were investigated using cells grown in the absence of T3 or high T3 concentration. The synchronization of L1210 cells into the cell cycle M-phase with subsequent release from colcemide block (as measured by flow cytometry), enabled us to detect the appearance of the T3 nuclear receptors during the G1/S and S-phases of the cell cycle.

Materials and methods

Materials

[125I]T3 (SA 44.4 TBq/g) was purchased from the Institute of Isotopes, Hungarian Academy of Sciences (Budapest, Hungary). [γ-32P]ATP (SA 110 TBq/mmol)
from Amersham (Aylesbury, UK). T₃, Triton X-100 and TRIS-(hydroxymethyl)-aminomethane from Sigma (St Louis, MO). Cell culture medium RPMI-1640 was from Flow Laboratories (Rickmansworth, UK), and fetal calf serum (FCS) from the Veterinary School (Brno, Czechoslovakia). Thymidine, colcemide, propidium iodide and Dowex 1-X8 were purchased from Serva (Heidelberg, Germany). Other reagent grade chemicals were from Lachema (Brno, Czechoslovakia).

Experimental procedures

Cell culture and synchronization procedure. Murine leukemia cells were grown as suspension culture in RPMI 1640 medium supplemented with 10% (in some series 2.5%) fetal calf serum. 100000 U/l penicillin and 68.6 μmol/l streptomycin at 37°C, in a Flow Laboratories humidified incubator IR 1500 (Rickmansworth, UK) in a 5% CO₂ atmosphere. The cells were inoculated into a fresh medium at a density of 3.0–4.0 × 10⁶ cells/l, then grown under control by cell countings with a doubling time of approximately 11 h after an initial lag. The cell numbers were not allowed to exceed 2.0 × 10⁹ cells/l. When estimating the effect of T₃ on the cell number doubling time, the cells were grown in the presence of T₃-depleted fetal calf serum at a concentration of 2.5%. T₃-depleted serum was prepared by Dowex 1-X8 treatment according to Samuels et al. (9). The growth of L1210 cells was chemically synchronized according to methods described previously (10,11). Cells in the exponentially growing phase (density approx. 5 × 10⁸ cells/l) were treated with 2 mmol/l thymidine and subsequently by colcemide at a concentration of 80 mmol/l to accumulate them in the S and M phases of the cell cycle, respectively. Thereafter, the cells were washed and resuspended in normal fresh medium with 10% FCS to release them from the arrest. Synchronization of the culture was followed by propidium iodide staining (12), and the DNA content was determined by flow cytometry in a FACSscan, Becton–Dickinson (Emm;bod giem, Belgium).

T₃ binding experiments. The exponentially growing cells (or cells in a particular phase of the cell cycle) were washed twice in serum-free RPMI-1640 medium and resuspended at a concentration of approximately 3 × 10⁸ cells/l or 1.0–2.0 × 10⁶ cells/tube. T₃ binding experiments were performed according to Barlow and De Nayer (13) with increasing concentrations of [¹²⁵I]T₃ (0.01–0.4 nmol/l). The non-specific nuclear T₃ binding was determined by incubation with a 1000-fold excess of unlabelled T₃ and was subtracted from the total to determine the receptor-specific bound T₃. The cells were incubated for 2 h at 37°C. After the binding period the cultures were chilled and washed once in serum-free RPMI-1640. The cell nuclei were isolated with Triton X-100 (0.25 mmol/l sucrose, 1.1 mmol/l MgCl₂, 20 mmol/l TRIS/HCl, pH 7.85; 0.5% Triton X-100) as previously described by Samuels et al. (14). The final pellet was quantified for specifically bound [¹²⁵I]T₃ in a Beckman model 4000 γ-spectrometer (Fullerton, CA).

Phosphorylation reaction and protein determination. In vitro phosphorylation of cytoplasmic proteins from L1210 cells was studied using [γ-³²P]ATP according to the method described previously by Knopp and Brtko (15). The protein concentration was determined by the method of Lowry et al. (16) using human albumin as a standard.

Results

The data from five independent experiments showed that the T₃ binding sites of L1210 murine leukemia cell nuclei are saturable. From the Scatchard plot (17) one class of high affinity receptors with an equilibrium association constant Kₛ = 2.187 × 10⁹ l/mmol is evident (Fig. 1). When relating maximum binding capacity (Bₘₐₓ) per 10⁶ cells, the value of 3.96 nmol T₃ bound to nuclear receptors is obtained, which represents 2385 T₃ receptor sites per nucleus. With the aim of elucidating the role of T₃ in total protein phosphorylation for L1210 cells grown in a medium containing T₃-depleted fetal calf serum at a concentration of 2.5%, experiments were performed at the high T₃ concentration (11.15 nmol/l). As shown in Table 1, T₃ was effective in enhancing protein phosphorylation. The effects of T₃ on growth rate were evaluated in a separate set of experiments. In a control group grown in a medium containing T₃-non-depleted fetal calf serum at a concentration of 2.5% the mean doubling time of the cell line was 14 h. However, after the addition of T₃ (11.15 nmol/l) to the suspension of cells grown in the medium containing the T₃-depleted calf serum, the doubling time was significantly (p < 0.05) decreased (Fig. 2).

![Fig. 1. Scatchard plot of [¹²⁵I]T₃ binding to L1210 cell nuclei (Kₛ = 2.187 × 10⁹ l/mmol; Bₘₐₓ = 3.96 nmol/10⁶ cells): effect of increasing concentrations of [¹²⁵I]T₃ (N = 5) on its specific binding to L1210 cell nuclei (inset).](image-url)
The L1210 cells grown in the medium supplemented with T4-non-depleted fetal calf serum were synchronized into the M phase of the cell cycle with 2 mmol/l thymidine and 80 mmol/l colcemide as described in the materials and methods section. After the release from the colcemide block, the concentrations of T3 receptors were observed to increase from the G1 to the late S phase of the cell cycle as evaluated by FACScan flow cytometry. In L1210 murine leukemia cell nuclei the synthesis of T3 receptors becomes evident during the late period of the G1 phase of the cell cycle (Table 2).

Discussion
The heterogeneity of the thyroid hormone actions in different tissues is based on the effect of T3 which, complexed with T3 receptors in cell nuclei, affects thyroid hormone responsive elements in the 5'-flanking region of T3-responsive genes (18).

In our experiments performed with the L1210 cell line, significantly lower numbers of T3 receptors were found when compared to data from recent literature dealing with the nuclei of pituitary cell lines (GH1, GH3, GC or GH3C1) or liver nuclei (19–20). However, Kd calculated from the Scatchard plot had a similar value to that found for a variety of other T3-receptor-rich tissues. As a matter of fact, the addition of T3 at a high concentration to the suspension of L1210 cells grown in a T3-depleted medium caused a significant enhancement of cell division; the doubling time of the cells was effectively decreased.

Phosphorylation of serine, threonine and tyrosine in cellular proteins is a common cellular mechanism for modulating diverse physiologic processes such as metabolic pathways, gene transcription, cell division, etc. (21). Among cellular responses related to thyroid hormone nuclear binding, the enhancement in cytosolic protein phosphorylation induced by T3 in rat liver has already been shown by Nakamura and DeGroot (22). In spite of low but evidently sufficient numbers of T3 receptors, similar stimulatory patterns representing the rate of protein phosphorylation in L1210 cells by T3 were observed.

Studies providing the first available information on nuclear T3 concentration as a function of the cell cycle stage in GC pituitary cell lines were reported by De Fesi et al. (23). Measurements obtained with synchronized GC cells suggested that newly dividing cells possess a full set of T3 receptors in cell nuclei. The results of the present study with L1210 cells as measured by flow cytometry indicate that the synthesis of the T3 receptors starts in the late G1 phase of the cell cycle which corresponds well with the conclusions based on experiments performed by De Fesi et al. (23).

Lymphocytic mouse leukemia L1210 was first described by Law et al. (24); in general, implantation of cultured cells into mice results in leukemia. Cultures of L1210 cells which grow in suspension are widely and advantageously used as models for prediction of anticancer effects of newly developed drugs. Evaluation of Kd and Bmax values of the T3 receptors in L1210 cells prompted us to focus our attention on investigating the effect of high T3 concentration on cell proliferation since

![Figure](image_url)

**Table 1.** Effect of thyroid hormone on total protein phosphorylation in cultured murine L1210 leukemia cells.

<table>
<thead>
<tr>
<th>Group</th>
<th>T3 Concentration (nmol/l)</th>
<th>Total protein phosphorylation (%)</th>
<th>sd</th>
<th>N</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.55</td>
<td>100</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>-T3</td>
<td>non-detectable</td>
<td>81.49</td>
<td>± 13.50</td>
<td>3</td>
<td>n.s.</td>
</tr>
<tr>
<td>+T3</td>
<td>11.15</td>
<td>153.06</td>
<td>± 5.99</td>
<td>3</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Table 2.** The dynamics of T3 receptor appearance in L1210 cell nuclei after the release from colcemide block.

<table>
<thead>
<tr>
<th>Group</th>
<th>Proportions of cells per cell cycle phase</th>
<th>T3 specific binding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1 (%)</td>
<td>S (%)</td>
</tr>
<tr>
<td>3 h</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>6 h</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>9 h</td>
<td>22</td>
<td>49</td>
</tr>
<tr>
<td>12 h</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Control</td>
<td>27</td>
<td>63</td>
</tr>
</tbody>
</table>

Values shown are representative data taken from one of the three independent experiments.
hormone receptors have been shown to be key elements in the dynamics of cellular control.

The results of the studies reported herein indicate that T3 has a general stimulatory effect on L1210 cells most probably mediated by nuclear receptors. Therefore, it seems reasonable to infer that the L1210 leukemia cell line may serve as a convenient tool in studies of T3 nuclear receptor and/or mechanism of action.

Acknowledgments. The authors are grateful to Mrs Dagmar Kubíková for secretarial service.

References
1. Glass CK, Holloway JM. Regulation of gene expression by the thyroid hormone receptor. Biochim Biophys Acta 1990; 1032:157-76
2. Halperin Y, Surks MI, Shapiro LE. L-Triiodothyronine (T3) regulates cellular growth rate, growth hormone production, and levels of nuclear T3 receptors via distinct dose-response ranges in cultured GC cells. Endocrinology 1990;126:2321–6
14. Samuels HH, Tsai JR. Thyroid hormone action: demonstration of nuclear receptors in intact cells and isolated nuclei. Proc Natl Acad USA 1973;70:3488–93
17. Scatchard G. The attraction of protein for small molecules and ions. Ann NY Acad Sci 1949;51:660–72
23. De Fisi CHR, Fels EC, Surks MI. Nuclear 3,5,3′-triiodothyronine receptor concentration increases during deoxyribonucleic acid synthesis in partially synchronized GC cell cultures. Endocrinology 1982;111:1156–65