T₃ Binding Properties of Nuclear Fibroblast Receptors from Patients Homozygous and Heterozygous for A Dominant Negative Mutation of C-erbAβ

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Abstract. This study represents the first measurements of T₃ binding to nuclear receptors in fibroblasts from a homozygous child with a dominant negative thyroid hormone receptor gene. Previously demonstrated in this child and kindred was a three base pair deletion in the c-erbAβ thyroid hormone receptor gene resulting in the loss of threonine codon 332. In this study using cultured skin fibroblasts from this homozygous child and his heterozygous father there was normal binding affinity and a two-fold less binding capacity in comparison to control fibroblasts. These data are consistent with loss of β-receptor T₃-binding activity, but the presence of normally functioning α-receptors in vivo.

Key words: Thyroid hormone resistance, T₃ binding, dominant negative mutation, c-erbAβ receptor, fibroblast

Introduction

Generalized resistance to thyroid hormone (GRTH) is a syndrome characterized by elevated serum levels of free thyroid hormones, resistance to thyroid hormone action, and inappropriately normal or elevated levels of thyroid stimulating hormone (TSH) [1]. Thyroid hormones regulate gene function through 1) triiiodothyronine (T₃) binding to the nuclear T₃ receptor (NT₃R) and 2) the T₃ and NT₃R complex binding to thyroid hormone response elements [2,3]. Diverse abnormalities in the c-erbAβ T₃-receptor are the cause of variable phenotypes of GRTH[4, 5]. Several studies of radio-labeled T₃ binding to nuclei from fibroblasts in patients with GRTH have been reported [6-8]. Some investigators have demonstrated small changes in T₃-binding affinity or number, whereas in most reports there did not appear to be any binding abnormalities [8].

We have previously described a unique child with a severe form of generalized resistance to thyroid hormones (GRTH); this
patient was homozygous for a mutation in the c-erbAβ thyroid hormone receptor gene [5,9]. This c-erbAβ mutation of kindred S is a three base-pair deletion in the T3-binding domain resulting in a single amino-acid deletion [5]. The mutant receptor synthesized in vitro using reticulocyte lysate had no detectable ability to bind T3 [5]. Here we describe the T3-binding characteristics of the in vivo nuclear receptors obtained from fibroblasts of the homozygous patient, a heterozygous parent and controls.

**Materials and Methods**

**Preparation of nuclear fibroblast receptors and T3-binding studies:**

The method was based on the procedure of Ichikawa et al [6] but [125I]-T3 was mixed in culture medium before harvesting cells. Fibroblast cultures were established from skin biopsy samples. Cells were grown in DMEM containing 10% fetal calf serum, penicillin (100 U/MI) and streptomycin (100 µg/Ml). All experiments were carried out 2 to 3 weeks after replanting, when cells were confluent. Before each experiment the medium was replaced twice with serum free Ham’s F-10 medium, at 48 and 24 hours. At the beginning of each experiment, the medium was removed and cells were incubated for two hours at 37°C with 0.005 × 10^-9 mol/L to 0.4 × 10^-9 mol/L [125I]-T3 (concentration > 3000 µCi/ug, Amersham, Arlington Heights, IL). To determine nonspecific binding, duplicate plates were added, containing an additional 3 × 10^-7 mol/L of unlabeled T3. Aliquots of incubation medium with [125I]-T3 were saved for free hormone determinations, and the cells were harvested by trypsinization and immediately cooled in an ice bath. Cells were collected by centrifugation and washed once with ice-cold 10 mmol/L PBS. Cells were resuspended in 1 ml of SMT buffer (0.25 mol/L sucrose, 1 mmol/L MgCl₂, 20 mmol/L Tris-HCl Ph 7.85) with 0.5% Triton X-100 and passed through a 25-gauge needle. The syringe was washed once with 1 ml of SMT buffer. Nuclei were collected by centrifugation at 12,000x g for ten minutes and washed with the same buffer, and radioactivity was counted. DNA concentration was determined by absorption of UV light at 260 angstroms. All data were corrected for DNA recovery. L-T3, calf thymus DNA Type 1 and Triton X-100 were purchased from Sigma Chemical (St. Louis, MO) and DMEM, fetal calf serum and trypsin-EDTA from GIBCO (New York, NY).

Linear regression analysis of Scatchard plots was performed. Specific binding was calculated by subtracting nonspecific binding from total binding. These studies were approved by the University of South Florida College of Medicine and All Children’s Hospital Institutional Review Boards, and written informed consent was obtained from the parents.

**Results and Discussion**

We determined the T3-binding affinity and total binding capacity of fibroblast nuclei from the child homozygous for the β-receptor deletion-mutation, his father who is heterozygous for the defect and two controls. The Scatchard plots of the data are shown in Figures 1 and 2. Total T3-binding capacity of both the child and his father was reduced, although T3-binding affinity of them was kept normal as shown in table.

Ichikawa et al have measured the T3-binding affinity and total binding capacity of whole cells, nuclei, and high-salt extracts from fibroblasts of the original Refetoff patient with GRTH [6, 10]. This patient has recently been shown to have a deletion of both c-erbAβ alleles and therefore only expresses α-forms of the thyroid hormone receptor [11]. Interestingly, there was no difference between control and patient fibroblasts in T3-binding affinity determined with whole cell incubations, isolated nuclei or with nuclear extracts. However, there was a reduction in binding capacity measured with nuclear extract, as one
T3 Binding in Generalized Resistance to Thyroid Hormone

Figure 1. Representative data from the homozygous child. Scatchard plot of the data. Binding affinity was not significantly reduced in the homozygous child.

Figure 2. Scatchard plot of the data of the heterozygous father. Binding affinity was not significantly reduced in the heterozygous father.

Table. Scatchard analysis of homozygous child and his heterozygous father

<table>
<thead>
<tr>
<th></th>
<th>Binding Affinity</th>
<th>Binding Capacity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$K_a \times 10^{4}$ mol/L</td>
<td>$B_{max}$ (fmol/100 μg DNA)</td>
</tr>
<tr>
<td>Exp. 1</td>
<td>6.73</td>
<td>3.26</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>3.56</td>
<td>4.23</td>
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<tr>
<td>Exp. 3</td>
<td>9.60</td>
<td>10.77</td>
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<tr>
<td>Father Control</td>
<td>5.12</td>
<td>10.76</td>
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<tr>
<td>Father Control</td>
<td>2.64</td>
<td>6.79</td>
</tr>
<tr>
<td>Father Control</td>
<td>5.75</td>
<td>11.89</td>
</tr>
</tbody>
</table>

Assays of child and father were done at the same time as control in each experiment. Exp.: experiment

might predict for the case of deleted c-erbAβ alleles. The findings in our homozygote are consistent with those found in the Refetoff patient. The kindred S receptor would not be expected to bind T3 in vivo given the in vitro results [5], and therefore the T3-affinity and binding capacity we measured would reflect only α-forms of the receptor as was the case for the Refetoff patient. We did not observe a difference between the homozygote and heterozygote in binding capacity, and this might be due to limitations in the sensitivity with whole cell incubations or the negative effect of mutant β-forms of the receptor on normal β-forms of the receptor.

Finally, dimerization of thyroid hormone receptors on DNA thyroid hormone response elements has been demonstrated [12], and perhaps dimerization between thyroid hormone receptor forms and other auxiliary proteins may also occur in the nucleus. The dominant negative kindred S receptor appears to be able to inhibit gene expression mediated by the wild-type α receptor [9]. However, the T3-binding affinity data with the homozygote did not demonstrate any antagonistic effect of the mutant receptor on the wild-type α-receptor T3-affinity. We conclude that, if
the mutant $\beta$-receptor form does dimerize with the $\alpha$-receptor form, this interaction does not affect the T3-binding affinity.

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