Nuclear Thyroid Hormone Receptors in a Human Breast Cancer Cell Line

Robert E. Burke and William L. McGuire

Department of Medicine, The University of Texas Health Science Center, San Antonio, Texas 78284

ABSTRACT

Proliferation of a human metastatic breast cancer cell line derived at the Michigan Cancer Foundation (MCF-7) was stimulated significantly (p < 0.05) by the addition of 3,3',5-triiodothyronine (T3) to the culture medium. An optimal effect was observed near 5 x 10^-10 M.

Thyroid hormone receptor was assayed by comparing the radioactive [125I]T3 incorporated by MCF-7 cells incubated in culture with and without unlabeled competitor. Bound [125I]T3 in the nuclei was determined directly by counting Triton X-100-purified nuclear pellets. Saturable effectiveness of unlabeled structural analogs to T3 as competitors for [125I]T3 binding was: T3 = 3,5-diiodo-3'-isopropyl-L-thyronine > 3,3',5-triiodothyronine > L-thyroxine = 3,3',5,5'-tetraiodo-L-thyroacetic acid > reverse 3,3',5'-triiodo-L-thyronine. The mono- and diiodotyrosines, bovine insulin, ovine prolactin, 17β-estradiol, prostaglandin F2α, indomethacin, and propylthiouracil did not compete for binding sites. Nuclear receptor levels were not altered by treatment of MCF-7 cells with these compounds or with T3 itself. Receptor levels also did not fluctuate with the growth phase.

Our data establish the presence of receptors for thyroid hormone in nuclei of cells derived from a human breast cancer.

INTRODUCTION

Controversy surrounds the role for thyroid hormone in both the etiology and the treatment of human breast cancer (4). Increased risk and incidence of breast cancer have been correlated with hypothyroidism (6, 15, 27) as well as with the use of supplements to treat the latter (8). Loeser (12) claimed a prophylactic role for thyroid hormone against the recurrence of breast cancer. However, arguments against an etiological role of thyroid dysfunction (16) and against the therapeutic value of thyroid hormone (3, 13, 17) have also been made. Nonetheless, objective tumor regressions in patients with advanced breast cancer have been attributed to the use of thyroid hormone in conjunction with surgical or other hormonal therapy (1, 11, 24). Although the intracellular mechanism(s) by which thyroid hormones act is not known with certainty, receptors for these hormones have been identified (19, 22, 28) and characterized (10, 24) in responsive, non-breast cells. Therefore, we considered it necessary to determine whether such specific receptors might be present to influence or mediate the proposed actions of thyroid hormone in breast neoplasms. We chose the well-defined (20, 26) human breast cancer cell line MCF-7 as a likely candidate to possess thyroid hormone receptor, since it has been shown to have other hormone receptors (5).

MATERIALS AND METHODS

Our MCF-7 cells were routinely grown in Eagle's autoclavable minimal essential medium, supplemented with 1% non-essential amino acids, 1% glutamine, 5% calf serum, bovine insulin (6 ng/ml; Sigma Chemical Company, St. Louis, Mo.), 0.5% saturated sodium bicarbonate, and gentamicin (25 µg/ml; Schering Corporation, Kenilworth, N. J.). The source of minimal essential medium, nonessential amino acids, glutamine, and calf serum was Grand Island Biological Co., Grand Island, N. Y. Cells were incubated at 37°C under a 5% CO2:95% air, high-humidity atmosphere in roller bottles that were subcultured weekly. Other human breast cancer cell lines were supplied by the Mason Research Institute (Rockville, Md.) as log-phase cultures growing in plastic 150-sq cm T-flasks. The growth medium was Leibovitz's L-15, supplemented with 10% fetal bovine serum, insulin (10 µg/ml), glutathione (16 µg/ml), 1% glutamine, 1% penicillin, and 1% streptomycin. Cells were changed to medium containing stripped serum 3 days before subculturing.

Cell Proliferation. Cell proliferation was followed by measuring the DNA content per flask of cells at various times during treatment. In all experiments vehicle was added to controls. Cells were removed by 1 mm EDTA-Hanks' balanced salt solution and then centrifuged at 1000 x g for 5 min. The cell pellets were frozen (-20°) until the experiment was completed. All pellets were then thawed and hydrolyzed with 0.56 N perchloric acid for 15 min at 90°C. DNA content was then determined by the diphenylamine method of Burton (2).

Stripped Calf Serum Preparation. Dextran-coated charcoal (0.25% Norit A-0.0025% dextran in 0.1 M Tris-HCl, pH 8, at 4°C) was sedimented at 1000 x g for 10 min. Calf serum (1 volume serum and 2 volumes dextran-coated charcoal) was added to the charcoal pellet, gently vortexed, and then incubated at 45° for 30 min in a shaking water bath. The resulting stripped calf serum was separated from the charcoal by centrifugation at 17,000 x g for 10 min. The supernatant was filtered through a Nalgene filter unit (0.2 µm) and frozen until use.

Assay for Thyroid Hormone Receptor. Thyroid hormone

1 This research was supported in part by the National Cancer Institute (Grants CA-13738, CA23862, and CA 09042), American Cancer Society Grant BC 23, and the Robert A. Welch Foundation.

2 To whom requests for reprints should be addressed.

Received June 27, 1977; accepted July 25, 1978.
binding was assayed by comparing the radioactivity incorporated by cells incubated in culture for a specified time in experimental growth medium containing \[^{125}\text{I}]\text{T3}\) with and without competitor. Following incubation, the culture flasks were inverted and chilled on ice, the medium was aspirated, and the cells were washed twice with 5 ml cold Hanks' balanced salt solution. Radiolabeled cells were scraped from the flasks at 4° with a 1-sq cm piece of perforated cellophane in STM buffer containing 0.5% Triton X-100. Greater than 90% of the cells were scraped off flasks in STM-Triton X-100 buffer were ruptured, as indicated by their failure to exclude trypan blue stain. In fact, many nuclei were seen to be devoid of cytoplasm at this stage of preparation. Cell harvests were then vigorously dispersed for 10 sec by a vortex mixer and centrifuged at 2000 x g for 15 min. Nuclear pellets were resuspended in 1 ml STM-Triton X-100, vortexed again, and recentrifuged.

Vortexing and sedimenting the cell harvest enriched it with “clean nuclei.” A second wash with Triton X-100 yielded a final nuclear pellet that was white and contained no whole cells or cellular debris. Nuclei appeared to be completely free of cytoplasm, immediately included trypan blue, and exhibited refractile halos. The rigor of this isolation procedure caused approximately 50% of the nuclei to rupture. Apparently, chromatin was released since these nuclei aggregated.

Total nuclear \[^{125}\text{I}]\text{T3}\) was determined by directly counting nuclear pellets in a Nuclear-Chicago gamma counter (78% efficiency). Total cellular binding was calculated as the sum of radioactivity in the nuclei plus that in the Triton-buffer washes. Comparable total \[^{125}\text{I}]\text{T3}\) binding could be observed when intact cells were isolated in the absence of Triton X-100 and counted directly. Specific binding was determined as the total bound radioactivity minus nonspecific binding (i.e., radioactivity bound but not competed by a 100-fold molar excess of unlabeled hormone).

**Hormone Analogs.** \(\text{T3 (95 to 99% pure), D-3,3',5-triiodothyronine, L-thyroxine, and 3,3',5,5'-tetraiodo-L-thyroacetic acid were purchased from Sigma. Reverse T3, 3,5,3'-diiodothyronine, 3,3'-isopropyl-L-thyronine, 3,5-diiodo-L-tyrosine, and 3,5-diiodo-L-tyrosine were gifts of Dr. Eugene C. Jorgensen, University of California School of Pharmacy (San Francisco, Calif.).}^{[125]}\text{T3 (98% pure, 2% iodide) was purchased from Abbott Laboratories (Chicago, Ill.).}\)

**Chemicals.** Propylthiouracil and bovine insulin were purchased from Sigma. Ovine prolactin (NIH-P-S-12) was a gift of the NIH (Bethesda, Md.); porcine relaxin was a gift of Dr. O. D. Sherwood, University of Illinois College of Medicine (Urbana, Ill.); and indomethacin and prostaglandin F\(_2\alpha\) were a gift of Dr. M. D. Lifshitz, University of Texas Health Science Center (San Antonio, Texas).

**RESULTS**

Chart 1A is representative of each of 3 dose-response experiments revealing that MCF-7 cell proliferation was stimulated by T3 with a mean optimal concentration of approximately \(1 \times 10^{-9} \text{ M}\). Differences between treated and untreated cells usually became apparent only after Day 8. A time course of MCF-7 response to treatment with \(5 \times 10^{-10} \text{ M T3}\) is compared to untreated controls in Chart 1B. Similar stimulation by T3 was obtained with cells grown in medium not containing bovine insulin (6 ng/ml), hydrocortisone \((10^{-8} \text{ M})\), and ovine prolactin (1 \(\mu\)g/ml), or in medium containing 1% hypothyroid calf serum (data not shown). Preliminary studies with hemocytometer cell counts verified the use of DNA content as an index of cell proliferation.

Saturable binding sites for \[^{125}\text{I}]\text{T3}\) were found in MCF-7 nuclei (Chart 2A), but not in the whole cells (Chart 2B). Nuclear binding was competed by a 100-fold molar excess of unlabeled ligand, whereas whole cell (or nonnuclear) binding was perhaps even increased by the presence of the excess unlabeled T3 (data not shown).

A time course (Chart 3A) demonstrated that the competitive nuclear binding of \[^{125}\text{I}]\text{T3}\) is rapid (80% maximal within 30 min at a saturating dose of labeled hormone), reaching equilibrium by 60 min.

Chart 3B is a Scatchard analysis showing that \[^{125}\text{I}]\text{T3}\) incorporation by MCF-7 cells. Total radioactivity incorporated by nuclei (A) and by the whole cells (B) is expressed as fmol of hormone bound per 100 \(\mu\)g DNA. Cells were incubated for 2 hr in culture with various concentrations of T3 on DNA content, expressed as a percentage above the untreated control. Data were pooled; bars, S.E. from 3 experiments. There are no bars at 0.5 \(\mu\)M T3 since this concentration was used in one experiment only. B, effect of T3 (O) at \(5 \times 10^{-10} \text{ M}\) on cell growth (DNA per flask), compared with controls (O). Bars, S.E.
Thyroid Hormone Receptors in Human Breast Cancer

Table 1
Relative affinities of thyroid hormone analogs for MCF-7 nuclear binding sites

<table>
<thead>
<tr>
<th>Analog</th>
<th>Relative binding affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-diiodo-3'-isopropyl-L-thyronine</td>
<td>1.1</td>
</tr>
<tr>
<td>T3</td>
<td>1.0</td>
</tr>
<tr>
<td>D-3,3',5-triiodothyronine</td>
<td>0.14</td>
</tr>
<tr>
<td>L-3,3',5,5'-tetraiodothyronine</td>
<td>0.05</td>
</tr>
<tr>
<td>3,5,5',5'-tetraiodo-L-thyroacetic acid</td>
<td>0.04</td>
</tr>
<tr>
<td>Reverse T3</td>
<td>0.002</td>
</tr>
<tr>
<td>3-iodo-L-tyrosine</td>
<td>0.0</td>
</tr>
<tr>
<td>3,5-Diiodo-L-tyrosine</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 2
Nuclear binding sites for T3 in several human breast cancer cell lines

Cells were washed in serum-free medium, and equal numbers were plated, 72 hr prior to assay, into Corning plastic 25-cm² flasks containing 5 ml Eagle's autoclavable minimal essential medium, supplemented with 1% stripped calf serum, 1% glutamine, 1% nonessential amino acids, 0.5% saturated sodium bicarbonate, and gentamicin (25 µg/ml). Cultures were incubated at 37° under a 5% CO₂-95% air, high-humidity atmosphere. This medium was renewed at 24 and 48 hr. At 72 hr 50 µl 115 nM [¹²⁵I]T3 with or without competitor in Hanks' balanced salt solution were added to each flask. Group A was exposed to radiolabeled ligand for 1.5 hr, in the presence of 200-fold molar excess T3. Group B was assayed after 6 hr, and binding was competed by a 100-fold molar excess of T3. Nuclear bound [¹²⁵I]T3 was determined directly by counting Triton X-100-purified nuclear pellets in a gamma counter. Specific binding was determined as the total bound radioactivity minus that which was not competed for by excess unlabeled ligand. The data represent single-dose competition assays.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell line</th>
<th>Specific binding (fmol/100 µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MCF-7</td>
<td>20 ± 2 a</td>
</tr>
<tr>
<td></td>
<td>MDA-MB 134</td>
<td>48 ± 5</td>
</tr>
<tr>
<td></td>
<td>MDA-MB 175</td>
<td>16 ± 1</td>
</tr>
<tr>
<td></td>
<td>SW 613</td>
<td>3 ± 1</td>
</tr>
<tr>
<td></td>
<td>T47 D</td>
<td>28 ± 7</td>
</tr>
<tr>
<td>B</td>
<td>MCF-7</td>
<td>20 ± 1</td>
</tr>
<tr>
<td></td>
<td>MDA-MB 231</td>
<td>43 ± 7</td>
</tr>
<tr>
<td></td>
<td>MDA-MB 361</td>
<td>27 ± 2</td>
</tr>
<tr>
<td></td>
<td>BT-20</td>
<td>35 ± 1</td>
</tr>
</tbody>
</table>

a Mean ± S.E. of triplicate determinations.

were calculated from these data. Other hormones or growth factors tested below in 9 separate experiments did not compete for [¹²⁵I]T3 nuclear binding. In every experiment each treatment was run in duplicate or triplicate; values for replicate flasks varied by less than 10%.

Pretreatment of MCF-7 cells with T3 (10⁻¹⁰ to 10⁻⁶ M) for 24 hr did not alter the number of nuclear receptor sites for thyroid hormone. Incubation of cells with radiolabeled ligand (1.1 × 10⁻⁸ M) from 0 to 30 hr also did not appreciably alter receptor binding affinity or number. Other treatments that did not alter receptor levels included: propylthiouracil (10⁻⁵ to 10⁻¹¹ M) for 2 days; bovine insulin (0 to 1 µg/ml),

...
porcine relaxin (0.05 to 2.0 μg/ml), or ovine prolactin (5 μg/ml) for 6 days; 17β-estradiol (10−7 to 10−11 M) from 1 to 14 days; and prostaglandin F2α (10−6 to 10−11 M) or indomethacin (10−8 to 10−11 M) for 9 days. In several experiments we also did not find any apparent fluctuations in thyroid hormone receptor levels throughout the log phase of cell growth or at confluence.

Several other human breast cancer cell lines were found to have compatible, nuclear binding sites for thyroid hormone (Table 2). Whole-cell binding of [125I]T3 was not compatible in any of these cell lines under identical conditions.

DISCUSSION

The normal development of the mammary gland epithelial cell is not known to be dependent upon thyroid hormones, but it certainly is influenced by them. Recent studies by Vonderhaar (29, 30) have shown that thyroid hormones stimulate at least a 3-fold increase in the synthesis of a differentiated product, α-lactalbumin, in murine mammary gland explants in culture. Thyroid hormones have also been shown (25) to be required for the development of certain estrogen-dependent rat tumors. Many human breast tumors have also been found to be estrogen dependent, so that the observation that thyroid hormone stimulates MCF-7 cell proliferation may be valuable.

The Kd for the nuclear T3 binding sites and the competition data correlate well with known characteristics of the nuclear bound or solubilized thyroid hormone receptor from rat liver (9, 10). MCF-7 nuclear receptor sites are not totally specific for T3 since many structural analogs compete effectively for binding. The relative affinities of these analogs in MCF-7 parallel those seen for other T3 receptors (7), reflecting a stereospecificity of binding that favors an unsubstituted 5′-position, may require both rings, and appears to be tightest at the phenolic hydroxyl end of the molecule. In other systems (14, 18) the relative binding affinities of these analogs parallel their biological potencies, supporting the biological relevance of the binding. Although the biological effectiveness of these other compounds has not been compared in MCF-7, the optimal concentration of T3 itself required for receptor saturation. In sum, these data are consistent with significance of the MCF-7 nuclear T3-bind-
ing sites as receptors in thyroid hormone intracellular action.

The failure of T3 or other hormones to affect the level of T3 receptor in MCF-7 cells does not parallel the action of thyroid hormones in the highly responsive rat pituitary tumor cell line GHI, where receptor depletion precedes the biological response (21). However, the number of MCF-7 nuclear T3 receptors is considerably lower than in GHI, cells which contain about 250 fmol/100 μg DNA (20). Indeed, the number of nuclear binding sites is low for all the human breast cancer cell lines assayed.

To our knowledge, receptor sites for thyroid hormone have not previously been reported for any breast-derived cells, malignant or otherwise. Their presence in a human breast cancer cell line is especially important since it offers one means by which thyroid hormone may influence breast cancer growth.

ACKNOWLEDGMENTS

We thank Dr. H. H. Samuels for helpful advice on the assay of thyroid hormone receptors, Dr. E. M. Jensen for donating the human breast cancer cell lines, Dr. E. C. Jorgensen for donating thyroid hormone analogs, and Dr. M. D. Lifshitz for donating indomethacin and prostaglandin F2α. We thank R. L. Dienglewicz for his expert technical assistance with growth studies.

REFERENCES


Nuclear Thyroid Hormone Receptors in a Human Breast Cancer Cell Line

Robert E. Burke and William L. McGuire


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/11_Part_1/3769

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.