Nuclear Thyroid Hormone Receptors in Rabbit Heart: Reduced Triiodothyronine Binding in Atrium Compared With Ventricle

Surath K. Banerjee, John M. Ulrich, and George J. Kaldor

Radiolabeled triiodothyronine (T3) binding to isolated nuclei was measured to compare the binding characteristics of the nuclear receptors in rabbit ventricular and atrial muscle cells. Scatchard analysis of the binding data yielded a maximum binding capacity of 170 ± 20 fmol per mg DNA and apparent dissociation constant of 525 ± 100 pM for ventricular nuclei. The binding capacity and the dissociation constant for the atrial muscle cell nuclei were 55 ± 10 fmol per mg DNA and 500 ± 75 pM, respectively. The results suggest that the binding capacity for T3 receptor in the atrium is considerably lower than that found in the ventricle. The reduced binding capacity of the T3 receptor in the atrium might reflect differences in the nuclear T3 receptors between ventricle and atrium. (Circulation Research 1988;63:267-271)

Thyroid hormone action in regulating cellular biological processes is generally believed to be mediated by its interaction with specific nuclear receptors. This has led many investigators to characterize triiodothyronine (T3) binding in isolated nuclei or nuclear extracts from various tissues responsive to thyroid hormone.

In recent years, thyroid hormone has been demonstrated to regulate expressions of myosin heavy chain genes. There is evidence that these genes respond differently in different tissues. For example, myosin responding to thyroid hormone in the ventricle is insensitive to the hormone in the atrium. It is clear that the properties of the nuclear T3 receptors may play a role in these processes.

A large body of literature has been accumulated on the binding characteristics of nuclear T3 receptors from liver tissue. However, relatively few studies have been reported on these receptors from myocardium. Two reports have utilized preparations from total heart that contained both myocardial and nonmyocardial nuclei. In the present study, we compared binding characteristics of T3 with nuclei prepared from ventricular and atrial muscle cell. The results show that the binding capacity for T3 receptor in the atrium is considerably lower than that found in the ventricle.

Materials and Methods

Atrial and ventricular muscle nuclei from rabbits were prepared by a combination of the procedures of Cutilletta et al and Liew et al. One hour prior to being killed, each rabbit was injected intraperitoneally with heparin (200 units/100 g body wt). Rabbits were anesthetized with Nembutal (0.06 ml/100 g body wt, 65 mg/ml). Following midline sternectomy, hearts were quickly removed and placed in cold Joklik medium for a few beats. The heart was then attached with surgical thread to a modified Langendorff perfusion apparatus. Aerated Joklik medium was allowed to perfuse through the heart (37°C, 10 ml/min) for 2 minutes before an enzyme solution containing 0.1 g collagenase, 2.0 g bovine serum albumin, and 50 μM CaCl2 in 100 ml Joklik medium was added. The perfusion time was between 45 and 60 minutes, depending on the heart size. At the end of perfusion, the heart was removed from the apparatus, the large vessels were removed, and the atria and ventricles were separated. The ventricles and atria were minced with scissors, placed in separate vacuum flasks with 1:1 dilution of the above enzyme solution and shaken in a water bath (37°C) while being aerated with oxygen for 10 minutes. Cold Joklik-50 (Joklik containing 50 μM Ca2+) was added to each suspension of cells. The suspension was then transferred to 50-ml centrifuge tubes and allowed to settle in ice for 15 minutes. The supernatant was drawn off and discarded. Cold Joklik-50 was added to the pellet, the cells were
suspended with the use of a disposable plastic pipet, and the suspensions were centrifuged at 50g for 5 minutes. The resulting pellets were resuspended in fresh, cold Joklik-50 and filtered through nylon mesh of 150-μm pore size. Cell number and viability were determined by the criterion of trypan blue exclusion under a microscope using a hemocytometer. The procedure yielded about 15±3x10⁶ ventricular cells per rabbit heart and over 80% of the cells excluded trypan blue.

To prepare nuclei, the cells were then collected by centrifugation and resuspended in cold isolation buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 1 mM MgCl₂, 0.1 mM phenylmethylsulfonyl fluoride [PMSF]), layered over a 2.3 M/2.7 M discontinuous sucrose density gradient mixture was incubated at 22°C for 2 hours. DNA concentration varied from 200 to 2,000 pM). This reaction mixture was incubated at 22°C for 2 hours. Following incubation, the reaction mixtures were cooled in an ice bath and then centrifuged at 1,000g for 5 minutes. From each sample, a 0.2 ml aliquot of suspension was then centrifuged at 800g for 10 minutes, and the resulting pellet was resuspended in buffer A (0.25 M sucrose, 1 mM MgCl₂, 0.1 mM PMSF) and sonicated for 10 seconds at setting 50 with a Fisher Sonic Dismembrator. This suspension was then centrifuged at 800g for 10 minutes, and the resulting pellet was resuspended in buffer A (0.25 M sucrose, 1 mM MgCl₂, 0.1 mM PMSF) and filtered through two layers of nylon mesh with pore sizes of 75 and 45 μm. Final suspensions were centrifuged at 800g for 10 minutes. The resulting pellets were washed once with 0.5% Triton X-100 in buffer A and once with fresh buffer A. The nuclear pellets were then resuspended in 2.2 M sucrose in buffer (10 mM Tris-HCl, 1 mM MgCl₂, 0.1 mM PMSF), layered over a 2.3 M/2.7 M discontinuous sucrose density gradient and centrifuged in a Beckman SW 25.1 rotor (Fullerton, California) at 90,000g for 90 minutes. The purified nuclei band at the 2.3 M/2.7 M interface was used in the T₃-binding studies. In a few preliminary experiments, the crude nuclei were also prepared using nonenzymatic procedures of Liew et al. Briefly, the ventricular and atrial tissues were homogenized in buffer A by "polytron," and the crude nuclei were collected by low-speed centrifugation. The crude nuclei were again homogenized in 10 volumes of fresh buffer A using a Teflon homogenizer, were filtered through two layers of nylon mesh (pore size 75 μm and 45 μm), and then pellets were collected by centrifugation. The resulting nuclear pellet was treated with 0.5% Triton X-100 and purified by discontinuous sucrose density gradient described above.

Nuclei from rat liver were prepared essentially as described by Spindler et al. Radiolabeled L-T₃ (1,200 μCi/μg; New England Nuclear, Boston, Massachusetts) binding was measured at room temperature according to Spindler et al. To 0.5 ml of nuclear suspension in buffer B (20 mM Tris-HCl, 0.25 M sucrose, 1 mM MgCl₂, 0.1 mM di-thiothreitol, 5% glycerol, pH 7.6) containing approximately 100 μg DNA, 0.4 ml of reaction buffer (20 mM Tris, 0.25 sucrose, 1.0 mM MgCl₂, 4.0 mM EDTA, 0.5 mM di-thiothreitol, 0.1 M NaCl, 5% glycerol) was added. A parallel tube containing a final concentration of 5 μM cold T₃ was also prepared. Then, 100 μl of reaction buffer containing [¹²⁵I]T₃ was added to the mixture (final concentration varied from 200 to 2,000 pM). This reaction mixture was incubated at 22°C for 2 hours. Following incubation, the reaction mixtures were cooled in an ice bath and then centrifuged at 1,000g for 5 minutes. From each sample, a 0.2 ml aliquot of supernatant was removed and assayed for radioactivity in a gamma counter to determine free [¹²⁵I]T₃ concentration. The remaining supernatant was then decanted and the pellets assayed for radioactivity. The nuclear pellets were washed and subsequently counted three times, using 0.5% Triton X-100 in buffer B for the first wash and fresh buffer B for the second and third washes. Wash buffers were ice-cold. The differences in radioactive counts per minute were obtained by subtracting counts of incubation containing excess cold T₃ from the counts of incubation without the competitor. These values were plotted against the number of washes and extrapolated to zero wash to give values for specific binding.

Competition assays to assess analogue specificity were carried out by incubation of nuclei with 2 nM [¹²⁵I]T₃ and varying concentrations of unlabeled

<table>
<thead>
<tr>
<th>Source</th>
<th>Bₘₐₓ (fmol/mg DNA)</th>
<th>Kᵣ (pM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat heart nuclei</td>
<td>600</td>
<td>...</td>
<td>5</td>
</tr>
<tr>
<td>Rat heart nuclear extract</td>
<td>84</td>
<td>420</td>
<td>14</td>
</tr>
<tr>
<td>Rabbit ventricle nuclei</td>
<td>170</td>
<td>525</td>
<td>This study</td>
</tr>
<tr>
<td>Rabbit atrium nuclei</td>
<td>55</td>
<td>500</td>
<td>This study</td>
</tr>
<tr>
<td>Rat liver nuclei</td>
<td>500-900</td>
<td>190-200</td>
<td>5,7,8</td>
</tr>
<tr>
<td>Rat liver nuclei</td>
<td>500</td>
<td>300</td>
<td>This study</td>
</tr>
</tbody>
</table>

Bₘₐₓ, maximal binding capacity; Kᵣ, apparent dissociation constant.
Banerjee et al  
Rabbit Heart Thyroid Hormone Receptors  

![Figure 2.](image1)

**Figure 2.** $[^{125}I]T_3$ binding in atrial nuclei as a function of varying $T_3$ concentration. Details of the experiments are described in "Materials and Methods." DNA concentration was 100 μg/ml.

compounds (100 pM to 5 μM) for 2 hours at room temperature.

DNA concentration was measured by the method of Burton\(^17\) and protein concentration was measured using Coomassie blue dye (Bio-Rad, Richmond, California) binding reagent and bovine serum albumin as standard.

**Results**

Initially, experiments were carried out with ventricular nuclei prepared by either enzymatic or nonenzymatic procedures to determine equilibrium conditions, linearity versus DNA concentration and pH conditions for the specific binding at room temperature (22 ± 2° C). There were no significant differences in the values for the two different preparations. However, the nuclei prepared by heart perfusion and discontinuous sucrose density gradient purification gave a better yield and were thus used routinely for most of our studies.

Because $T_3$ binding to rat liver nuclei has been described by several workers, we first investigated the binding of $T_3$ using liver nuclear preparation under similar conditions described above. Table 1 compares the data obtained in this study with those obtained by several other workers, and it is seen that our results are in good agreement with those previously reported.

Figures 1–3 describe detailed analysis of $T_3$ binding to rabbit atrial and ventricular muscle cell nuclei. The binding data followed Michalis-Menten saturation profile with a single class of high affinity binding site (Figure 3). Figure 4 shows Scatchard\(^18\) plots of the binding, which include data shown in Figure 3 as well as results from other experiments. Binding parameters $B_{\text{max}}$ (the maximal binding capacity) and $K_d$ (the apparent dissociation constant) were 170 ± 20 fmol/mg DNA and 525 ± 100 pM, respectively, in the ventricle. $B_{\text{max}}$ and $K_d$ in the

![Figure 3.](image2)

**Figure 3.** Equilibrium binding data for $T_3$ binding to atrial (○) and ventricular (○) muscle nuclei from rabbit heart. DNA concentration was 100 μg/ml. Experimental details are given in "Materials and Methods."

![Figure 4.](image3)

**Figure 4.** Scatchard representation of the binding data. $B$, mol×10^2 specifically bound $T_3$/mg DNA; $F$, molar concentration×10^3 free hormone.
atrium were 55 ± 10 fmol/mg DNA and 500 ± 75 pM, respectively.

The $B_{\text{max}}$ for ventricular nuclei reported here is considerably lower than that found for rat heart nuclei. $B_{\text{max}}$ for ventricular nuclei is considerably lower than that found in rat liver and is in agreement with the findings by others. The ratio of binding capacity of heart nuclei to liver nuclei is about 0.34 compared with a value of 0.65 reported previously. The results differ considerably from the report of Ladenson et al., who used nuclear extract of rat heart and described multiple classes of binding sites with high and low affinity and a much lower binding capacity of 84 fmol/mg DNA. These authors, however, recognized that nuclear extract may have been contaminated with cytosolic elements giving low affinity $T_3$ binding. The $K_d$ value of 525 pM is comparable with that found by others.

Table 1 shows that the binding affinity for $T_3$ to atrial muscle nuclei is comparable to that found for ventricular nuclei. However, $B_{\text{max}}$ is about one third that found for the ventricle, suggesting that nuclear binding capacity is considerably less in the atrium than in the ventricle.

Competitive binding assays (Figure 5) using unlabeled $T_3$, thyroxine, reverse $T_3$, and diiodothyronine show the normal characteristics of the thyroid hormone receptor described by others.

**Discussion**

To our knowledge, this study represents the first characterization of the binding reaction of $T_3$ by rabbit heart nuclear receptors. The results indicate that the binding capacity of the myocardial nuclear thyroid hormone receptor from rabbit heart differs, at least qualitatively, from the values reported for rat heart and liver $T_3$ receptor(s). The binding affinities, on the other hand, are similar irrespective of the animal species or tissue differences.

The present data also demonstrate that the relative $T_3$ binding capacity in the atrium is about one third of that found in the ventricle, indicating that the binding sites for $T_3$ vary between tissues from different regions of the heart. Since these measurements were carried out in nuclei obtained from ventricular and atrial muscle cells, the differences in the maximum binding capacity may be related to the differences in the receptors' properties in these specific cell types.

Although the molecular mechanisms for the different effects of thyroid hormone in mediation of a particular protein expression in different tissues remain to be clarified, the reduced binding capacity of the nuclear receptors in the atrium compared with the ventricle may account in part for the differential response of myosin expression in these tissues by thyroid hormone.

It is not clear whether the differences in the binding capacities of nuclear receptors between ventricles and atria are due to the presence of different $T_3$ binding proteins. Using the techniques of photoaffinity labeling with underivatized thyroid hormones and competition binding assay, Dozin et al. have shown that there are two nuclear thyroid hormone receptor proteins in the rat liver of molecular masses 56 and 45 kDa, respectively. In addition, several recent studies have shown evidence for the existence of isoforms of thyroid hormone receptor proteins.

To date, there is little information regarding heart $T_3$ receptor proteins. The molecular mass of the rat heart nuclear binding protein has been estimated to be between 50 and 55 kDa. It is not known whether the heart protein is homologous to the 56 kDa rat liver protein. Moreover, there is evidence that multiple $T_3$ binding proteins with varying molecular sizes and hormone binding activities exist in different subcellular compartments. Thus, future studies must characterize each of these individual proteins from various cell types in order to understand the contribution these proteins play in the overall biochemical response of thyroid hormone.

**References**

1. Oppenheimer JH: Thyroid hormone action at the cellular level. Science 1979;203:971-979
4. Samuels HH, Tsai JS: Thyroid hormone action in cell cultures: Demonstration of nuclear receptors in intact cells and isolated nuclei. Proc Natl Acad Sci USA 1973; 70:3488-3492


10. Izumo S, Nadal-Ginard B, Mahdavi V: All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. *Science* 1986;231:597-600


16. Liew CC, Jackowski G, Ma T, Sole MJ: Nonenzymatic separation of myocardial cell nuclei from whole heart tissue. *Am J Physiol* 1983;244:C3-C10


19. Dozin B, Cahnmann HJ, Nikodem V: Comparative characterization of thyroid hormone receptors and binding proteins in rat liver nuclei, plasma membrane and cytosol by photoaffinity labeling with 1-thyroxine. *Biochemistry* 1985; 24:5203-5208


**KEY WORDS** • receptor binding • atrium • ventricle • rabbit • triiodothyronine • nuclear receptor
Nuclear thyroid hormone receptors in rabbit heart: reduced triiodothyronine binding in atrium compared with ventricle.

S K Banerjee, J M Ulrich and G J Kaldor

doi: 10.1161/01.RES.63.1.267

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/63/1/267