Influence of Thyroid Status on Intracellular Distribution of Cardiac Adrenoceptors

Catherine Limas and Constantinos J. Limas

Previous studies have suggested that thyroid hormones influence the number of membrane-bound cardiac adrenoceptors, but their effect on the intracellular distribution of adrenoceptors has not been examined. A plasma cell membrane and a vesicular fraction devoid of membrane markers were prepared from hearts of euthyroid and hyperthyroid rats and were used to compare β- and α-adrenoceptors. During daily injection of l-thyroxine, cardiac hypertrophy developed within 4 days and remained unchanged thereafter. The number of membrane-bound β-receptors increased progressively and plateaued within 2 weeks of thyroxine administration. Vesicular β-receptors, on the other hand, increased more gradually and to a lesser extent so that after 2 weeks of l-thyroxine injection, they constituted a smaller proportion of the total β-receptor population compared to normal rats. In contrast, the number of cardiac α,-adrenoceptors declined rapidly to about 80% of that in euthyroid animals and did not change further for the duration of the study. Membrane-bound and vesicular α,-adrenoceptors were affected to the same extent in hyperthyroidism. During regression of cardiac hypertrophy following cessation of thyroxine administration, α,-adrenoceptors rose rapidly (within 2 days) to normal values while β-receptors declined more gradually to normal levels within 2 weeks. In hypothyroid rats, there was a significant decline in the density of both α,- and β-adrenoceptors, with a shift away from the vesicular fraction. These results indicate that both the total numbers of cardiac adrenoceptors and their distribution between the plasma membrane and vesicular fraction are influenced by the thyroid status. (Circulation Research 1987;61:824–828)

I

It has been long known that cardiac hypertrophy induced by thyroid hormone administration, in contrast to other hypertrophy models, is associated with an increase in the number of membrane-bound β-adrenoceptors and enhanced β-agonist effects.1-3 Conversely, hypothyroidism results in a decline in both cardiac β-adrenoceptor numbers and β-agonist mediated inotropism.4-8 Changes in cardiac α,-adrenoceptor numbers under the influence of altered thyroid status are less firmly established; most investigators agree that hyperthyroidism is associated with a decline in cardiac α,-receptors while both an increase9-11 and a decrease12,13 have been described in hypothyroidism.

Despite the intense interest in the modulation of cardiac β-adrenoceptors by thyroid hormones, several issues remain unresolved. For example, the temporal relation of the adrenoceptor changes to the development and regression of cardiac hypertrophy has not been delineated. In addition, it has not been examined whether or not receptor shifts from intracellular pools contribute to the increase in membrane-bound β-adrenoceptor numbers following thyroid hormone administration. The existence of a pool of preformed intracellular receptors previously established for transferrin,16 acetylcholine,11 and a-sialoglycoprotein12 has recently been extended to the β-adrenergic receptors.13,14 Furthermore, changes in the relative size of the two pools have been described under the influence of short-term exposure to isoproterenol,15 ischemia,16 and cardiac hypertrophy in the spontaneously hypertensive rat.17 In the present study, we report that the time course of receptor change during the establishment and repression of thyroxine-induced cardiac hypertrophy is different for β- and α,-adrenoceptors. Furthermore, the relative distribution between the membrane and intracellular fraction is influenced by the thyroid status.

Materials and Methods

Hyperthyroidism was induced in 5–6-month-old male Sprague-Dawley rats weighing 350–400 g by daily injections of l-thyroxine (0.5 mg/kg i.p.) for 2–14 days. In studies involving regression of cardiac hypertrophy, rats were injected daily for 14 days; thyroxine administration was then discontinued, and the animals were killed 2, 4, 6, 9, 11, or 14 days later. For induction of hypothyroidism, methimazole 0.025% was added to the drinking water for 4 weeks.

Preparation of Membrane and Vesicular Fractions

Preparation of membrane and vesicular fractions followed previously described techniques14,15 with slight modifications. Briefly, hearts from the experimental groups (euthyroid, hyperthyroid, and hypothyroid rats) were minced in 10 volumes of cold 50 mM Tris-HCl-5 mM EDTA (pH 7.5) solution and homogenized with a Polytron PT-20 homogenizer at half-maximal speed for 20 seconds. The homogenates were centrifuged at 500g for 10 minutes, and the supernatants were passed through gauze prior to centrifuging at 40,000g for 15 minutes. The resultant pellet was

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washed twice and was used as the membrane fraction. The supernatant was centrifuged at 150,000g for 1 hour, and the resultant pellet was used as the vesicular fraction. The vesicular fraction is devoid of plasma membrane markers such as Na⁺, K⁺-ATPase, and 5'-nucleotidase and contains sequestered receptors uncoupled from adenylate cyclase and not subject to GTP regulation. Receptors in the vesicular fraction are inaccessible to hydrophilic ligands and dicyclohexylcarbodiimide, which inactivates surface-bound receptors. These properties suggest that receptors recovered in the vesicular fraction are intracellular, although the precise location in the intact cell has not been conclusively identified.

Assay of β- and α₁-Adrenoceptors

For determination of β-adrenoceptors in the membrane and vesicular fractions, the assay medium contained 50 mM Tris-HCl-5 mM MgCl₂ (pH 7.5), 1–20 nM [³H]dihydroalprenolol (DHA) (specific activity 105 Ci/mol, New England Nuclear Co., Boston, Mass.), and 0.1–0.2 mg protein (determined by the method of Lowry et al) in a total volume of 0.5 ml. Incubations were carried out at 37° C for 15 minutes and terminated by filtering through Whatman GF/C filters, washing 3 times with 5 ml cold Tris-Mg buffer, and drying the filters before counting. Nonspecific binding was determined in the presence of 1 μM propranolol.

α₁-Adrenergic receptors were determined using subcellular fractions. Incubations were carried out at 37° C for 15 minutes in a medium containing 50 mM Tris-HCl-5 mM MgCl₂ (pH 7.5), 0.025–10 nM [³H]prazosin (specific activity 24.4 Ci/mmol, Amersham/Searle, Arlington Heights, Ill.), and 0.1–0.3 mg membrane protein in a total volume of 0.5 ml. At the end of the incubation time, 2 ml cold buffer were added to each tube and the contents passed through Whatman GF/C filters. The filters were washed 3 times with 5 ml cold Tris-Mg buffer, and dried and transferred to scintillation vials for counting. Nonspecific binding was determined in the presence of 10⁻⁵ M phenolamine and averaged 5% of the total. Maximal numbers of receptors and affinities were calculated from Scatchard plots of the binding data.

Results

Administration of thyroxine resulted in a rapid cardiac hypertrophy as evidenced by increased heart-weight-to-body-weight ratios (Figure 1). Heart weights were significantly higher in hyperthyroid animals (1.18 ± 0.12 g versus 0.92 ± 0.07 g in controls, p<0.01) while body weights were lower (324 ± 31 g versus 369 ± 40 g, p<0.05).

Cardiac adrenoceptors were compared in the membrane and vesicular fractions of euthyroid and hyperthyroid rats. [³H]Dihydroalprenolol was used to identify cardiac β-adrenoceptors. As shown in Table 1, the affinity of the β-receptor for dihydroalprenolol is similar in all preparations. For both control and hyperthyroid animals, however, the density of β-adrenoceptors is higher in the vesicular than in the membrane fraction. This is compatible with the proposed function of the vesicular fraction as a site of sequestered receptors. In agreement with previous studies, the density of membrane-bound β-adrenoceptors is higher in hyperthyroid animals; in addition, there is a corresponding increase in the vesicular fraction of hyperthyroid rats.

We then determined the influence of hyperthyroidism on the number of β-adrenoceptors recovered per heart. After 2 weeks of thyroxine administration, there was approximately 48% increase in the total β-adrenoceptor number in hyperthyroid rats (Table 2). This was accounted for primarily by a substantial (65%) increase in membrane-bound receptors and a much smaller increase (about 22%) in the vesicular fraction. The time course of β-adrenoceptor changes during the evolution of thyroxine-induced cardiac hypertrophy is shown in Figure 2. There is a steep rise in β-adrenoceptors recovered from the membrane fraction, which plateaus at about 11 days, while the rise in the vesicular fraction is much more gradual and of smaller magnitude. As a result, the proportion of β-adrenoceptors in the vesicular fraction declines from the

| Table 1. Density and Kᵦ of β- and α₁-Adrenoceptors in the Membrane and Vesicular Fractions From Hearts of Euthyroid and Hyperthyroid Rats |
|---|---|---|---|
| | Bmax (fmol/mg prot) | Kᵦ (nM) |
| | β-Adrenoceptors | α₁-Adrenoceptors | β-Adrenoceptors | α₁-Adrenoceptors |
| Euthyroid | | | | |
| Membrane | 47 ± 0.04 | 51 ± 0.04 | 2.35 ± 0.02 | 0.23 ± 0.03 |
| Vesicular | 68 ± 0.06 | 63 ± 0.04 | 2.51 ± 0.03 | 0.25 ± 0.04 |
| Hyperthyroid | | | | |
| Membrane | 61 ± 0.05* | 34 ± 0.03* | 2.40 ± 0.03 | 0.19 ± 0.04 |
| Vesicular | 89 ± 0.07* | 53 ± 0.04* | 2.48 ± 0.04 | 0.22 ± 0.03 |

Data calculated from Scatchard plots of the [³H]prazosin binding data represent mean ± SEM for 6 comparisons. *p<0.01 compared with the respective fraction from euthyroid animals.
control of 40% to 35% after 2 weeks of thyroxine administration.

Changes in the distribution of cardiac \( \alpha_1 \)-adrenoceptors in response to thyroxine were also determined. As shown in Table 1, the density of membrane-bound \( \alpha_1 \)-adrenoceptors was significantly lower in hyperthyroid rats. Although in both experimental groups the density of the \( \alpha_1 \)-receptors was higher in the vesicular compared with the membrane fractions, a decline in receptor numbers was also noted in the vesicular fraction of hyperthyroid compared with euthyroid rats. Receptor affinities were similar in both experimental groups.

The recovery of \( \alpha_1 \)-adrenoceptors in the membrane fraction per heart was again lower in the hyperthyroid animals (Table 2), although the extent of the decline in the density of receptors was partly compensated by an increase in membrane protein per heart. In addition, there was a significant decline in the total number of \( \alpha_1 \)-adrenoceptors recovered in the vesicular fraction.

The time course of the changes in the distribution of \( \alpha_1 \)-adrenoceptors as a function of length of thyroxine administration was then studied (Figure 3). In contrast to the pattern seen in the \( \beta \)-adrenoceptors, \( \alpha_1 \)-receptors declined rapidly (within 2 days) and remained essentially unchanged thereafter. Also, the time course was similar for the membrane and vesicular fractions so that \( \alpha_1 \)-adrenoceptors in the vesicular fraction represented the same percent of the total in both experimental groups (43% in controls, 41% in hyperthyroid rats).

The time course of adrenoceptors' recovery following cessation of thyroxine administration was then studied. Rats were injected with thyroxine daily for 2 weeks, and then hormone administration was stopped. Animals were killed 2, 4, 7, 9, 11, or 14 days later, and the time course of hypertrophy regression and changes in total adrenoceptor recovery and redistribution between the membrane and vesicular fractions were followed. As shown in Figure 4, the time course of \( \beta \)-adrenoceptor reversal was different from that of \( \alpha_1 \)-adrenoceptor. While the decline in \( \beta \)-adrenoceptors toward the control levels followed a linear course parallel to that of the regression of cardiac hypertrophy, \( \alpha_1 \)-adrenoceptors recovered within 4 days (when significant hypertrophy was still present) and did not change thereafter. The distribution of adrenoceptors followed a pattern similar to that of the total receptor numbers (data not shown).

The influence of hypothyroidism on the distribution of cardiac adrenoceptors, induced by adding methimazole to the drinking water for 4 weeks, was then examined. Cardiac \( \beta \)-adrenoceptors recovered in both the membrane and vesicular fractions were decreased in hypothyroid animals (Table 3). The proportion of \( \beta \)-receptors in the vesicular fraction was significantly lower (28.5 ± 0.3%) in the hypothyroid compared with euthyroid (37 ± 0.4%) rats. Similar changes were found for \( \alpha_1 \)-adrenoceptors: both

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Table 2. Recovery of \( \beta \)- and \( \alpha_1 \)-Adrenoceptors in the Membrane and Vesicular Fractions From Heart of Hyperthyroid and Euthyroid Animals

<table>
<thead>
<tr>
<th></th>
<th>( \beta )-Adrenoceptors (pmol/heart)</th>
<th>( \alpha_1 )-Adrenoceptors (pmol/heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>0.65 ± 0.04</td>
<td>1.01 ± 0.08</td>
</tr>
<tr>
<td>Vesicular</td>
<td>0.46 ± 0.03</td>
<td>0.78 ± 0.05</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>1.08 ± 0.07*</td>
<td>0.77 ± 0.06*</td>
</tr>
<tr>
<td>Vesicular</td>
<td>0.57 ± 0.04*</td>
<td>0.59 ± 0.04*</td>
</tr>
</tbody>
</table>

[\( ^{3} \text{H} \)]Dihydroalprenolol of [\( ^{3} \text{H} \)]prazosin binding was carried out using 0.1–0.2 mg protein from the appropriate fraction and the total number of adrenoceptors was estimated from the receptor density (fmol/mg prot from Scatchard plots) and the total amount of protein recovered in each fraction. Results represent mean ± SEM for 6 comparisons.

* \( p < 0.01 \) compared with the respective fraction from euthyroid animals.

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FIGURE 2. Time course of \( \beta \)-adrenoceptor changes in the cardiac membrane and vesicular fractions of animals injected with 1-thyroxine. [\( ^{3} \text{H} \)]Dihydroalprenol binding was determined in injected and control animals, as described in the text. Results are expressed as percent increase over euthyroid controls (0.65 ± 0.04 pmol/heart for the membrane and 0.41 ± 0.03 pmol/heart for the vesicular fraction) and represent mean ± SEM for 6 comparisons at each time point.

FIGURE 3. Time course of \( \alpha_1 \)-adrenoceptor changes in the cardiac membrane and vesicular fractions of rats injected with 1-thyroxine. Results are expressed as percent decline over control animals (1.05 ± 0.07 pmol/heart for the membrane and 0.72 ± 0.05 pmol/heart for the vesicular fraction) and represent mean ± SEM for 6 comparisons at each time point.
Adrenergic Receptors and Thyroid Status

Limas and Limas

The effect of thyroid hormones is probably not a mere reflection of enhanced protein synthesis since it shows tissue specificity, e.g., hyperthyroidism is associated with lower β-receptor numbers in hepatocytes but higher in cardiac myocytes.

For several receptors, an intracellular pool has been identified that may be available for rapid enrichment of the complement of plasma membrane-bound receptors in response to functional demands. We, as well as others, have reported that about one third of all cardiac β-adrenoceptors are located intracellularly. Short-term exposure to isoproterenol induces a shift from the plasma membrane to a vesicular fraction while ischemia induces redistribution in the opposite direction. Cardiac hypertrophy in the spontaneously hypertensive rat is associated with a decline in plasma membrane-bound β-receptors and a concomitant expansion of the vesicular pool. It was of interest, therefore, to examine the possibility that thyroid hormones also influence the distribution of cardiac β-adrenoceptors between the two intracellular pools.

The results of our study demonstrate that hyperthyroidism changes this distribution because it expands preferentially the membrane pool so that the number of β-receptors in the vesicular fraction is a smaller proportion of the total than in euthyroid animals. The total number of cardiac β-receptors is significantly higher in hyperthyroidism, and the increase is not accounted for solely on the basis of cardiac hypertrophy as reflected in increased ventricular weight. It is likely, therefore, that the synthesis of β-receptors is regulated by thyroid hormones, in part, independently of other cellular components. Although the relative distribution of cardiac β-adrenoceptors between the membrane and vesicular fractions is altered in hyperthyroidism, this is secondary to a preferential expansion of the membrane pool, perhaps through de novo receptor synthesis. It is not a priori apparent, however, why such an increase in receptor synthesis should involve predominantly the membrane receptor pool. It is likely that this apparent redistribution reflects an effect of hyperthyroidism on the rate of receptor cycling between the two pools (i.e., either decreased rate of internalization or enhanced transit from the vesicular to the membrane compartment). This possibility is currently under investigation in our laboratory.

The time course of the increase in β-receptor number is interesting in that there is a progressive rise in membrane-bound β-receptors even after the extent of hypertrophy has plateaued. In contrast, the increase in the vesicular fraction is much more gradual. Conversely, hypothyroidism is associated with a loss of β-receptors in both the membrane and vesicular fraction and a consequent decline in the total β-receptor numbers per heart.

The effect of hypothyroidism on cardiac α₁-adrenoceptors shows a different pattern from that on β-

**Discussion**

Previous studies have clearly demonstrated that the number of membrane-bound β-adrenoceptors is under the control of thyroid hormones; concordant changes in isoproterenol-stimulated adenylyl cyclase activity and β-agonist inotropism document the functional significance of the alterations in β-adrenoceptor numbers. The effect of thyroid hormones is probably not a mere reflection of enhanced protein synthesis since it shows tissue specificity, e.g., hyperthyroidism is associated with lower β-receptor numbers in hepatocytes but higher in cardiac myocytes.

**FIGURE 4.** Recovery of cardiac adrenoceptor changes following cessation of l-thyroxine administration. Rats were injected with 0.5 mg/kg l-thyroxine daily for two weeks, after which T₄ administration was stopped. Thyroxine-pretreated and control animals were killed 2–14 days later, and the number of β- and α₁-adrenoceptors in the membrane and vesicular fractions were determined as described in the text. Results are expressed as a ratio of total (membrane and vesicular) β- or α₁-adrenoceptors per heart in thyroxine-pretreated over control animals and represent mean±SEM for 6 comparisons at each time point. Total β-adrenoceptor numbers were 1.15 ± 0.08 pmol/heart for controls and 1.87 ± 0.09 pmol/heart for the thyroxine-treated animals. For α₁-adrenoceptors, the respective numbers were 1.82 ± 0.09 pmol/heart and 1.37 ± 0.05 pmol/heart. Heart-weight-to-body-weight ratios were 2.51 ± 0.01 × 10⁻³ for controls and 3.62 ± 0.02 × 10⁻³ for thyroxine-treated animals.

**Table 3.** Effect of Hypothyroidism on the Recovery of β- and α₁-Adrenoceptors in the Membrane and Vesicular Fractions

<table>
<thead>
<tr>
<th>Condition</th>
<th>β-Adrenoceptors (pmol/heart)</th>
<th>α₁-Adrenoceptors (pmol/heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>0.76 ± 0.06</td>
<td>1.21 ± 0.09</td>
</tr>
<tr>
<td>Vesicular</td>
<td>0.42 ± 0.03</td>
<td>1.00 ± 0.06</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>0.48 ± 0.03*</td>
<td>0.92 ± 0.06*</td>
</tr>
<tr>
<td>Vesicular</td>
<td>0.31 ± 0.02*</td>
<td>0.59 ± 0.04*</td>
</tr>
</tbody>
</table>

Assay conditions are in legend to Table 2. Results represent mean±SEM for 6 comparisons.

*p<0.01 compared with the respective fraction from euthyroid animals.
receptors. First, the time course is different with a rapid decline within 2–4 days and a plateau thereafter in contrast to the continued rise in β-adrenoceptors. Second, membrane-bound and vesicular α₁-adrenoceptors are affected to the same extent so that the percent of α₁-receptors located intracellularly is not changed compared to controls. It is likely, therefore, that the regulation of β- and α₁-adrenoceptors in the heart involves different mechanisms. No support can be given by our data to the suggestion by Kunos that hyperthyroidism induces an interconversion of α₁- and β-adrenoceptors.

Much less agreement exists on the influence of hypothyroidism on cardiac α₁-adrenoceptors, some reporting lower and others higher numbers. Several factors are responsible, including the use by some investigators of nonselective ligands that do not differentiate between α₁- and α₂-receptors, and the observation that variable effects of hypothyroidism on cardiac α₁-adrenoceptors is decreased in both the membrane or the vesicular fraction. However, the decline in total α₁-receptor numbers parallels the decrease in cardiac weight in hypothroid animals, which suggests that myofibrillar components are preferentially affected. It is, furthermore, evident that the enhancement in α-agonist effects reported in hypothyroid rats must be regulated at a postreceptor step.

Several models of cardiac hypertrophy and failure are associated with altered regulation of β- and, possibly, α₁-adrenoceptors, which may have important functional implications (for review, see Limas and Limas). Our results indicate that this regulation involves, in part, shifts in the relative distribution of receptors between the plasma membrane and intracellular pools. The factors controlling the size of these pools have not been characterized and are currently under investigation in our laboratory.

**References**

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**Key Words** • adrenoceptors • hypothyroidism • internalization • hypothyroidism • hyperthyroidism • hyperthyroidism • adenylyl cyclase
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