Brief Communications

Disparate Effects of Colchicine on Thyroxine-Induced Cardiac Hypertrophy and Adrenoceptor Changes

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Short-term (5 days) administration of L-thyroxine (0.1 mg/kg i.p. daily) to adult Sprague-Dawley rats induces a modest degree of cardiac hypertrophy (22% increase in heart weight/body weight ratios) and directionally opposite changes in cardiac adrenoceptors (24% increase in β-adrenoceptors and 20% decline in α₁-adrenoceptors). Pretreatment with colchicine did not affect the ability of L-thyroxine to induce cardiac hypertrophy but prevented its effects on both β- and α₁-adrenoceptors. These results suggest a selective involvement of microtubules in the action of thyroid hormones on the expression of cardiac adrenoceptor genes. (Circulation Research 1991;68:309–313)

Administration of thyroid hormones induces both cardiac hypertrophy and an increase in the number of cardiac β-adrenoceptors, which involves both the membrane-bound and the intracellular receptor pools.1 The time course of β-receptor increase during thyroxine administration follows that of cardiac hypertrophy, but its rate is proportionately higher so that the number of β-receptors per gram heart is increased in the hyperthyroid animals.1,2 This is consistent with the view1,2 that the synthesis of β-adrenergic receptors is preferentially induced by thyroid hormone. In contrast, thyroxine induces a prompt decline in the number of α₁-adrenoceptors in the heart1,2 through mechanisms that are, at present, unclear.

In addition to the rate of synthesis, the steady-state levels of membrane-bound β-receptors are influenced by the rates of receptor cycling to and from intracellular sites.4 Both aspects of receptor cycling depend on the presence of intact microtubules and, therefore, are expected to be influenced by inhibition of microtubule assembly.4,5 In the presence of β-agonists, such inhibitors exert their influence predominantly on the endocytotic phase and inhibit agonist-induced β-receptor downregulation. In the absence of β-agonists, however, their effects are less predictable; under conditions of induced enhancement of β-receptor synthesis, microtubules might be expected to influence two events: 1) the transduction of signals to the nucleus that initiate cardiac hypertrophy or β-receptor synthesis and 2) the insertion of newly synthesized β-receptors from the Golgi apparatus (where they are first glycosylated) to the plasma membrane. These alternatives were examined during short-term thyroxine administration to adult, euthyroid Sprague-Dawley rats.

Materials and Methods

Experiments were carried out on adult male Sprague-Dawley rats (Bio-Labs, St. Paul, Minn.). Group IA (n=9) consisted of euthyroid controls; group IB (n=9) was injected with L-thyroxine (0.1 mg/kp i.p. daily) for 5 days. Group IIA (n=8) was injected with colchicine (0.05 mg/kg i.p. daily) for 8 days; group IIB (n=8) was injected with colchicine (0.05 mg/kg i.p. daily) for 8 days and, starting at day 4, with L-thyroxine (0.1 mg/kg i.p. daily) for 5 days. Finally, group III (n=6) consisted of rats injected with colchicine (0.05 mg/kg i.p. daily) for 14 days.

Preparation of Membrane and Vesicular Fractions

Preparation of membrane and vesicular fractions followed previously described techniques1,6 with slight modifications. Briefly, hearts from the experimental groups (euthyroid and hyperthyroid) were minced in 10 vol cold 50 mM Tris-HCl plus 5 mM EDTA (pH 7.5) solution and homogenized with a Poltron homogenizer (model PT-20, Brinkmann Instruments, Inc., Westbury, N.Y.) at half-maximal speed for 20 seconds. The homogenates were centrifuged at 500g for 10 minutes, and the supernatant was centrifuged at 40,000g for 30 minutes at 4°C. The pellet was washed twice and 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 seques-tered receptors uncoupled from adenylate cyclase and not subject to GTP regulation.\(^6\) Comparisons of these membrane enzyme markers did not reveal any significant differences among the experimental groups (results not shown).

**Assay of \(\beta\)- and \(\alpha_2\)-Adrenoceptors**

For determination of \(\beta\)-adrenoceptors in the membrane and vesicular fractions, the assay medium contained 50 mM Tris-HCl plus 5 mM MgCl\(_2\) (pH 7.5), 1–20 nM \(^{3}H\)dihydroalprenolol (specific activity, 105 Ci/mol; New England Nuclear Co., Boston), and 0.1–0.2 mg protein (determined by the method of Lowry et al\(^7\)) in a total volume of 0.5 ml. Incubations were carried out at 37°C for 15 minutes and terminated by filtering through GF/C filters (Whatman Inc., Clifton, N.J.), washing three times with 5 ml cold Tris-magnesium buffer, and drying the filters before counting. Nonspecific binding was determined in the presence of 1 \(\mu\)M propranolol.

\(\alpha_2\)-Adrenergic receptors were determined using subcellular fractions at 37°C for 15 minutes in a medium containing 50 mM Tris-HCl plus 5 mM MgCl\(_2\) (pH 7.5), 0.025–10 nM \(^{3}H\)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 was added to each tube, and the contents were passed through Whatman GF/C filters. The filters were washed three times with 5 ml cold buffer, 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\(^8\) of the binding data.

**Statistical Analysis**

Results are presented as mean±SEM. Analysis of variance and paired or unpaired Student’s \(t\) test were used to compare the results. Differences were considered significant at \(p<0.05\).

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**Table 1. Effect of Colchicine on the Induction of Cardiac Hypertrophy by Thyroxine**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Heart weight (g)</th>
<th>Body weight (g)</th>
<th>Heart wt/body wt×10(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA ((n=9))</td>
<td>0.994±0.02</td>
<td>382±6.8</td>
<td>2.60±0.07</td>
</tr>
<tr>
<td>IB ((n=9))</td>
<td>1.126±0.03*</td>
<td>360±7.5†</td>
<td>3.17±0.08*</td>
</tr>
<tr>
<td>IIA ((n=8))</td>
<td>0.832±0.03</td>
<td>337±6.7</td>
<td>2.47±0.08</td>
</tr>
<tr>
<td>IVB ((n=8))</td>
<td>1.054±0.03*</td>
<td>328±3.2†</td>
<td>3.21±0.09*</td>
</tr>
<tr>
<td>III ((n=6))</td>
<td>1.007±0.03</td>
<td>406±8.3</td>
<td>2.47±0.08</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Group IA, euthyroid control rats; group IB, rats injected with \(L\)-thyroxine for 5 days; group IIA, rats injected with colchicine for 8 days; group IB, rats injected with colchicine for 8 days and, starting at day 4, with \(L\)-thyroxine for 5 days; group III, rats injected with colchicine for 14 days.

\(*p<0.01\) compared with respective control group.

\(†p<0.05\) compared with respective control group.

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**Results**

In agreement with previous studies,\(^1–3\) short-term administration of thyroxine results in cardiac hypertrophy, as evidenced by increased heart weight/body weight ratios and higher ventricular weights (Table 1). Rats treated with colchicine alone had somewhat smaller body weights but normal heart weight/body weight ratios. In the presence of colchicine, thyroxine was still able to induce significant cardiac hypertrophy as well as a small decrease in body weight.

The influence of hyperthyroidism on the cardiac \(\beta\)-adrenoceptors is shown in Figure 1. As expected, there was a significant (24%) increase in membrane-bound \(\beta\)-adrenoceptors in hyperthyroid compared with euthyroid rats. In colchicine-treated rats, however, thyroxine was considerably less effective, resulting in only 10% increase in \(\beta\)-receptor numbers. The affinity of the \(\beta\)-receptor for the ligand, as reflected

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**Figure 1. Bar graph showing effect of thyroxine and colchicine on the density of membrane-bound and vesicular \(\beta\)-adrenoceptors.** Group IA, euthyroid control rats; group IB, rats injected with \(L\)-thyroxine for 5 days; group IIA, rats injected with colchicine for 8 days; group IB, rats injected with colchicine for 8 days and, starting at day 4, with \(L\)-thyroxine for 5 days; group III, rats injected with colchicine for 14 days. Results represent mean±SEM for six to nine rats in each experimental group. Differences in \(\beta\)-adrenoceptor numbers are significant (\(p<0.01\)) for group IB vs. group IA and for group III vs. group IA for both membrane and vesicular fractions.
in the $K_d$ values obtained from Scatchard plots of the binding data, was unaltered by the experimental interventions (data not shown). The number of the $\beta$-receptors recovered in the vesicular pool was also increased by thyroxine (Figure 2), so that the number of vesicular $\beta$-receptors as a proportion of the total was unchanged. In the colchicine group, however, the proportion of vesicular $\beta$-receptors was higher both with and without concomitant thyroxine administration. This difference reached statistical significance only after 2 weeks of colchicine, when a substantial decline in the density of membrane-bound $\beta$-receptors is almost exactly counterbalanced by an increase in the vesicular receptors.

In contrast to the $\beta$-adrenoceptors, the number of the $\alpha_1$-adrenoceptors declined after thyroxine administration to 80% of the controls (Table 2). In colchicine-treated rats, however, this decline in $\alpha_1$-adrenoceptor numbers was totally prevented. As shown in Table 3, the total number of $\beta$-adrenoceptors was significantly increased by thyroxine, and this increase was attenuated by colchicine. Reciprocal changes were induced in cardiac $\alpha_1$-adrenoceptors. After 2 weeks of colchicine, there were only minor (about 10%) changes in the total number of cardiac adrenoceptors, in contrast to the evidence for redistribution (Figure 2).

### Discussion

Although significant progress has been made in the last few years in elucidating the biochemical and structural consequences of cardiac hypertrophy, the early events in its induction remain elusive and may differ depending on the initiating stimulus. The effects of thyroxine on the heart are thought to result from its interaction with nuclear receptors and consequent modification of specific gene transcription. The sequence and regulation of the steps leading to the nuclear localization of thyroxine and its interaction with gene control elements are not well understood. It is possible that microtubules are involved in the transduction of signals to the nucleus, which result in either repression or enhancement of specific gene transcription. Examples of repression are provided by the $\alpha_1$-adrenoceptor, $\beta$-myosin, and thyrotropin genes, whereas enhanced transcription is thought to explain the effects on $\beta$-adrenoceptors and $\alpha_1$-myosin gene expression.

The most striking aspect of the present study is the observed dissociation induced by colchicine between the effects of thyroxine on cardiac hypertrophy and on the adrenoceptor numbers. Since the percent increase in ventricular weight and heart weight/body weight ratios in response to thyroxine was unaltered by colchicine, a generalized inhibition of protein synthesis is unlikely. This contrasts with the profound inhibition of the thyroxine effects on the cardiac $\beta$- and $\alpha_1$-adrenoceptors despite their opposite direction. Since both membrane and vesicular fractions were affected by colchicine, the predominant effect

### Table 2. Effect of Colchicine on the Thyroxine-Induced Decline of Membrane-Bound $\alpha_1$-Adrenoceptors

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>$n$</th>
<th>$\alpha_1$-Adrenoceptors (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>9</td>
<td>35.4±0.2</td>
</tr>
<tr>
<td>IB</td>
<td>9</td>
<td>28.7±0.3*</td>
</tr>
<tr>
<td>IIA</td>
<td>8</td>
<td>29.9±0.3</td>
</tr>
<tr>
<td>IIB</td>
<td>8</td>
<td>34.5±0.4</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>25.6±0.4*</td>
</tr>
</tbody>
</table>

*Values are mean±SEM. Group IA, euthyroid control rats; group IB, rats injected with L-thyroxine for 5 days; group IIA, rats injected with colchicine for 8 days; group IIB, rats injected with colchicine for 8 days and, starting at day 4, with L-thyroxine for 5 days; group III, rats injected with colchicine for 14 days. *p<0.01 compared with control group.

### Table 3. Effect of Thyroxine and Colchicine on the Total Number of Cardiac $\beta$- and $\alpha_1$-Adrenoceptors

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>$\beta$-Adrenoceptors (pmol/heart)</th>
<th>$\alpha_1$-Adrenoceptors (pmol/heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>0.94±0.08</td>
<td>1.09±0.09</td>
</tr>
<tr>
<td>IIB</td>
<td>1.28±0.10*</td>
<td>0.88±0.08*</td>
</tr>
<tr>
<td>IIA</td>
<td>0.80±0.07</td>
<td>0.83±0.09</td>
</tr>
<tr>
<td>IIB</td>
<td>1.06±0.09*</td>
<td>1.02±0.10*</td>
</tr>
<tr>
<td>III</td>
<td>0.89±0.07</td>
<td>0.98±0.10</td>
</tr>
</tbody>
</table>

*Values are mean±SEM for six to nine rats in each experimental group. Group IA, euthyroid control rats; group IB, rats injected with L-thyroxine for 5 days; group IIA, rats injected with colchicine for 8 days; group IIB, rats injected with colchicine for 8 days and, starting at day 4, with L-thyroxine for 5 days; group III, rats injected with colchicine for 14 days. The total number of adrenoceptors was calculated from the receptor density (fmol/mg protein) and the total amount of membrane protein. *p<0.01 compared with respective control group.
appears to be through inhibition of β-receptor synthesis. We noted, however, that the percentage of β-receptors recovered in the vesicular fraction was somewhat higher in the colchicine-treated rats (group II), suggesting that inhibition of intracellular receptor traffic—in particular, impaired insertion of newly synthesized receptors into the plasma membrane—may also play a role. This is substantiated by the striking redistribution of the β-adrenoceptors after 2 weeks of colchicine administration (Figure 2), which suggests an intracellular receptor “traffic jam” due to, perhaps, reduced membrane incorporation. The reason that redistribution of β-adrenoceptors is more striking after 2 weeks of colchicine administration may be because, at earlier stages, the inhibitory effect of colchicine on β-receptor synthesis induced by thyroxine predominates and prevents a significant intracellular accumulation of β-receptors. This issue remains to be clarified in future experiments measuring directly the rate of β-receptor gene transcription in control and colchicine-treated rats. A similar redistribution also appears to be responsible for the apparent decline in the density of membrane-bound α1-adrenoceptors (Table 2) in rats treated with colchicine for 2 weeks (results not shown). This is supported by the fact that the total adrenoceptor numbers were not significantly altered (Table 3).

The mechanism of the inverse regulation of β- and α-adrenoceptors by thyroid hormones remains controversial. Reciprocal changes are found using either membranes1–3 or isolated cardiac myocytes13 and are most probably determined at the level of transcription of the responsible receptor genes. The functional interconversion of α- and β-receptor responses described in several tissues in response to thyroid hormones appears to be regulated at a postreceptor step (for a review, see Kunos and Ishac4).

It is interesting that the pattern of adrenoceptor redistribution after colchicine (i.e., reciprocal changes in β- and α1-adrenoceptor density and the disproportionate increase in the vesicular receptor pool) resembles that seen in the hypertrophied myocardium of spontaneously hypertensive rats.6 It is possible, therefore, that microtubule dysfunction may, in part, determine the β-adrenoceptor abnormalities in this and, perhaps, other models of cardiac hypertrophy.

Microtubule dysfunction has been shown to interfere with hormone action at several levels (for a review, see Hall15). Little information exists, however, on the potential role of microtubules on specific gene expression. For example, it has been recently shown that the expression of the casein gene by prolactin16 or α1-glycoprotein mRNA by the hepatocyte-stimulating factor17 are prevented by microtubule inhibitors, whereas the expression of a heat-shock protein in the mammalian nervous system is strongly influenced by agents affecting microtubule stability.18 A perinuclear microtubule system has been demonstrated in the cardiac myocyte and undergoes striking proliferation and rearrangement during the induction of cardiac hypertrophy.19 However, no data are yet available on the possible involvement of microtubules in cardiac gene expression. In addition, it should be stressed that we have, as yet, no direct evidence that transcription of adrenoceptor genes is altered by either thyroxine or colchicine (although this remains the primary working hypothesis). An alternative explanation would be a colchicine effect on a posttranscriptional step, for example, on the stability of mRNA.20 At least in the case of the α1-glycoprotein mRNA,17 however, the inhibitory effect seems to be exerted at the transcriptional level. Further experiments are needed to clarify this issue.

References


**KEY WORDS** • colchicine adrenergic receptors • thyroxine • cardiac hypertrophy
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