Triiodothyronine-induced Thyrotoxicosis Increases Mononuclear Leukocyte β-Adrenergic Receptor Density in Man

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ABSTRACT β-Adrenergic receptors are increased in some tissues of experimentally thyrotoxic animals but are reported to be unchanged in mononuclear leukocytes of spontaneously thyrotoxic humans. We examined the effects of triiodothyronine (100 μg/d for 7 d) and placebo on high-affinity mononuclear leukocyte β-adrenergic receptors in 24 normal human subjects, using a double-blind design. β-Adrenergic receptors were assessed by specific binding of the antagonist (−)[^{3}H]dihydroalprenolol. Triiodothyronine administration resulted in objective evidence of moderate thyrotoxicosis and an increase in mean (−)[^{3}H]-dihydroalprenolol binding from 25±3 to 57±9 fmol/mg protein ($P < 0.001$). The latter was attributable, by Scatchard analysis, to an increase in β-adrenergic receptor density (967±134 to 2250±387 sites per cell, $P < 0.01$); apparent dissociation constants did not change. Placebo administration had no effects. Marked inter- and intraindividual variation in mononuclear leukocyte β-adrenergic receptor density was also noted. Because this was approximately threefold greater than analytical variation, it is largely attributable to biologic variation. Thus, we conclude: (*a*) The finding of a triiodothyronine-induced increase in mononuclear leukocyte β-adrenergic receptor density in human mononuclear leukocytes, coupled with similar findings in tissues of experimentally thyrotoxic animals, provides support for the use of mononuclear leukocytes to assess receptor status in man. (*b*) There is considerable biologic variation in β-adrenergic receptor density in man. (*c*) The findings of thyroid hormone-induced increments in β-adrenergic receptor density provide a plausible mechanism for the putative enhanced responsiveness to endogenous catecholamines of patients with thyrotoxicosis.

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INTRODUCTION

Many manifestations of thyrotoxicosis, such as increased myocardial contractility, tachycardia, enhanced thermogenesis, diaphoresis, tremor, lid-lag, and hyperkinesis, resemble those produced by catecholamines. It is now established that sympathoadrenal catecholamine release is not increased in thyrotoxic patients; urinary excretion rates (1), static plasma concentrations (2, 3) and turnover rates (4, 5) of norepinephrine and of epinephrine are not increased. Thus, the possibility has been raised that thyroid hormone excess might result in increased responsiveness to endogenous catecholamines.

Available evidence that thyrotoxic patients exhibit generalized hypersensitivity to catecholamines is not, in our judgment, compelling. Although early studies suggested enhanced systolic pressor responsiveness to intravenous norepinephrine (6) and epinephrine (7), subsequent more extensive studies (8, 9) did not document increased cardiovascular sensitivity. More recently, however, Guttler et al. (10) reported increased heart rate-pulse pressure products and urinary cyclic AMP responses to intravenous epinephrine in patients with thyrotoxicosis; Seino et al. (11) reported that thyrotoxic patients have increased serum gastrin levels with exaggerated increments in gastrin in response to isoproterenol and decrements in gastrin in response to propranolol. Thus, the latter studies in thyrotoxic patients, coupled with the clinical similarities between catecholamine and thyroid hormone excess noted above and the animal data cited below, are consistent with the hypothesis that thyroid hormone excess causes enhanced responsiveness to catecholamines.

Although not all studies in experimentally thyrotoxic animals have demonstrated enhanced responsiveness to catecholamines in vivo (12–14) or in vitro (15),

several have demonstrated such an effect. These include studies reporting enhanced catecholamine-stimulated myocardial contractility (16, 17) protein kinase activation (16), phosphorylase *a* activation (17) and cyclic AMP accumulation (18), and enhanced catecholamine-stimulated thermogenesis (19), lipolysis in adipose tissue (20) and cyclic AMP accumulation in (avian) erythrocytes (21). Further, incubation of fetal mouse hearts with triiodothyronine in organ culture increases the chronotropic response to norepinephrine (22) and incubation of cultured myocardial cells with triiodothyronine increases the cyclic AMP response to epinephrine (23). Of particular note is the fact that several of these studies (16, 21, 22) demonstrate that thyroid hormone excess results in increased responsiveness to submaximal, but not maximal, catecholamine concentrations, i.e., that thyroid hormone excess causes a shift in the dose-response curve with an increase in tissue sensitivity to catecholamines.

Thyroid hormone-induced hypersensitivity to catecholamines could be due to alterations of adrenergic receptors and/or of any of the steps distal to the receptors. Alterations of erythrocyte β-adrenergic receptors were not found in thyroid hormone-treated turkeys (21) and rat adipose tissue β-adrenergic receptors have been reported to be unchanged (24) or increased (25) by thyroid hormones. There is, however, considerable evidence that experimental thyrotoxicosis in animals causes an increase in myocardial β-adrenergic receptor numbers (26, 27). Indeed, thyroid hormones have been shown to increase β-adrenergic receptor number in isolated ventricle slices (28) and in cultured heart cells (23). In humans, Williams et al. (29) found mononuclear leukocyte β-adrenergic receptors to be unaltered in spontaneously thyrotoxic patients when compared with those of euthyroid controls.

To explore further the status of β-adrenergic receptors in human thyrotoxicosis, we examined the effect of triiodothyronine and placebo administration on mononuclear leukocyte β-adrenergic receptors in 24 normal human subjects, using a double-blind design. Triiodothyronine administration for 1 wk, in doses causing moderate objective (but not subjective) thyrotoxicosis, resulted in a more than twofold increase in mononuclear leukocyte β-adrenergic receptor density in these subjects.

METHODS

Subjects. This study was approved by the Washington University Human Studies Committee. All subjects gave their written, informed consent. 24 normal young adults consented to participate and were assigned alternately to either a triiodothyronine treatment group or a placebo treatment group. The triiodothyronine treatment group consisted of six women and six men; their mean (±SE) age was 25.2±0.6 yr (range 22 to 30 yr) and their mean body weight was 66.8±3.9 kg (range 51.8 to 93.0 kg). The placebo group consisted of seven women and five men; their mean age was 25.1±0.5 yr (range 22 to 29 yr) and their mean body weight was 64.0±2.7 kg (range 54.2 to 78.0 kg).

Study protocol. Each subject was studied on two occasions: before (day 1) and after (day 8) the ingestion of either triiodothyronine (Cytomel, Smith, Kline & French Laboratories, Div. of SmithKline Corp. Philadelphia, Pa.), 25 μg 4 times/d for 7 d, or placebo. Neither the subjects nor the investigator who performed the binding studies knew the drug assignments (triiodothyronine vs. placebo groups).

All studies were performed on the Washington University School of Medicine Clinical Research Center. After an overnight fast, the subject arrived at ~0700 h and was weighed, and the resting heart rate and blood pressure were recorded. The subject then assumed the supine position and maintained that position until all sampling was completed. An intravenous sampling needle was inserted and, after 20 min, three blood samples for plasma norepinephrine and epinephrine determinations were drawn at 5-min intervals. The values reported here are the means of these three determinations. Blood samples for serum triiodothyronine, thyroxine, triiodothyronine-uptake test, cholesterol, and glucose determinations; and for total and differential leukocyte counts were then obtained and 100 ml of blood was removed for mononuclear leukocyte isolation and subsequent β-adrenergic receptor measurements.

Analytical methods. Plasma norepinephrine and epinephrine were measured with a single isotope derivative method (30, 31). Serum triiodothyronine and thyroxine were determined by radioimmunoassay; serum cholesterol and glucose and blood leukocyte counts were determined with standard methods.

Mononuclear leukocytes were isolated from heparinized whole blood (100 ml) and prepared as previously described (32). Mononuclear cells isolated on day 1 (pretreatment) were resuspended in 50 mM Tris/HCl (pH 7.7), 10 mM $MgCl_2$ (incubation buffer) in a concentration of ~10^8 cells/ml, quick-frozen in a dry ice/ethanol bath, and stored at −70°C. On day 8 these cells were thawed and prepared along with posttreatment (day 8) cells for β-adrenergic receptor assay. Preliminary experiments showed that freezing and storage of mononuclear leukocytes for up to 10 d had no effect on β-adrenergic receptor number or affinity.

β-Adrenergic receptors on mononuclear leukocyte preparations were assessed by binding studies using the antagonist (−)[^{3}H]dihydroalprenolol [(−)[^{3}H]DHA],[1] 34–49 Ci/mmol, New England Nuclear, Boston, Mass.) as previously described (32). Specific binding was defined as that displaceable by 1.0 μM (−)propranolol (Ayerst Laboratories, Div. American Home Products Corp., New York) and averaged 85–90% of total binding. This preparation contains (−)[^{3}H]DHA binding sites that exhibit the characteristics of β-adrenergic receptors, in that binding is rapid, reversible, and saturable, and in that competition for binding by agonists (and other antagonists) is stereospecific and exhibits the potency sequence isoproterenol > epinephrine > norepinephrine. The preparation is responsive to down-regulation (a decrease in the number of available binding

[1] *Abbreviation used in this paper*: (−)[^{3}H]DHA, (−)[^{3}H]-dihydroalprenolol.

sites) during incubation with isoproterenol in vitro and has been shown to exhibit sequential up-regulation and then down-regulation during exposure to agonists in vivo (32).

Although the methods used to assess mononuclear leukocyte β-adrenergic receptors in our laboratory are essentially those described by Williams et al. (33), one fundamental difference warrants emphasis. In the mononuclear leukocyte binding studies reported here (and in those reported earlier [32]), we have used four or five (−)[^{3}H]DHA concentrations with a maximum (−)[^{3}H]DHA concentration of <5.0 nM to construct binding curves for each sampling point. Others (29, 33), in contrast, have used (−)[^{3}H]DHA concentrations up to 90 nM. In preliminary experiments employing a broad range of (−)[^{3}H]DHA concentrations (from <0.5 nM to 50.0 nM), we observed that the binding curves were discontinuous with an initial plateau at <5.0 nM (−)[^{3}H]DHA; these data yielded curvilinear Scatchard plots (data not shown). Bishopric et al. (34) and Davies and Lefkowitz (35) have recently pointed out that use of the higher (−)[^{3}H]DHA concentrations may result in binding to low affinity leukocyte binding sites that do not exhibit the characteristics of β-adrenergic receptors. They have, therefore, advocated use of lower (−)[^{3}H]DHA concentrations, similar to those used in our studies, to assess leukocyte β-adrenergic receptors. Parenthetically, Winek and Bhalla (36) have made a similar observation in studies of β-adrenergic receptors in myocardium. Thus, we are examining high-affinity (apparent dissociation constants of ~1 nM), low-capacity (~1,000 sites/cell) binding sites which exhibit the characteristics of a β-adrenergic receptor.

Statistical methods. Student's *t* test for paired data was used to compare pretreatment values with post-triiodothyronine or postplacebo values. The coefficient of variation is the standard deviation divided by the mean, expressed as a percent. Correlation coefficients (*r*) were determined by linear regression analysis. Throughout this manuscript data are expressed as the mean±SEM.

RESULTS

Nonreceptor effects of triiodothyronine administration. As shown in Table I, the ingestion of triiodothyronine in a dose of 25 μg 4 times/d for 7 d resulted in elevation of the serum triiodothyronine concentration from a mean (±SE) control value of 145±10 to 310±20 ng/dl ($P < 0.001$). The latter corresponds to serum triiodothyronine levels that occur commonly in patients with spontaneous thyrotoxicosis (37–39). Serum thyroxine concentrations declined appropriately.

Evidence of the moderate thyrotoxic state produced by triiodothyronine included a small but significant reduction in body weight, and highly significant decrements in serum cholesterol and increments in resting heart rate. Although an apparent increase in mean pulse pressure did not achieve statistical significance, the

TABLE I
Effects of Triiodothyronine (T_3) in Normal Human Subjects

	Control → T_3		Control → Placebo	
	n = 12		*n = 12*	
Triiodothyronine, *ng/dl*	145±10	310±20*	135±8	141±5
Thyroxine, *μg/dl*	7.0±0.5	4.1±0.4*	6.4±0.5	6.6±0.4
T_3 uptake test, %	42±1	42±1	42±1	42±1
Cholesterol, *mg/dl*	149±7	111±6*	146±5	146±5
Glucose, *mg/dl*	77±3	90±2*	85±5	82±4
Norepinephrine, *pg/ml*	184±26	165±16	184±24	184±14
Epinephrine, *pg/ml*	21±3	24±4	31±5	31±6
Heart rate, *per min*	75±2	88±4*	74±4	73±4
Pulse pressure, *mm Hg*	37±2	43±4	37±2	37±2
Systolic blood pressure, *mm Hg*	112±3	115±4	106±3	107±3
Diastolic blood pressure, *mg Hg*	75±3	72±3	69±3	70±2
Double product‡	8,400±450	10,200±594§	7,770±377	7,780±405
Body weight, *kg*	66.8±3.9	66.3±4.0‖	64.0±2.7	64.2±2.8
Leukocyte count, *per mm^3*	4,800±400	4,400±300	4,800±200	4,600±200
Lymphocytes, %	38±4	36±3	36±4	37±3
Monocytes, %	6±1	7±1	6±1	7±1
Neutrophils, %	52±4	51±4	54±4	50±3

* $P < 0.001$.
‡ Product of the heart rate and systolic blood pressure.
§ $P < 0.01$.
‖ $P < 0.05$.

"double product" (heart rate times systolic blood pressure) increased significantly after triiodothyronine ingestion. Despite these objective changes, the subjects were not able to identify their medication. Indeed, most thought they had been assigned to the placebo group.

Plasma norepinephrine and epinephrine concentrations, indices of sympathoadrenal catecholamine release, were not significantly altered by triiodothyronine. Total and differential leukocyte counts were also unaffected by triiodothyronine.

Variation in β-adrenergic receptor density. There was considerable inter- and intraindividual variation in mononuclear leukocyte β-adrenergic receptor density (calculated by Scatchard analysis) in the subjects studied. From the control (pretreatment) determinations for the entire group of 24 subjects the interindividual coefficient of variation was 50%. The intraindividual coefficient of variation, calculated from the determinations performed before and after placebo administration (see below), was similar, 58%. These can be contrasted with an analytical coefficient of variation of 16% from replicate determinations on a single large blood sample. Thus, the biologic variation (estimated as the difference between total variation and analytical variation) was 34–42%.

No significant correlations were detected between mononuclear leukocyte (−)[^{3}H]DNA binding and basal plasma norepinephrine ($r = -0.096$) or epinephrine ($r = 0.164$) concentrations nor between binding and serum triiodothyronine ($r = -0.070$) or thyroxine ($r = -0.207$) levels in the pretreatment samples from the entire group of 24 subjects.

Effect of triiodothyronine on mononuclear leukocyte β-adrenergic receptors. Despite marked intraindividual variation in receptor density, the placebo had no significant effect on specific (−)[^{3}H]DHA binding to mononuclear leukocyte preparations (Fig. 1). In contrast, specific (−)[^{3}H]DHA binding increased from 25±3 to 57±9 fmol/mg protein ($P < 0.001$) after triiodothyronine ingestion (Fig. 2). As shown in Fig. 3, this effect of triiodothyronine was attributable, by Scatchard analysis, to an increase in β-adrenergic receptor density (967±134 to 2,250±387 sites/cell, $P < 0.01$). Apparent dissociation constants (K_d) did not change (1.1±0.2–1.1±0.3 nM).

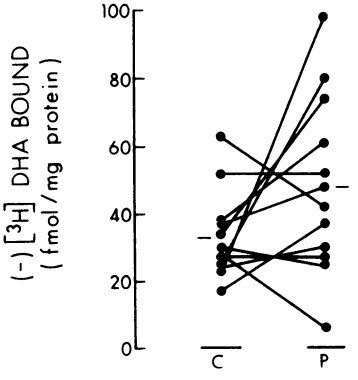

FIGURE 1 Specific (−)[^{3}H]DHA binding to a mononuclear leukocyte preparations obtained before (C, control) and after placebo (P) ingestion for 7 d in 12 normal human subjects.

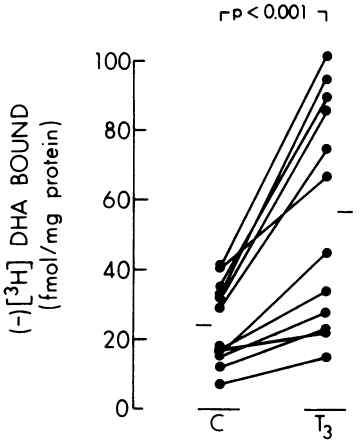

FIGURE 2 Specific (−)[^{3}H]DHA binding to mononuclear leukocyte preparations obtained before (C, control) and after the ingestion of triiodothyronine (T_3), 25 μg 4 times/d for 7 d, in 12 normal human subjects.

DISCUSSION

These findings demonstrate that elevation of serum triiodothyronine to levels that occur commonly in thyrotoxic patients (37–39) results in a marked change in mononuclear leukocyte β-adrenergic receptors in man. By Scatchard analysis, this change can be attributed to an increase in β-adrenergic receptor density.

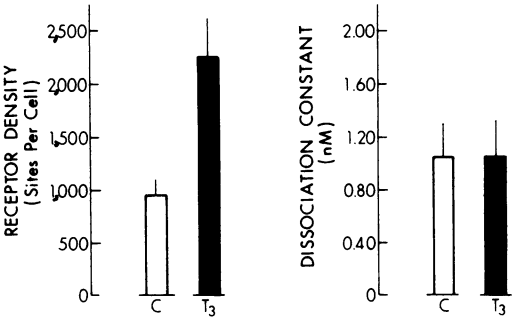

FIGURE 3 Mean (±SE) mononuclear leukocyte β-adrenergic receptor densities (left) and apparent dissociation constants (right) before (C, control, open columns) and after the ingestion of triiodothyronine (T_3, closed columns), 25 μg 4 times/d for 7 d, in 12 normal human subjects.

It is reasonable to conclude that the observed triiodothyronine-induced increase in mononuclear leukocyte β-adrenergic receptor density represents a direct effect of triiodothyronine. Since plasma norepinephrine and epinephrine concentrations were not significantly altered by triiodothyronine administration, it is unlikely that the increase in β-adrenergic receptor density was due to suppression of sympathoadrenal activity (2). Further, increments in myocardial β-adrenergic receptor numbers have been produced by triiodothyronine in vitro (23, 28). Triiodothyronine administration did not alter lymphocyte or monocyte counts. Although we cannot exclude triiodothyronine-associated changes in lymphocyte subpopulations, Bishopric et al. (34) found no differences in β-adrenergic receptor densities or affinities between T and B lymphocytes.

This study also demonstrates considerable inter- and intraindividual variation in human mononuclear leukocyte β-adrenergic receptor density, as assessed by specific (−)[^{3}H]DHA binding, even when such variables as time of day, food intake, and body position are controlled and when subjects of similar ages are examined. Similar variation can be calculated from the data of Davies and Lefkowitz (35). Because this variation is approximately three times greater than analytical variation, it reflects, in large part, biologic variation and is further evidence that β-adrenergic receptors are substantially modulated in man (32). As exemplified by the present findings, this biologic variation may well include heterologous hormonal regulation, as well as homologous regulation by adrenergic agonists. In this regard, however, it should be noted that (−)[^{3}H]DHA binding was not significantly correlated with basal plasma norepinephrine or epinephrine concentrations or with serum triiodothyronine or thyroxine levels in the pretreatment data from the 24 normal subjects studied.

The use of circulating mononuclear leukocytes to assess human receptors is based on the premise that mononuclear leukocyte receptors reflect the status of receptors outside the circulation. In view of the several reports of increased β-adrenergic receptor numbers in tissues of experimentally thyrotoxic animals (23, 25–28), the report of Williams et al. (29) that mononuclear leukocyte β-adrenergic receptors from spontaneously thyrotoxic patients did not differ from those of euthyroid controls seriously undermined that premise. Why do our findings differ from those of Williams et al. (29)? (*a*) It is conceivable, but in our judgment not likely, that the effects of experimental thyrotoxicosis induced by the relatively short-term administration of exogenous thyroid hormone—the model used in this study in human subjects and in the animal studies reporting increased β-adrenergic receptor densities (25–27)—are fundamentally different from those produced by relatively long-term endogenous overproduction of thyroid hormones. (*b*) We did not induce more severe thyrotoxicosis than that which occurs spontaneously; our subjects took triiodothyronine for only 1 wk, had no subjective manifestations of thyrotoxicosis, and had serum triiodothyronine concentrations lower than those of most spontaneously thyrotoxic patients (37–39). (*c*) In view of the marked biologic variation in mononuclear leukocyte β-adrenergic receptor density which we have observed, it may be that the experimental design we used—a double-blind design in which each subject served as his or her own control—allowed us to find a treatment-related difference not perceptible when data from a heterogeneous group of spontaneously thyrotoxic patients were compared with data from a group of euthyroid controls. (*d*) The use of different (−)[^{3}H]DHA concentrations to assess mononuclear leukocyte β-adrenergic receptors may well explain the difference. Whereas we used (−)[^{3}H]-DHA concentrations of <5.0 nM, Williams et al. (29) used (−)[^{3}H]DHA concentrations of 10 and 40 nM. The latter may result in some binding to low affinity sites that do not exhibit the characteristics of β-adrenergic receptors (34, 35).

Available evidence (10, 11, 16–22), discussed earlier, suggests that thyroid hormone excess results in enhanced tissue responsiveness to endogenous catecholamines, an effect plausibly explained by increased β-adrenergic receptor densities in those target tissues. The present finding that thyroid hormone excess results in increased β-adrenergic receptor density on circulating mononuclear leukocytes does not, of course, establish that a similar increase occurs in human extravascular target tissues. When coupled, however, with similar findings in some tissues of experimentally thyrotoxic animals (23, 25–27), our finding suggests that this does occur in man. Further, proof that thyroid hormone-enhanced tissue sensitivity to catecholamines is due to increased β-adrenergic receptor density will require demonstration of a cause-and-effect relationship between the increase in β-adrenergic receptor density and the increase in biologic responsiveness to catecholamines in human target cells.

In conclusion, (*a*) the finding of a triiodothyronine-induced increase in mononuclear leukocyte β-adrenergic receptor density in human mononuclear leukocytes, coupled with similar findings in the tissues of experimentally thyrotoxic animals, provides support for the use of mononuclear leukocytes to assess receptor status in man. (*b*) There is considerable biologic variation in β-adrenergic receptor density in man. (*c*) The findings of thyroid hormone-induced increments in β-adrenergic receptor density

provide a plausible mechanism for the putative enhanced responsiveness to endogenous catecholamines of patients with thyrotoxicosis.

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