Increased Fat and Skeletal Muscle β-Adrenergic Receptors but Unaltered Metabolic and Hemodynamic Sensitivity to Epinephrine In Vivo in Experimental Human Thyrotoxicosis

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Abstract

Based largely on evidence of increased target tissue β-adrenergic receptor densities and responsiveness in animal and, to a lesser extent, human tissues, it is often assumed that thyroid hormone excess results in increased sensitivity to catecholamines in vivo, thus explaining several clinical manifestations of thyrotoxicosis. To test the hypothesis that thyrotoxicosis results in increased target tissue β-adrenergic receptor densities and correspondingly increased metabolic and hemodynamic sensitivity to epinephrine in vivo, we measured these in 10 normal humans before and after administration of triiodothyronine (100 μg daily) for 10 d. Thyrotoxicosis increased β-adrenergic receptor densities in fat ($\sim$ 60%) and skeletal muscle ($\sim$ 30%). Despite increments in β-adrenergic receptor densities in these and probably other target tissues, metabolic and hemodynamic sensitivity to epinephrine in vivo was unaltered. An apparently adaptive increase in insulin secretion plausibly explains normal glycemic, glycogenolytic/glycolytic, lipolytic, and ketogenic sensitivity to epinephrine in the thyrotoxic state. In view of this striking homeostatic efficiency of the intact individual, the finding of altered adrenergic receptors, even in relevant target tissues, should not be extrapolated to altered sensitivity to catecholamines in vivo in the absence of direct testing of that hypothesis. With respect to the clinical issue, these data suggest that increased sensitivity to catecholamines does not explain clinical manifestations of thyrotoxicosis in humans.

Introduction

Several manifestations of thyrotoxicosis resemble those of catecholamine excess and are ameliorated by administration of a β-adrenergic antagonist (1). If these are catecholamine mediated, they must be the result of increased sensitivity to catecholamines since sympathochromaffin catecholamine release is not increased (2, 3). In addition to a body of evidence from animal studies (reviewed in references 4 and 5), several in vitro studies using tissues from thyrotoxic humans are consistent with this possibility. Increased mononuclear leukocyte

β-adrenergic receptor densities (5–7) and adenylate cyclase responses to isoproterenol (7) have been reported, although these findings have not been universal (8–10). Similarly, there are reports of increased β-adrenergic receptor densities and cAMP and lipolytic responses to agonists of fat cells removed from thyrotoxic humans (11, 12). The impact of such thyroid hormone-induced changes in catecholamine target tissues on sensitivity to catecholamines in vivo is unclear. Seemingly well-controlled studies of thyrotoxic humans have not disclosed increased hemodynamic sensitivity to infused catecholamines (13, 14) although increased responses have been noted in other studies (15, 16). With respect to metabolic actions, increased plasma FFA responses to catecholamines were noted in some, but not all, of a small number of thyrotoxic patients (17). Increased metabolic rate responses to epinephrine have been reported (16). However, plasma glucose responses to epinephrine have been reported to be decreased (18). To our knowledge plasma catecholamine concentration-response curves have not been used to measure sensitivity to catecholamines in vivo in thyrotoxic humans, nor have in vivo sensitivity and β-adrenergic receptor status of target tissues been assessed in the same individuals.

The apparent contradiction between the in vitro and in vivo findings just summarized could be reconciled if increased target tissue sensitivity to catecholamines induced by thyroid hormone excess, demonstrable in vitro, were offset by adaptive changes in physiologic regulatory systems in the intact individual. To test this hypothesis we assessed the impact of experimental thyrotoxicosis on β-adrenergic receptors and adenylate cyclase in two catecholamine target tissues, fat and skeletal muscle, studied in vitro, and on metabolic and hemodynamic responses to epinephrine in vivo in the same human subjects.

Fat and muscle were selected for in vitro study because they play important roles in the lipolytic and ketogenic responses and the glycemic and glycogenolytic/glycolytic responses to epinephrine, respectively, in vivo (19, 20), and because it is feasible to biopsy these in humans. The direct actions of epinephrine to increase glucose production and limit glucose use are largely, perhaps exclusively, mediated through β_2-adrenergic mechanisms in humans. The major indirect hyperglycemic action is α_2-adrenergic limitation of insulin secretion. Epinephrine also stimulates lipolysis, ketogenesis, and glycogenolysis (and in muscle, glycolysis), largely through β-adrenergic mechanisms (19). Although both β_1-adrenergic receptors that mediate an increase in lipolysis and α_2-adrenergic receptors that mediate a decrease in lipolysis are present on fat cells, the net effect of epinephrine is to increase lipolysis (21). Insulin, on the other hand, suppresses all of these metabolic processes. Acting through β-adrenergic receptors, epinephrine also increases heart rate and widens pulse pressure.

Methods

Study design. 10 healthy subjects (2 females, 8 males ages 22–33 yr) of normal height and weight participated in two identical study sessions. The control study was performed while the subjects were taking no medications and the thyrotoxic study was performed after the subjects had taken oral triiodothyronine (100 μg/d in four divided doses) for 10 d. After an overnight fast the subjects rested supine for 30 min and then, under local anesthesia, an open fat biopsy was performed, followed by a percutaneous skeletal muscle needle biopsy. The subjects then had two intravenous lines inserted (one for sampling and one for epinephrine infusion) and rested for 45 min, at which time a 3.5-h epinephrine infusion study was begun. These studies were approved by the Washington University School of Medicine Human Studies Committee and all subjects gave written informed consent.

Fat cells. Fat (0.75–1.0 g wet wt) was obtained from 1-cm incisions in the upper gluteal region after four-quadrant local anesthesia was established with 0.5% bupivicaine. At no time was the anesthetic injected directly into the fat to be biopsied. The duration of anesthesia for both fat and skeletal muscle biopsies was > 8 h; thus, the subjects experienced no pain from the biopsy sites during the subsequent epinephrine infusions. Fat cells were isolated by a method similar to that of Rodbell (22). The common buffer for fat cell studies was Krebs-Ringer phosphate (128 mM NaCl, 1.4 mM MgSO$_4$, 1.4 mM CaCl$_2$, 5.2 mM KCl, 10 mM Na$_2$HPO$_4$, and 0.2 g/100 ml dextrose) (KRP).[1] Fat was minced and placed in 5.0 ml of KRP supplemented with 3.0 g/100 ml BSA and 0.5 mg/ml crude collagenase, shaken in a rotating bath at 50 cycles/s for 60 min at 37°C, and the cells filtered through 300-μm nylon mesh. Collagenase from the same pretested batch was used in all experiments. The fat cells were washed in assay buffer (collagenase-free KRP with 0.5 g/100 ml BSA) three times by resuspension and 10-s centrifugations. The typical final suspension was 8 ml with a concentration of ~ 3 × 10^5 cells/ml. Portions of this suspension were then used for β-adrenergic receptor binding studies and cAMP studies described below. Cell number was determined from DNA analysis by the fluorometric bisbenzimide method (23) using calf thymus DNA for standards and assuming 5 pg of DNA/cell.

Skeletal muscle membranes. After 0.5% bupivicaine subcutaneous anesthesia a Bergstrom biopsy needle (Depuy Manufacturing, Warsaw, IN) was used to obtain ~ 200 mg of skeletal muscle from the lateral gastrocnemius muscle. The method of biopsy and preparation of skeletal muscle membranes was as described recently (24). The muscle was frozen in liquid nitrogen and stored at −70°C until processing, at which time homogenization with a polytron (Brinkmann Instruments, Inc., Westbury, NY) in cold 10 mM Tris/5 mM EDTA buffer was performed. The suspension was filtered once through nylon mesh, washed twice by centrifugation at 45,000 g for 20 min, and resuspended. The final resuspension was in 75 mM Tris/25 mM MgCl$_2$, 5 mM EDTA buffer (skeletal muscle assay buffer). Protein concentration was determined by the method of Bradford using BSA as standards (25).

Receptor binding studies. Both intact fat cell and skeletal muscle membrane β-adrenergic receptors were studied with [^{125}I]pindolol (IPIN) with propranolol (1 μM) used to define nonspecific binding. As described by Doyle et al. (26), the specific activity of IPIN was assumed to be constant during decay of ^{125}I. The conditions described below provided the appropriate properties for ligand receptor interactions (saturability, reversibility, stereospecificity, rank order potency, and typical kinetics) as determined by preliminary studies. Intact fat cells (~ 4 × 10^4) were incubated in assay buffer (final vol 0.5 ml) in triplicate with eight concentrations of IPIN (10–500 pM) in the absence or presence of 1 μM propranolol for 30 min at 37°C. The reaction was terminated by addition of 10 ml ice-cold 154 mM NaCl and the con-

tents filtered over glass fiber filters (type GF/C; Whatman Laboratory Products Inc., Clifton, NJ). The tubes and filters were washed with 10 ml additional NaCl and the filters counted in a gamma counter at 80% efficiency. The binding of IPIN to fat cells was similar to that described for [^{125}I]iodocyanopindolol (11). Nonspecific binding represented 15 and 40% of total binding at 10 and 500 pM IPIN, respectively. Skeletal muscle membranes (~ 100 μg) were incubated in triplicate with eight concentrations of IPIN (5–300 pM) in a final vol of 0.25 ml with or without 1 μM propranolol for 90 min at 25°C (24). GTP (100 μM) was included to eliminate retained agonist binding. The tubes and filters were washed with ice-cold skeletal muscle assay buffer in the same manner as described for fat cells, and the filters counted.

cAMP and adenylate cyclase studies. For intact fat cells the accumulation of cAMP was studied by incubating cells in assay buffer with 1 mM ascorbic acid and 0.1-mM concentrations of the phosphodiesterase inhibitors RO-20-1724 and 3-isobutyl-1-methylxanthine (IBMX) in the presence or absence of various agents for 5 min at 37°C. The reaction was terminated by addition of perchloric acid, neutralized with potassium bicarbonate, centrifuged, and an aliquot of the supernatant acetylated with acetic anhydride and triethylamine (27). cAMP was measured by RIA using ^{125}I-succinyl cAMP by the method of Steiner et al. (28). For assessment of maximal β-adrenergic receptor-mediated cAMP production cells were incubated with 10 μM isoproterenol. For α-adrenergic receptor stimulation (which causes an inhibition of adenylate cyclase) cells were incubated with 10 μM isoproterenol plus 10 μM epinephrine. (Preliminary studies showed that the α$_2$-adrenergic antagonist yohimbine blocked this inhibitory effect of epinephrine and that the α$_2$-adrenergic agonist clonidine had a similar but less potent inhibitory effect.)

Adenylate cyclase activities of skeletal muscle membranes were assessed by incubating membranes (5–20 μg) with 0.2 mM ATP, 0.1 mM GTP, 1.0 mM IBMX, 10 mM MgCl$_2$, 1.0 mM EDTA, 50 mM Tris, 1.0 mM ascorbic acid, 25 mM phosphocreatine, and 1.0 mg/ml creatine phosphokinase, in the presence or absence of 10 μM isoproterenol for 15 min at 37°C (24). The reaction was stopped by placing the tubes in a boiling water bath for 3 min. cAMP was measured by RIA as described above.

Epinephrine infusions. After 45 min of supine rest after the biopsies measurements of blood pressure and heart rate (Dynamap model 1846; Critekon Inc., Tampa, FL) and blood sampling were performed every 10 min for 210 min. At each time point 10 ml of blood was sampled and aliquotted to appropriate tubes for measurements of epinephrine and norepinephrine by a single isotope derivative (radioenzymatic) method (29), and glucose, FFA, β-hydroxybutyrate, glycerol, lactate and alanine and insulin, C-peptide, glucagon, cortisol, growth hormone, and pancreatic polypeptide by methods used previously (20, 30). The first 30 min constituted baseline measurements without epinephrine infusion. Thereafter, epinephrine (diluted in saline with 0.5 mg/ml ascorbic acid) was infused by Harvard pump (model 22; Harvard Apparatus Co., Inc., South Natick, MA) over sequential 30-min intervals in doses of 1.4, 7, 14, 28, 42, and 70 ng/kg per min. The intravenous lines were maintained with infusions of normal saline at a rate that matched blood loss.

Statistical analysis. The receptor densities (B_{max}) and apparent dissociation constants (K_d) for fat cell and skeletal muscle membrane β-adrenergic receptors were obtained by Scatchard analysis (31) of the saturation binding studies described above. Data are presented as the mean±SE. Comparisons of control and thyrotoxic receptor properties, cAMP content, or adenylate cyclase activities were performed by paired *t* tests. For summary purposes epinephrine plasma concentration-response curves are presented as plots of the means±SE for a given response vs. the means±SE of the epinephrine concentrations at the end of each 30-min infusion period. Methods described previously (20, 32) were used to determine the presence of differences between control and thyrotoxic concentration-response curves. Individual curves were normalized to baseline (time 0) values and then the data were fit to a fourth degree polynomial. The coefficients from these equations were then compared by multivarate analysis of variance (MANOVA) using

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1. *Abbreviations used in this paper:* IBMX, 3-isobutyl-1-methylxanthine; IPIN, [^{125}I]pindolol; KRP, Krebs-Ringer phosphate; MANOVA, multivariate analysis of variance.

SAS version 6.02 (SAS Institute, Cary, NC). For t tests and MANOVA significance was defined as $P < 0.05$.

Chemicals. [^{125}I]Pindolol (2,200 Ci/mmol) was purchased from New England Nuclear (Boston, MA) and Na^{125}I from Amersham Corp. (Arlington Heights, IL). ^{125}I-succinyl cAMP (tyrosine methylesther) was made by the method of Steiner et al. (28). (−)Propranolol was a gift from Ayerst Laboratories (New York, NY) and RO-20-1724 was a gift from Hoffman-La Roche (Nutley, NJ). Crude collagenase (204 U/mg, lot No. 67155M) was purchased from Worthington Biochemical Corp. (Freehold, NJ). Triiodothyronine was supplied as 25-μg tablets (Cytomel; Smith, Kline and French, Philadelphia, PA). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Results

Triiodothyronine administration resulted in elevated serum T_3 levels (126±11 to 349±43 ng/dl, $P < 0.002$), decreased thyroid-stimulating hormone levels (1.27±0.27 to 0.15±0.05 μU/ml, $P < 0.002$), and decreased serum thyroxine levels (6.4±0.4 to 3.3±0.3 μg/dl, $P < 0.0001$). Resting heart rate increased $\sim$ 30% (58±4 to 75±4 beats/min, $P < 0.02$). Baseline plasma catecholamine concentrations were unaltered. Plasma epinephrine levels were 31±8 and 37±12 pg/ml and norepinephrine levels were 119±14 and 131±17 pg/ml, respectively, before and during triiodothyronine administration.

Thyrotoxicosis was associated with an increase in β-adrenergic receptor densities in both intact fat cells and skeletal muscle membranes (Fig. 1). Fat cell receptor density increased $\sim$ 60%, from 6,230±970 to 9,920±1,590 sites/cell ($P < 0.05$) without a change in IPIN binding affinity (K_d = 77±8 vs. 102±31 pM). Skeletal muscle β-adrenergic receptor density increased $\sim$ 30%, from 7.9±1.0 to 10.2±0.9 fmol/mg protein ($P < 0.05$), with an increase in IPIN binding affinity (K_d = 23±1 vs. 10±1 pM, $P < 0.0001$) after thyrotoxicosis. Neither basal (47±10 vs. 41±8 pmol cAMP/10^7 cells) nor β-adrenergic receptor-mediated maximal stimulation (310±37 vs. 333±35 pmol cAMP/10^7 cells) of fat cell cAMP content was altered by thyrotoxicosis. Basal (336±53 vs. 452±90 pmol cAMP/mg protein per 15 min) and iosproterenol-stimulated (501±95 vs. 749±134 pmol cAMP/mg protein per 15 min) skeletal muscle adenylate cyclase activities were not different statistically. The α_2-adrenergic-mediated inhibition of cAMP production in fat cells was unchanged (74±10 vs. 86±4% inhibition).

In vivo plasma epinephrine concentration-response curves for FFA, β-hydroxybutyrate, glucose, and lactate are illustrated in Fig. 2. There were no significant differences in the responses of these to epinephrine during thyrotoxicosis. The epinephrine concentration-response curves for heart rate and blood pressure are shown in Fig. 3. Although baseline heart rates were elevated after thyrotoxicosis, the response to infused epinephrine was the same as in the euthyroid state. Systolic and diastolic blood pressure responses were also unaltered. In contrast, insulin, C-peptide, and pancreatic polypeptide secretory responses to epinephrine were clearly increased ($P < 0.05$, MANOVA) during thyrotoxicosis as depicted in Fig. 4. Glucagon levels were unchanged (Fig. 4). In data not shown the cortisol, growth hormone, alanine, and norepinephrine responses were not altered significantly although plasma norepinephrine appeared to rise to higher levels during epinephrine infusions after triiodothyronine administration. As is apparent from Figs. 2–4, plasma epinephrine concentrations were comparable in the control and triiodothyronine studies.
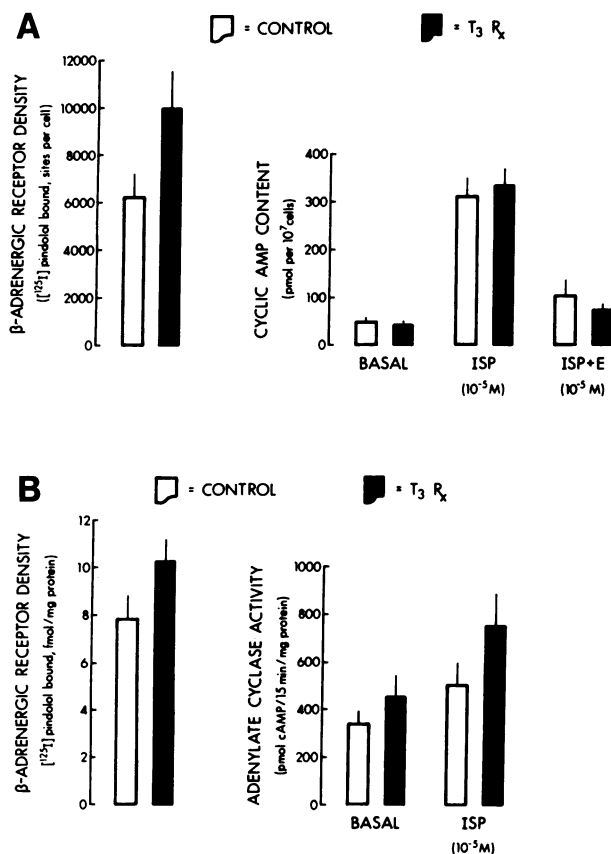

Figure 1. Effects of thyrotoxicosis on fat cell (A) and skeletal muscle (B) β-adrenergic receptor densities, fat cell basal, isoproterenol (*ISP*)-stimulated, and epinephrine (E) inhibition of ISP-stimulated cAMP contents and basal and ISP-stimulated skeletal muscle adenylate cyclase activities. Control = before and T_3 = during triiodothyronine-induced thyrotoxicosis.

Discussion

To test the hypothesis that thyrotoxicosis results in increased target tissue β-adrenergic receptor densities and correspondingly increased metabolic and hemodynamic sensitivity to epinephrine in vivo, we measured these in 10 normal humans before and after administration of triiodothyronine (100 μg daily) for 10 d. This dose, administered for only 7 d, was previously shown to result in objective thyrotoxicosis and increased mononuclear leukocyte β-adrenergic receptor density in a placebo-controlled study (5). It raised serum triiodothyronine concentrations nearly threefold, suppressed serum thyroid-stimulating hormone levels, and increased resting heart rate in the present study. Thyrotoxicosis was associated with significant increments in fat cell and skeletal muscle β-adrenergic receptor densities averaging $\sim$ 60 and 30%, respectively. Basal and maximal isoproterenol-stimulated cAMP contents (fat) and adenylate cyclase activities (muscle) were unaltered by triiodothyronine administration, but sensitivity to agonist was not tested because of limited tissue availability. Thus, the sensitivity of individual tissues to epinephrine may well have been increased.

Since triiodothyronine administration has been shown previously to increase mononuclear leukocyte β-adrenergic receptor density (5–7), and the latter correlates with myocar-
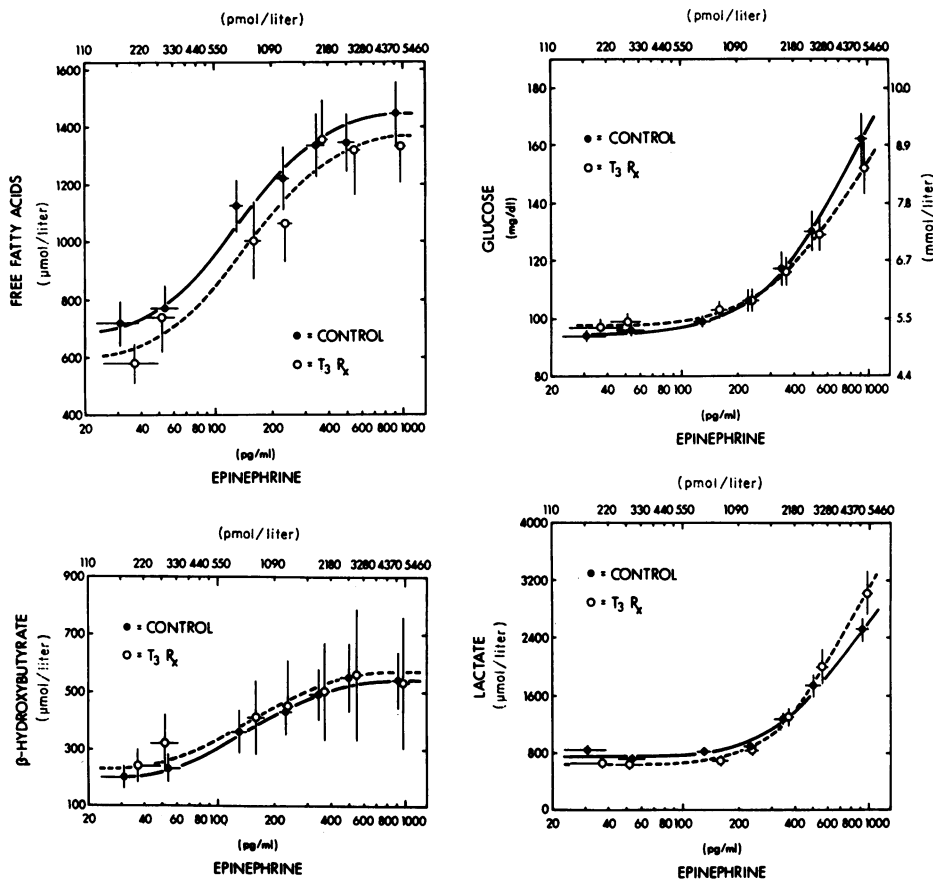
Figure 2. In vivo plasma epinephrine concentration-response curves for FFA, β-hydroxybutyrate, glucose, and lactate before (control) and during (T_3 Rx) triiodothyronine-induced thyrotoxicosis.
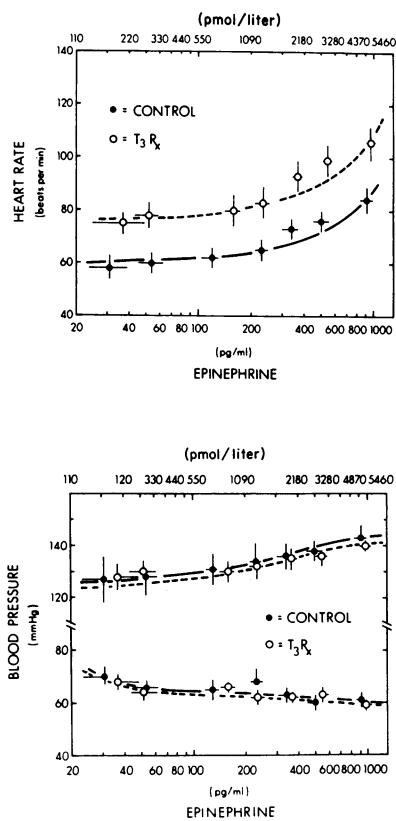

Figure 3. Heart rate and systolic and diastolic blood pressure plasma epinephrine concentration-response curves before (control) and during (T_3 Rx) triiodothyronine-induced thyrotoxicosis.

dial (33) and lung (34) receptor densities, thyroid hormone excess probably resulted in increased β-adrenergic receptor densities in diverse catecholamine target tissues in addition to fat and skeletal muscle.

Despite these increments in target tissue β-adrenergic receptor densities, metabolic and hemodynamic responses to epinephrine in vivo were unaltered in the thyrotoxic state. Epinephrine was infused intravenously in six doses that resulted in steady-state plasma epinephrine concentrations that spanned the physiologic range (19) before and during experimental thyrotoxicosis. The epinephrine plasma concentrations were similar on both occasions. Plasma epinephrine concentration-response curves for plasma glucose, blood lactate, serum FFA, and blood β-hydroxybutyrate concentrations, as well as those for systolic and diastolic blood pressure and heart rate, were virtually identical under both conditions. Complete epinephrine concentration-response curves were obtained for FFA and β-hydroxybutyrate levels. Neither sensitivity nor responsiveness to epinephrine was altered. Although complete concentration-response curves for the other variables mentioned cannot be constructed in humans, there was no suggestion of increased sensitivity of the changes in these to epinephrine.

Why was sensitivity to epinephrine in vivo unaltered despite increased target tissue β-adrenergic receptor densities during triiodothyronine administration? One possibility is that the increase in receptor density was not of sufficient magnitude to alter cellular responses to the agonist. Alternatively,
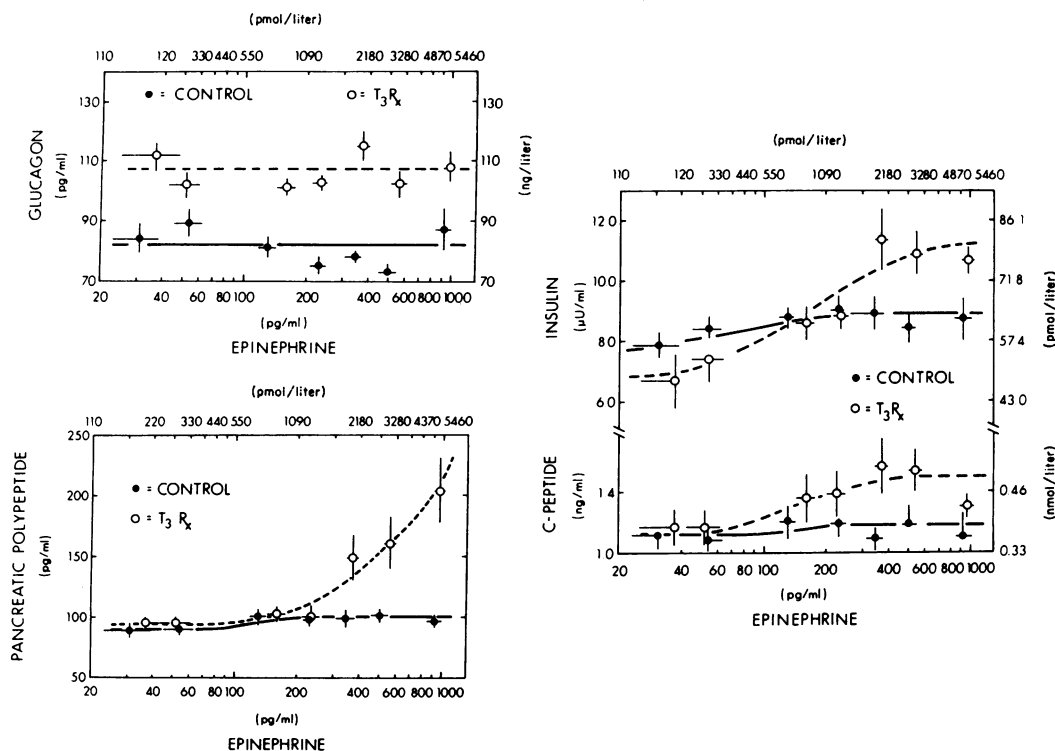
Figure 4. In vivo plasma epinephrine concentration-response curves for glucagon, pancreatic polypeptide, insulin, and C-peptide, before (control) and during (T₃ Rx) triiodothyronine-induced thyrotoxicosis. The responses for insulin, C-peptide, and pancreatic polypeptide were significantly increased during thyrotoxicosis as assessed by MANOVA.

offsetting local (intracellular, autocrine, or paracrine) or systemic regulatory compensation may have occurred. Although we cannot exclude roles for the other possibilities, we suggest that systemic regulatory compensation accounts, at least in part, for these findings based on our in vivo data. The absence of increased glycemic (plasma glucose), glycogenolytic/glycolytic (blood lactate), lipolytic (serum FFA), and ketogenic (blood β-hydroxybutyrate) sensitivity to epinephrine in vivo despite triiodothyronine-induced increments in target tissue β-adrenergic receptor densities is plausibly explained by the observed, apparently compensatory, increase in the insulin secretory response during epinephrine infusions, as noted previously by Perez et al. (18). The increased insulin secretory response to epinephrine in thyrotoxicosis could have been nonspecific, the result of increased β₂-adrenergic sensitivity (stimulatory), decreased α₂-adrenergic sensitivity (inhibitory), or both of insulin secreting pancreatic β-cells. However, direct exposure of perfused pancreatic rat islets to triiodothyronine has been reported to decrease (35) or have no effect (36) on the insulin secretory response to glucose. Furthermore, in the present study there was not a generalized change in islet sensitivity to epinephrine since plasma glucagon levels were unchanged during thyrotoxicosis. Finally, the precise matching of the glucose, lactate, FFA, and β-hydroxybutyrate responses to epinephrine in vivo before and after triiodothyronine administration suggests feedback regulation of insulin secretion, i.e., that the enhanced insulin secretory response was a homeostatic regulatory event. Thus, we cannot conclude that it was the result of increased β-cell sensitivity to epinephrine per se.

With respect to the unaltered blood pressure and heart rate responses to epinephrine during thyrotoxicosis, one could speculate that enhanced β-adrenergic sensitivity was offset in part by augmented parasympathetic activity. We have no direct measure of the latter, but observed an increased plasma pancreatic polypeptide response to epinephrine during thyrotoxicosis. The plasma pancreatic polypeptide response to hypoglycemia is mediated by the parasympathetic nervous system; however, it is not clear whether or not the response to epinephrine (37, 38) is a marker of parasympathetic function.

In a related study in a larger group of normal subjects we found no relationships between mononuclear leukocyte (β_2), platelet (α_2), fat cell (β_1), and skeletal muscle (β_2) adrenergic receptor densities or their corresponding basal, stimulated (β), or inhibited (α_2) cAMP contents/adenylate cyclase activities, and metabolic or hemodynamic sensitivity to epinephrine in vivo (39). Thus, interindividual variation in adrenergic receptor densities and their linked adenylate cyclase systems is not sufficient to alter in vivo sensitivity to epinephrine, at least with respect to the endpoints assessed. As a direct corollary, measurements of these receptor/adenylate cyclase parameters do not appear predictive of the responses to the hormone in vivo. In the present study we found no effect of induced changes in catecholamine target tissue adrenergic receptor densities, documented here in fat and skeletal muscle and probably present in other tissues (5, 33, 34), on the metabolic and hemodynamic responses to epinephrine in vivo. Again, these measurements were not predictive of the response to the hormone in vivo.

Obviously we cannot exclude the possibility that differences in adrenergic receptor densities of greater magnitude than we produced might result in altered sensitivity to epinephrine in vivo. However, reports of relationships between measured adrenergic receptors and in vivo responses to agonists have shown conflicting results (40–45). In view of the striking homeostatic efficiency of the intact individual demonstrated in the current study, the finding of altered adrenergic receptor characteristics, even in target tissues, should not be extrapolated to altered sensitivity to catecholamines in vivo.

Rather, it should serve as the basis of a hypothesis that must then be tested in vivo before it is incorporated into concepts of regulatory physiology and pathophysiology.

Finally, with respect to the clinical issue, these data suggest that increased sensitivity to catecholamines does not explain clinical manifestations of thyrotoxicosis in humans. However, we emphasize that we studied normal humans before and during short-term, experimental thyrotoxicosis that clearly differed in duration, and may have differed in other important ways, from spontaneous thyrotoxicosis.

Acknowledgments

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