Contributions of Plasma Triiodothyronine and Local Thyroxine Monodeiodination to Triiodothyronine to Nuclear Triiodothyronine Receptor Saturation in Pituitary, Liver, and Kidney of Hypothyroid Rats

FURTHER EVIDENCE RELATING SATURATION OF PITUITARY NUCLEAR TRIIODOTHYRONINE RECEPTORS AND THE ACUTE INHIBITION OF THYROID-STIMULATING HORMONE RELEASE

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ABSTRACT Injections of triiodothyronine (T_3) and thyroxine (T_4) into chronically hypothyroid rats were used to evaluate the contribution of intracellular T_4 to T_3 conversion to nuclear T_3 in pituitary, liver, and kidney, and to correlate the occupancy of pituitary nuclear T_3 receptors with inhibition of thyroid-stimulating hormone (TSH) release. Injection of a combination of 70 ng T_3 and 400 ng T_4/100 g body wt resulted in plasma T_3 concentrations of 45±7 ng/dl (mean±SD) and 3.0±0.4 μg/dl T_4 3 h later. At that plasma T_3 level, the contribution of plasma T_3 to the nuclear receptor sites resulted in saturation of 34±7% for pituitary, 27±5% for liver, and 33±2% for kidney. In addition to the T_3 derived from plasma T_3, there was additional T_3 derived from intracellular monodeiodination of T_4 in all three tissues that resulted in total nuclear occupancy (as percent saturation) of 58±11% (pituitary), 36±8% (liver), and 41±11% (kidney), respectively. The percent contribution of T_3 derived from cellular T_4 added 41% of the total nuclear T_3 in the pituitary which was significantly higher than the contribution of this source in the liver (24%) or the kidney (19%). 3 h after intravenous injection of increasing doses of T_3, the plasma T_3 concentration correlated well with both the change in TSH and the nuclear occupancy, suggesting a linear relationship between the integrated nuclear occupancy by T_3 and TSH release rate. The contribution of intrapituitary T_4 to T_3 conversion to nuclear T_3 was accompanied by an appropriate decrease in TSH, supporting the biological relevance of nuclear T_3. Pretreatment of the animals with 6-*n*-propylthiouracil before T_4 injection decreased neither the nuclear T_3 derived from intrapituitary T_4 nor the subsequent decrease in TSH.

These results indicate that intracellular monodeiodination of T_4 contributes substantially to the nuclear T_3 in the pituitary of the hypothyroid rat, and suggest a linear inverse relationship between nuclear receptor occupancy by T_3 in the pituitary and TSH release rate. The data further indicate that T_4 to T_3 monodeiodination is considerably more important as a source of nuclear T_3 in the pituitary than in the liver and kidney. This provides a mechanism whereby the TSH secretion could respond promptly to a decrease in thyroid secretion (predominantly T_4) before a decrease in plasma T_3 would be expected to lead to significant metabolic hypothyroidism.

INTRODUCTION

There is considerable indirect evidence suggesting that interaction of triiodothyronine (T_3)[1] with a specific

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[1] *Abbreviations used in this paper*: αGPD, α-glycerophosphate dehydrogenase; MBC, maximal binding capacity; N/P, nuclear/plasma ratio; PTU, 6-*n*-propylthiouracil; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone.

nuclear thyroid hormone receptor is a critical event in the mechanism by which thyroid hormones produce many of their effects (1–9). This event has specifically been demonstrated for induction of growth hormone synthesis and inhibition of prolactin production in growth hormone (GH-1) cells and for stimulation of hepatic mitochondrial α-glycerophosphate dehydrogenase (αGPD) and malic enzyme by T_3 in rats (10–13). We have recently observed (14) that there is a similar excellent chronological and quantitative correlation between nuclear occupancy by T_3 and subsequent suppression of thyroid-stimulating hormone (TSH) after intravenous injection of 70 ng/100 g body wt T_3 to hypothyroid rats. When 800 ng thyroxine (T_4)/100 g body wt was injected, the identical TSH response was correlated with the same nuclear T_3 content as after 70 ng T_3/100 g body wt. Negligible quantities of T_4 were found bound to the pituitary nuclei. These data suggested that nuclear occupancy by T_3 may be the initial event in the process leading to the acute suppression of TSH release in hypothyroid animals after thyroid hormone administration (14–16). Furthermore, because negligible amounts of T_3 were found in the plasma during the first few hours after T_4 injection, the data suggested that there was a very active conversion of T_4 to T_3 in the pituitary with subsequent binding of the T_3 produced to the pituitary nuclei.

Because our laboratory has recently demonstrated (17) that some thyromimetic effects of T_4 do not appear to be associated with appropriate increases in serum T_3, the question of whether or not there is a significant quantity of intracellular T_4 monodeiodination to T_3 has arisen. The T_3 produced therefrom could then be bound to critical sites in the cell and produce hormone effects not manifested by an increase in the concentration of plasma T_3. The present studies were performed to determine the relative quantitative importance of this source of nuclear T_3 in three thyroid hormone-responsive tissues of hypothyroid rats.

METHODS

Male Sprague-Dawley rats were obtained from Zivic-Miller (Allison Park, Pa.) where they had thyroidectomy with parathyroid gland reimplantation 2–3 months before performance of all experiments. Hypothyroidism was established by plateau of weight gain, serum T_3 concentrations <10 ng/dl (normal rats, mean 38±19 ng/dl), and plasma or serum TSH concentrations >1,500 μU/ml (normal range, 50–150 μU/ml). ^{131}I-T_3 was obtained from Amersham Corp. (Arlington Heights, Ill.; 30 μCi/μg), ^{125}I-T_4 from Cambridge Nuclear Corp. (Billerica, Mass.; ≅5,600 μCi/μg). T_3 contamination of T_4 was <1% as estimated by affinity chromatography using specific T_3 antibody coupled to Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.) by a method we recently described (18). All injections were given to animals in a small aliquot of hypothyroid rat serum. In general, the volume of injection was <0.1 ml/100 g body wt. T_4 T_3 (both in free acid form) were obtained from Sigma Chemical Co. (St. Louis, Mo.). T_4 was <0.5% T_3 by weight as determined by radioimmunoassay (19, 20).

Estimation of plasma or serum T_3, T_4, and TSH. Plasma basal T_3 concentrations were measured by radioimmunoassay (19). The method previously used in our laboratory has been modified to use 25-μl aliquots of rat serum or plasma. Because of differences in nonspecific binding between rat and human serum, the standard curve included 25 μl of T_3-free rat serum prepared using a CG-400 anion-exchange resin (Mallinckrodt Inc., St. Louis, Mo.). Plasma or serum T_4 was measured as described previously using human serum in the standard curve (20). Serum TSH was assayed using reagents provided by the Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolic, and Digestive Diseases using the modifications for tracer preparation and purification which we have recently described (15). All other estimates of plasma or serum T_3 and T_4 concentrations were based on the specific activity of injected hormones (see below).

Preparation of cell nuclei. Nuclei from pituitary, hepatic, and renal tissues were prepared by centrifugation through 2.4 M sucrose, 3 mM $MgCl_2$ (21, 22). Before the 2.4 M sucrose centrifugation, the 1,000-g pellet was washed once with 0.32 M sucrose, 3 mM $MgCl_2$ containing 0.5% Triton X-100. Also, the 2.4 M sucrose nuclear pellet was washed once with 0.14 M unbuffered NaCl to remove loosely bound protein. This procedure minimized the nonspecific binding and decreased the specifically bound T_3 by <10% (9.5±1.0%, SD). The purity of nuclear preparations was verified by phase-contrast microscopy. In general, 2-g aliquots of liver and both kidneys were used for analyses. DNA was measured by Burton's method (23) modified by Giles and Myers (24). The yield of pituitary nuclear DNA averaged 6±1.4 (mean ±SD, n = 15) mg/g wet wt with recoveries ranging from 75 to 83%. Pituitary glands weighed from 8 to 12 mg. Hepatic nuclear DNA was 2.0±0.3 mg/g wet wt (recovery of 50±10%), and kidney nuclear DNA was 3.9±0.7 mg/g wet wt (recovery 58±10%).

Determination of the quantity of trapped plasma in pituitary tissues. The contribution of trapped plasma to tissue T_3 radioactivity was negligible in agreement with previous reports (25). In T_4-injected animals, however, the contamination by plasma ^{125}I-T_4 was significant. In studies of pituitary T_3 and T_4 content, the trapped plasma volume was evaluated by simultaneous injection of ^{131}I-bovine serum albumin labeled in our laboratory (14, 25). The contribution of plasma T_4 to pituitary nuclear radioactivity was shown to be eliminated by perfusion of the intact animals with 30 ml of cold saline (retrograde) through the abdominal aorta immediately after exsanguination. The plasma ^{125}I-T_4 still provided a considerable contribution to the extranuclear radioactivity. However, because the goal of the present studies was to evaluate the intracellular T_3 (not T_4), and this was not significantly influenced by trapped plasma, this perfusion technique was used throughout. This allowed us to inject ^{131}I-T_3 and ^{125}I-T_4 simultaneously. Our previous studies have shown that from 0 to 10% of nuclear radioactivity in the pituitary gland was T_4 2–4 h after ^{125}I-T_4 administration (14).

Specificity of nuclear thyroid hormone binding. The specificity of nuclear binding was assessed in pilot experiments. These showed that in the nuclei from pituitary, liver, and kidney, the binding of tracer T_3 was decreased to <2.5% of its value by simultaneous injection of 20 μg T_3/100 g body wt. In similar experiments performed with ^{125}I-T_4-injected animals, the pituitary nuclear radioactivity was reduced to <1.5% of the value found with tracer T_4 alone by injection of 20 μg of T_3. In liver and kidney, injection of 20 μg T_3 with

^{125}I-T_4 led to a reduction of total radioactivity to 37 and 30%, respectively, of the control values, but chromatographic analysis of the nuclear radioactivity showed that this was virtually all T_4, i.e., all the ^{125}I-T_3 was specifically bound to limited capacity binding sites. The ^{125}I-T_4 found in the nuclear preparation under these circumstances presumably represents contamination of the nuclei with plasma and/or cytosol. Assuming that this did represent plasma contamination, it corresponded to 0.25 μl of plasma/mg DNA in the liver (0.52 μl/g wet wt) and 0.12 μl/mg DNA in the kidney (0.39 μl/g wet wt).

Identification of labeled compounds in nuclei, plasma, and injected ^{125}I-T_4. Radioactivity was extracted from nuclei using acid-butanol as previously described (14). T_3 was separated from T_4 and identified by paper chromatography in tertiary amyl alcohol/hexane/2-N NH_4OH (6:1:5), as we have previously described (26, 27). Nuclear radioactivity was also chromatographed in *n*-butanol saturated with 2 N NH_4OH in some studies. The locations of ^{125}I-T_3 and ^{125}I-T_4 were determined by staining unlabeled T_3 and T_4 (which had been added to the origin) with diazotized sulfanilic acid. ^{131}I-T_3, either injected simultaneously or added at the beginning of the extraction procedure, was used as an additional marker and to monitor recovery.

2–3 h after ^{125}I-T_4 injection, the plasma ^{125}I-T_4/^{125}I-T_3 ratio was very high (150–200:1). Accurate determination of the ^{125}I-T_3 present required prior elimination of most of the ^{125}I-T_4 because chromatographic separation of T_3 and T_4 on paper generates 0.6–0.8% of the T_4 as T_3 (27). Elimination of T_4 was achieved by extracting 100 μl of plasma with specific anti-T_3 antibody conjugated to Sepharose as has been described previously (18). In the washed Sepharose pellet, ^{125}I-T_4 was reduced to <1% of the original quantity while >30% of the T_3 was retained as judged by the ^{131}I-T_3 present (Appendix). The anti-T_3-antibody-Sepharose conjugate was then extracted with methanol:2 N NH_4OH (90:10) and chromatographed on paper in tertiary amyl alcohol: hexane:2-N NH_4OH as above. The contribution of artifactual T_4 to T_3 conversion during chromatography to the measured T_3 was therefore negligible. The center of the ^{131}I-T_3 peak on paper chromatography was used for quantitation of ^{125}I-T_3. The fraction of the original ^{131}I-T_3 present in this area was assessed by comparison with standards appropriately corrected for geometry and counting efficiency. The ^{125}I-T_3 originally present could then be estimated from this ^{131}I-T_3 recovery figure.

Inasmuch as the goal of the study was to estimate what fraction of nuclear ^{125}I-T_3 was derived from intracellular T_4 to T_3 conversion, that portion of nuclear ^{125}I-T_3 coming from plasma had to be quantitated and subtracted. The sources of plasma ^{125}I-T_3 were two: one was a fraction of the ^{125}I-T_3 contaminating the injected ^{125}I-T_4 still present in plasma, and the second was ^{125}I-T_3 derived from intracellular monodeiodination of ^{125}I-T_4 which had entered the plasma compartment. Because ^{131}I-T_3 was injected simultaneously with ^{125}I-T_4, in most experiments, and because the percent contamination of each ^{125}I-T_4 tracer with T_3 was known, it was possible to determine the relative contribution of each of these two sources to the plasma ^{125}I-T_3 present. At 2 h after injection of ^{125}I-T_4, >95% of the ^{125}I-T_3 was derived from contamination (14); at 3 h, contaminating ^{125}I-T_3 accounted for ≅40% of the plasma ^{125}I-T_3. To determine what fraction of the nuclear ^{125}I-T_3 was derived from the total plasma ^{125}I-T_3, the simultaneously injected ^{131}I-T_3 was used to determine the nuclear ^{131}I-T_3 (counts per milligram DNA) to plasma ^{131}I-T_3 (counts per milliliter) ratio. The total plasma ^{125}I-T_3 (counts/milliliter) × ^{131}I-T_3 nuclear/plasma ratio gave the number of ^{125}I-T_3 (counts per milligram DNA) present in the nucleus that was derived from plasma ^{125}I-T_3. These counts were subtracted from the total nuclear ^{125}I-T_3 and the locally generated T_3 was estimated as follows: nuclear ^{125}I-T_3/total ^{125}I-T_4 × 2 × dose of T_4 (nanograms) × 651/777) based on previous considerations (14). A complete example of these calculations is presented in the Appendix.

Specific experimental protocols

Assessment of the maximal nuclear T_3-binding capacity (MBC). MBC was measured in vivo in hypothyroid rats using an isotopic approach (3). In previous experiments it was found that from 2 h on after the injection of 70 ng T_3/100 g body wt the nuclear/plasma (N/P) ratio of T_3 was maximal and constant representing equilibrium of T_3 between receptor sites and plasma (14). Thus, plasma T_3 concentration is proportional to the concentration of T_3 to which the nuclear binding sites are exposed in animals with negligible plasma T_4 concentrations. Therefore, the bound T_3 is in rapid exchange with the free T_3 around the receptor, and between this T_3 pool and plasma. This type of equilibrium is a requisite for analysis of the nuclear T_3 content by isotopic techniques (28). 3 h was thus chosen as a reliable time-point mainly because a constant N/P ratio was clearly established and because it allowed enough time for plasma TSH to reflect the final result of integrated changes in the rate of TSH release. After suitable pilot experiments, groups of three hypothyroid rats were injected intravenously with ^{125}I-T_3 containing increasing doses of unlabeled T_3. The doses used were 19, 70, 210, and 630 ng/100 g body wt. An additional dose of 20 μg/100 g body wt was injected into another group to allow calculation of nondisplaceable (nonspecific) binding. At 3 h after injection, plasma, pituitary, liver, and kidney tissue was obtained, and nuclei were prepared as described. Plasma samples were obtained at time 0, and TSH measured in the same assay (using ^{131}I-TSH) as those samples obtained at the time the animals were sacrificed.

Based on the principles of reversible binding, and the law of mass action, the reaction of T_3 with nuclear receptor (N) can be viewed as:

$$[T_3] + [N] \underset{k2}{\overset{k1}{\rightleftarrows}} [NT_3], \tag{1}$$

and at equilibrium

$$Kd = \frac{[T_3]\cdot[N]}{[NT_3]}, \tag{2}$$

where N represents the number of unoccupied sites and NT_3 the number of occupied sites or bound T_3, and Kd the dissociation constant or half-maximal saturation concentration of free T_3 at the receptor. Because the in vivo system is in a rapid exchange at equilibrium, and assuming no rapid variations of dialyzable fraction of the hormone, the total plasma T_3 concentration that saturates half of the sites is proportional to the concentration of free hormone around the receptors so that Kd can be replaced by $[T_3]_{50}$. On the other hand, since N is unknown and the goal is to know the total number of sites, i.e. the MBC, N can be replaced by [MBC − T_3N]. Substitution in Eq. 2 yields the following expression:

$$[T_3]_{50} = \frac{[T_3]\cdot[\mathrm{MBC} - NT_3]}{[NT_3]}, \tag{3}$$

which on rearrangement gives a linear equation:

$$NT_3 = \mathrm{MBC} - [T_3]_{50}\cdot\frac{NT_3}{[T_3]}. \tag{4}$$

Thus the experimental data can be fitted to a straight line with the y intercept being the MBC and the slope the half-maximal saturation plasma concentration (29). Further rearrangements of the above give

$$\frac{NT_3}{MBC} = \frac{[T_3]}{[T_3] + [T_3]_{50}}, \quad (5)$$

which allows the estimation of the nuclear saturation at any plasma concentration.

Analysis of the correlations of nuclear T_3 receptor occupancy and suppression of TSH release in hypothyroid rats. A plot of the plasma T_3 and TSH concentrations 3 h after T_3 injection and the fact that there is a short latency between thyroid hormone injection and TSH suppression (14, 15) suggested that a saturable system might be involved relating plasma T_3 and the TSH release rate. If so, the data could then be fitted in the following equation as has been done for αGPD and malic enzyme (13):

$$\Delta TSH = \frac{[T_3]}{[T_3] + [T_3]_{50}}, \quad (6)$$

where ΔTSH is the decrease in TSH at 3 h after T_3 and $[T_3]$ is plasma T_3. $[T_3]_{50}$ is the plasma $[T_3]$ at 3 h after injection of a T_3 dose which causes 50% decrease in TSH release. To determine the maximal attainable ΔTSH(ΔMAX), Eq. 6 can be written

$$\frac{\Delta TSH}{\Delta MAX} = \frac{[T_3]}{[T_3] + [T_3]_{50}}, \quad (7)$$

and this can be rearranged to yield

$$\Delta TSH = \Delta MAX - \frac{\Delta TSH}{[T_3]} \cdot [T_3]_{50}. \quad (8)$$

Again the data could be fitted to this linearized relationship and ΔMAX and $[T_3]_{50}$ can be obtained.

To compare the plasma T_3 concentration at 3 h with both the fraction of maximal suppressible TSH and of nuclear occupancy, both NT_3 and ΔTSH/ΔMAX were plotted against the log of $[T_3]$. The $[T_3]_{50}$s determining the shapes of the two curves were obtained by the least squares method for Eq. 4 and 7 for NT_3 and ΔTSH, respectively.

Effect of 6-n-propylthiouracil (PTU) on nuclear T_3 derived from intracellular deiodination of T_4. PTU was given to hypothyroid rats in a dose of 1 mg/100 g body wt 16 and 2 h before the experiments. Two groups of animals, control and PTU treated, were injected with 800 ng/100 g body wt ^{125}I-T_4 and killed 2 h later. Pituitary glands and livers were removed and analyzed for nuclear T_3 as described. This dose of PTU has been shown previously to significantly decrease plasma T_3 concentrations in euthyroid rats 14 and 24 h after injection, and to abolish most of the mitochondrial αGPD response of hypothyroid rats to 800 ng/100 g body wt T_4/day for 12–13 days (17). Statistical analyses were performed by Student's t test on paired or unpaired samples as stated (30).

RESULTS

MBC for T_3 in the nuclei of pituitary, liver, and kidney tissues from hypothyroid rats. The nuclear content of specifically bound T_3 in pituitary, liver, and kidney tissues was significantly related to the plasma T_3 concentration. When nuclear T_3 was plotted against the N/P T_3 ratio, a linear relationship was apparent with $r = -0.90$ for pituitary ($P < 0.001$), -0.93 for liver ($P < 0.001$), and -0.87 for kidney ($P < 0.001$; Fig. 1). The MBC were 0.96, 0.43, and 0.13 ng T_3/mg DNA for pituitary, liver, and kidney, respectively (Fig. 1). In the same figure, the $[T_3]_{50}$ was 0.92, 0.98, and 0.66 ng T_3/ml for pituitary, liver, and kidney, respectively. Both pituitary and liver nuclei have a lower apparent affinity for T_3 than do kidney nuclei.

To emphasize the relationships between plasma T_3 and nuclear T_3 content, in Fig. 2 both variables were plotted for the three tissues examined. The dotted lines represent the 95% confidence limits of the regression coefficient ($[T_3]_{50}$). The curves are quite similar considering the dispersion obtained. The results indicate that over 90% of nuclear T_3 saturation is obtained with plasma levels of 10 ng/ml, and one-half maximal occupancy is obtained with plasma T_3 concen-

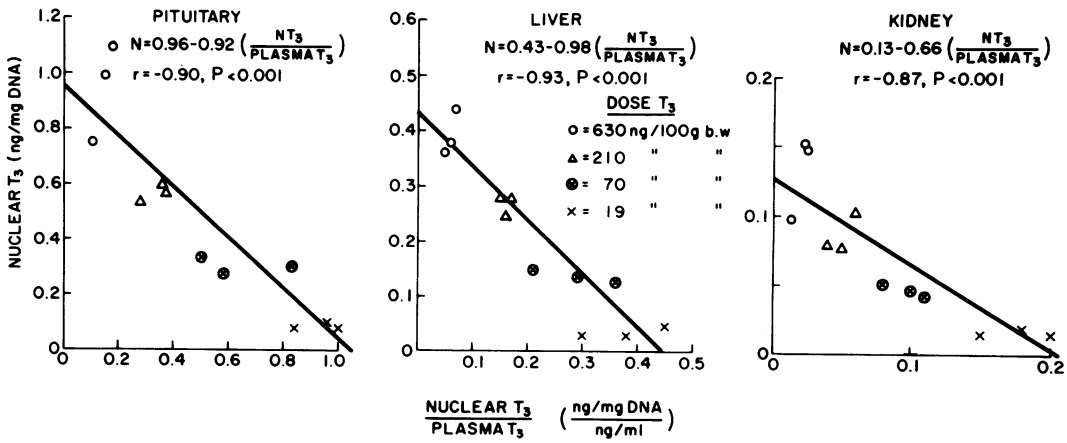

FIGURE 1 Nuclear T_3 vs. the nuclear T_3/plasma T_3 ratio 3 h after injection of various doses of T_3 to chronically hypothyroid rats. Lines were constructed by least squares regression analysis. Each point represents results of a single animal.

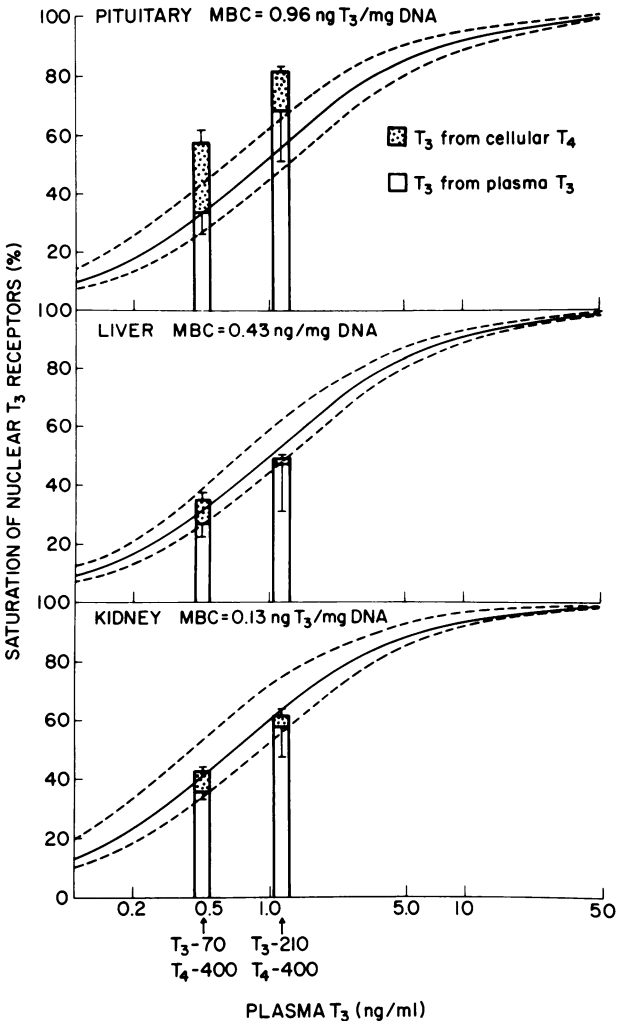

FIGURE 2 Comparison of the saturation of nuclear T_3 receptors in pituitary, liver, and kidney 3 h after injection of various doses of T_3 with that found after injection of 400 ng T_4 + 70 or 210 ng T_3/100 g body wt in chronically hypothyroid rats. The bar graphs depicting total nuclear T_3 (from plasma T_3 and T_3 derived from local T_4 to T_3 conversion) are superimposed on the curves obtained from the data presented in Fig. 1. The brackets indicate mean 1 SD. Plasma and nuclear T_3 were calculated from the known specific activity of injected ^{131}I-T_3 or ^{125}I-T_4.

trations of 0.92 ng/ml in the pituitary, 0.98 in the liver, and 0.66 in the kidney. The bar graphs superimposed on this figure are discussed below.

Relative contribution of injected T_4 to the nuclear T_3 in hypothyroid animals given 70 ng/100 g body wt T_3. When 400 ng/100 g body wt ^{125}I-T_4 was given together with 70 or 210 ng ^{131}I-T_3, the degree of nuclear saturation obtained for T_3 alone was similar for all of these tissues (bar graph in Fig. 2). When the contribution of ^{125}I-T_3 derived from local ^{125}I-T_4 tissue conversion is considered, it can be appreciated that in the pituitary it raised the nuclear saturation beyond the expected 95% confidence limits, whereas in the liver and kidney the increments were within the expected experimental variation (although by paired t test at the 70 ng T_3-400 ng T_4 dose this contribution was significant for both tissues (Table I)). The plasma concentrations of T_4 and T_3, 3 h after intravenous injection of 70 ng T_3 + 400 ng T_4/100 g body wt were in the physiological range (0.45±0.07 ng T_3/ml and 3.0±0.4 μg T_4/dl, mean±SD) as estimated by the specific activity of injected isotopes.

The results of this experiment are analyzed in greater detail in Tables I–III. In Table I it is shown that at both T_3 dose levels, ^{125}I-T_4 contributed more T_3 to the pituitary nuclear T_3 receptors than it did in the liver and kidney ($P < 0.005$). Thus the degree of saturation of the pituitary nuclei was increased from 34 to 58% by injection of 400 ng/100 g body wt of T_4, whereas this source of T_3 only results in an increase in saturation from 27 to 36% in the liver and from 33 to 41% in the kidney. All contributions of T_4 to nuclear T_3 are reduced proportionately when the dose of T_3 given is trebled, suggesting that the T_3 derived from these two sources can mix in an intracellular compartment before binding to the nuclear receptor.

An evaluation of the N/P T_3 ratios in the three tissues is presented in Table II. The pituitary N/P ratio for T_3 derived from plasma alone was 0.73±0.22 but when the contribution of T_3 from T_4 was added, this was significantly higher, 1.03±0.26 ($P < 0.005$). The increase in the N/P for liver nuclei due to T_3 derived from T_4 was also significant ($P < 0.025$) but modest (0.25–0.27), and there was no significant effect in the kidney.

Because the contribution of T_4 to nuclear T_3 from intracellular monodeiodination is likely to be dependent on the T_4 concentration within the cell, and this in turn is related to plasma T_4 concentration, it was of interest to evaluate the nuclear ^{125}I-T_3 to plasma ^{125}I-T_4 ratio under the same circumstances (Table III). This ratio, although smaller than the N/P T_3 ratio by a factor of 100, is significantly higher for pituitary nuclei than it is for those of either liver or kidney at either dose of T_3 given ($P < 0.005$). As expected, the nuclear T_3/plasma T_4 ratio decreased in all three tissues when the dose of T_3 was increased, reflecting the limited binding capacity of the nuclear T_3 receptors.

Correlation of TSH suppression with nuclear and plasma T_3. Severely hypothyroid rats seemed suitable models to evaluate the quantitative relationships between nuclear T_3 and TSH suppression because the component of nuclear T_3 derived from local T_4 to T_3 conversion is negligible. Fig. 3 shows the correlation between ΔTSH and ΔTSH/[T_3] as discussed in Methods. The maximal theoretical suppression obtained 3 h after the T_3 injection was 95%, and the $[T_3]_{50}$ was 0.62 ng/ml ($r = -0.91$, $P < 0.001$).

TABLE I

Source of Nuclear T_3 in Pituitary, Liver, and Kidney of Hypothyroid Rats 3 h after Intravenous ^{131}I-T_3 (70 or 210 ng/100 g Body wt) and 400 ng/100 g Body wt ^{125}I-T_4

Dose			Plasma hormone concentrations 3 h after dose		Source of nuclear T_3 in pituitary (P), liver (L), and kidney (K) % of MBC																
					T_3 from plasma						T_3 from intracellular T_4 to T_3 monodeiodination						Nuclear T_3 from both sources % of MBC					
								P values‡						*P* values‡						*P* values‡		
								P vs.	P vs.	L vs.				P vs.	P vs.	L vs.				P vs.	P vs.	L vs.
T_3	T_4		T_3*	T_4*	P	L	K	L	K	K	P	L	K	L	K	K	P	L	K	L	K	K
ng/100 g body wt			*ng/dl*	*μg/dl*																		
70	400	Mean	45	3.0	34	27	33	NS	NS	NS	24	8.5	7.8	<0.005	<0.005	NS	58	36	41	<0.005	<0.05	NS
		SD	7	0.4	7	5	2				4	2.0	0.5				11	8	2			
210	400	Mean	112	2.4	68	46	55	<0.05	NS	<0.025	13	3.5	3.5	<0.001	<0.005	<0.025	81	50	59	<0.025	<0.05	NS
		SD	34	0.4	15	14	11				1	0.6	0.6				15	13	10			

* Estimated from the specific activity of the respective injected iodothyromine.
‡ Paired *t* test.

Fig. 4 was generated by plotting the 3-h ΔTSH/ΔMAX (i.e. the fraction of maximal attainable suppression) and nuclear T_3 as a function of plasma T_3. The regression lines were obtained from Eqs. 4 and 7 (Figs. 1 and 3). The 95% confidence limit (dotted lines) of nuclear T_3 as a function of plasma T_3 is plotted as an index of the expected experimental variation. It can be appreciated that the plasma T_3 concentration at 3 h bears an almost superimposable relationship with both the nuclear occupancy and the maximal suppression of TSH obtained 3 h after each dose. 95% nuclear saturation was obtained with a plasma T_3 concentration of approximately 20 ng/ml, similar to that which produces 95% inhibition of TSH release.

TABLE II

N/P T_3 Ratios 3 h after Injection of ^{131}I-T_3 (70 or 210 ng/100 g Body wt) and 400 ng/100 g Body wt ^{125}I-T_4 into Hypothyroid Rats

Iodothyronine dose		N/P T_3 ratios (ng T_3/mg DNA)/(ng T_3/ml plasma) Mean±SD		
T_3	T_4	Pituitary	Liver	Kidney
ng/100 g body wt				
Nuclear T_3 from plasma T_3 only (^{131}I-T_3)				
70	400	0.73±0.22	0.25±0.04	0.10±0.01
210	400	0.59±0.05	0.18±0.03	0.07±0.01
Total nuclear T_3 (^{131}I-T_3 + ^{125}I-T_3)				
70	400	1.03±0.26*	0.27±0.04‡	0.10±0.01
210	400	0.70±0.07*	0.19±0.03‡	0.07±0.01

* $P < 0.005$ or $P < 0.025$ (‡), for difference from nuclear T_3/plasma T_3 ratio calculated using the plasma T_3 contribution (^{131}I-T_3) alone.

Effect of the increment in nuclear T_3 provided by T_4 to the inhibition of pituitary TSH release. In Table IV are shown the biological effects of administration of 70 ng T_3/100 g body wt with or without 400 ng/100 g body wt T_4. The percent saturation of the nuclear T_3 receptors by T_3 from both plasma and intracellular T_4 to T_3 conversion is also presented. 70 ng T_3 alone resulted in 32% saturation of the nuclear T_3 receptors at 3 h after administration, and a 39% decrease in plasma TSH. Injection of T_3-70 + T_4-400 ng/100 g body wt resulted in a higher nuclear receptor occupancy of 58% ($P < 0.025$), with a concomitant reduction in plasma TSH equivalent to 61% of the basal TSH concentration ($P < 0.025$ compared with T_3 alone). At a dosage level of 210 ng T_3/100 g body wt, the nuclear T_3 saturation was 60% and the decrease in plasma TSH was equivalent to 77% of the basal TSH concentration, probably reflecting a higher previous nuclear occupancy as has been discussed (14). Addition of 400 ng T_4/100 g body wt did not result in a significantly higher nuclear saturation, nor in a further decrease in plasma TSH. However, changes at this level of nuclear T_3 and ΔTSH are more difficult to detect.

Effect of PTU pretreatment on the acute contribution of injected T_4 to pituitary and hepatic nuclear T_3. The results of T_4 injections given to control and PTU-treated hypothyroid rats are presented in Table V. In the pituitary, 800 ng T_4/100 g body wt increased nuclear T_3 0.42 ng/mg DNA. This was identical to the quantity of nuclear T_3 derived from 800 ng T_4/100 g body wt in PTU-pretreated rats. Thus, the lack of effect of PTU pretreatment on T_4-induced TSH suppression was reflected in the absence of effect on nuclear T_3 as well. Under the acute conditions of the

TABLE III

Nuclear ^{125}I-T_3/Plasma ^{125}I-T_4 Ratios in Pituitary, Liver, and Kidney of Hypothyroid Rats after Administration of ^{131}I-T_3 (70 or 210 ng/100 g Body wt) and 400 ng/100 g Body wt ^{125}I-T_4

Iodothyronine dose		Nuclear ^{125}I-T_3/plasma ^{125}I-T_4 ratios (ng/mg DNA)/(ng/ml)					
		Mean±SD			*P* values*		
T_3	T_4	Pituitary × 10^{-3}	Liver × 10^{-3}	Kidney × 10^{-3}	P vs. L	P vs. K	L vs. K
ng/100 g body wt							
70	400	7.9±1.3	1.2±0.2	0.4±0.1	<0.005	<0.005	<0.01
210	400	5.3±1.0	0.6±0.2	0.2±0.1	<0.005	<0.005	<0.025
P values (unpaired *t* test) 70–400 vs. 210–400		<0.025	<0.005	NS			

* *P* (unpaired *t* test).

experiment, PTU pretreatment did not alter the small contribution of local T_4 monodeiodination to T_3 to hepatic nuclear T_3.

DISCUSSION

To analyze the relationship between nuclear content of T_3 in the pituitary and the resulting suppression of TSH, the maximal T_3-binding capacity of the pituitary nuclei must be known. Using an approach similar to that of Oppenheimer et al. (3, 4), we have observed that the MBC of the pituitary nuclei of hypothyroid rats is 0.96 ng T_3/mg DNA, whereas it is 0.43 in hepatic nuclei and 0.13 in nuclei from kidney tissue (Fig. 1). The results for MBC and apparent *Kd* in hypothyroid animals differ slightly in some tissues (liver and kidney) from those previously reported in euthyroid animals (21), but these differences do not affect the conclusions of the present study. Because the T_3 specifically bound to the nucleus either coming from plasma or from local T_4 to T_3 conversion can be fully displaced by an excess of cold T_3, we may conclude that all the nuclear binding sites are available to both sources of T_3. It follows that the approach of saturation analysis gives a good estimation of the MBC. How-

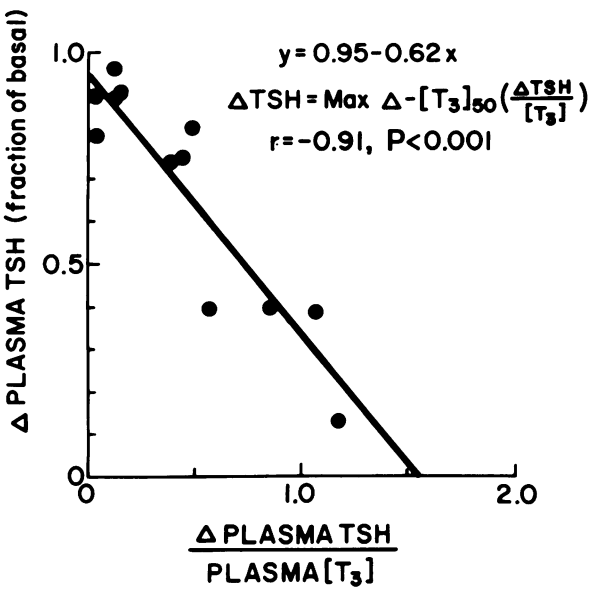

FIGURE 3 Δ Plasma TSH as a function of ΔTSH/[T_3] ratio in hypothyroid animals given the quantities of T_3 depicted in Fig. 1 3 h before analysis. Each point is the result of the response of a single animal. The maximum ΔTSH in the equation corresponds to the maximal obtainable suppression of TSH, and the $[T_3]_{50}$ is the plasma T_3 concentration associated with the 50% decrease in plasma TSH concentration.

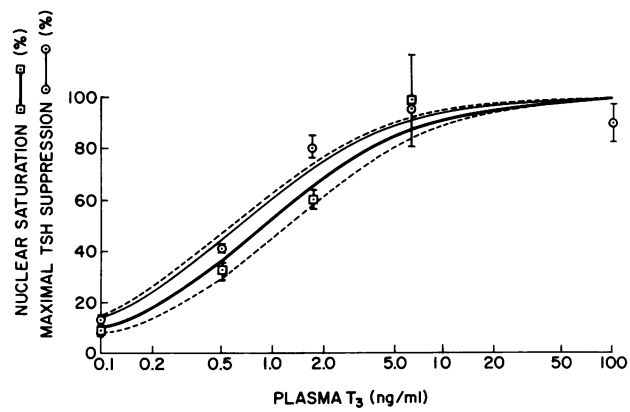

FIGURE 4 Pituitary nuclear saturation and maximal TSH suppression plotted as a function of plasma T_3 concentration in chronically hypothyroid rats injected with various doses of T_3 3 h before analysis of pituitary T_3 and plasma TSH. The heavy line (□) is the curve describing nuclear saturation vs. plasma T_3, and the dotted lines indicate the 95% confidence limits of the regression coefficient. The thin line (○) relates TSH suppression to plasma T_3 in the same animals.

TABLE IV

Biological Effects of Nuclear Pituitary T_3 Generated Intracellularly from T_4 in Hypothyroid Rats 3 h after Intravenous Administration of Iodothyronine(s)

Dose				
T_3	T_4	n	Nuclear T_3 (% saturation)	Decrease in plasma TSH (% of basal TSH concentration)
ng/100 g body wt				
70	0	3	32±3	39±1
70	400	4	58±11	61±10
P			<0.025	<0.025
210	0	3	60±4	77±4
210	400	4	81±15	78±9
P			NS	NS

Mean±SD.

ever, as discussed below, this approach may not give an accurate estimate of the degree of saturation at endogenous levels of plasma T_3 in euthyroid animals.

For reasons stated in the Introduction, we have speculated that a local mechanism for generation of T_3 from T_4 should be quantitatively more important as a source of nuclear T_3 in the pituitary than in the liver of the rat. The results in Fig. 2 and Tables I–III indicate that when T_4 is given together with T_3, the increment in the pituitary nuclear T_3 content is three- to fourfold that found in the liver and kidney. Tables I–III show in detail the relative importance of this mechanism for T_4 deiodination in these three tissues. Several "artifactual" explanations can be readily excluded for the presence of T_3 in the nuclei of these tissues soon after T_4 injection. The quantity of locally derived T_3 in the nuclei is determined from the net ^{125}I-T_3 present over that which can be accounted for by the small quantities of ^{125}I-T_3 present in plasma. Therefore, the accurate determination of plasma ^{125}I-T_3 is quite critical. Inasmuch as the ^{125}I-T_3 can be followed throughout the identification and quantitation procedure using the injected ^{131}I-T_3 (see Appendix for calculations), we are unable to postulate a mechanism by which the recoveries of the two T_3 molecules, labeled with different isotopes, could be different. Because the ^{131}I-T_3 N/P ratios determined for at least pituitary and liver are quite similar to those reported by other laboratories (21), it seems unlikely that both could be underestimated. If, in some way, plasma ^{125}I-T_3 were underestimated, it would result in an overestimation of locally produced nuclear T_3 from T_4. This overestimation would be greatest in pituitary nuclei due to the higher N/P ratio present in this tissue in comparison with liver and kidney. It would be impossible to interpret the present experiments accurately without a precise method for specific isolation of small amounts of ^{125}I-T_3 from the large quantities of plasma ^{125}I-T_4 such as the T_3-antibody-Sepharose conjugate technique used here (18). The possibility of a rapid and disproportionately greater ^{125}I-T_4 monodeiodination (with a subsequent peak of plasma ^{125}I-T_3) on the first passage of T_4 through the tissues can also be eliminated. Because the plasma ^{125}I-T_3 derived from this reaction would disappear at the same rate as the initially injected ^{131}I-T_3, we should have seen fivefold higher quantities of ^{125}I-T_3 than were actually observed 2–3 h after ^{125}I-T_4 injection. As the unlabeled T_4 contained even less contaminating T_3 than did the tracer (<1% as opposed to <0.5%), no significant physiological effect can be attributed to this source.

TABLE V

*Lack of Effect of 6-N-Propylthiouracil (PTU) Pretreatment on the Acute Contribution of Tissue T_4 to Pituitary and Hepatic Nuclear T_3**

Pituitary			Liver		
Control	PTU	*P*	Control	PTU	*P*
Nuclear T_3, *ng/mg DNA*					
0.42±0.04	0.42±0.08	NS	0.07±0.02	0.06±0.01	NS
Plasma TSH response, *% of basal concentration*					
67.5±8.1	59.6±15.3	NS	—	—	—

Mean±SD.

* Hypothyroid rats received 800 ng/100 g body wt T_4 with or without intraperitoneal injections of 1 mg/100 g PTU 16 and 2 h previously. Animals were killed 2 h after T_4 injection.

The data demonstrating an apparently rapid rate of T_4 to T_3 conversion in pituitary tissue is at variance with recent in vitro studies. These have indicated that the rat kidney and liver are the most active on a weight basis (31), and, in fact, in vitro T_4 to T_3 conversion has not been previously demonstrated in pituitary tissue (32) despite suggestive in vivo data in other species (33–36). However, using improved technology (18), we have recently observed T_4 to T_3 conversion in pituitary homogenates at rates at least similar to the rat liver on a weight basis[2] under certain in vitro circumstances.

Of the total nuclear T_3 in animals receiving 70 ng T_3 and 400 ng T_4, 41% is derived from local T_4 monodeiodination in pituitary, whereas only 23 and 19% of nuclear T_3, respectively, is derived from this source in liver and kidney. In rats given 210 ng T_3 plus 400 ng T_4/100 g body wt, the relative contribution of T_4 is less, but again, much higher in the pituitary than in the liver. Correspondingly, the N/P

[2] Silva, J. E., T. E. Dick, and P. R. Larsen. Manuscript submitted for publication.

ratio for T_3 after T_4 is markedly higher for pituitary nuclei than it is for those of the liver and kidney. Although the biological significance of this T_3 is apparent in terms of pituitary nuclei, because it is associated with TSH suppression (14 and Table IV), there are no known rapid effects of T_3 on the hepatic or renal tissues to allow early estimates of the possible physiological significance of the T_3 derived from T_4 in these tissues. Thus, despite the in vitro capacity of hepatic and renal tissues to produce T_3 from T_4, the T_3 generated does not appear to contribute substantially to the nuclear T_3 in these tissues.

Because the apparent affinity of nuclear receptors for T_3 has been shown in vitro to be approximately the same in all tissues (21, 37), a fact we have also confirmed in vivo for the pituitary and liver in the present studies, the higher nuclear T_3 content after T_4 in the pituitary has to be explained on a different basis. The higher MBC of the pituitary (0.96 ng T_3/mg DNA) compared to the liver (0.43 ng T_3/mg DNA) cannot account for the difference either because expressing nuclear T_3 derived from T_4 as a percent of the MBC, i.e., in terms of saturation, the contribution of T_4 to nuclear T_3 in the pituitary is still significantly higher (threefold) than in the liver.

It can also be proposed that local T_4 to T_3 conversion and subsequent binding to the nucleus reaches equilibrium at very different intervals after T_4 injection in the tissues studied. This possibility also seems unlikely because it has been estimated that 10 min are required for establishment of an equilibrium between total tissue T_4 and plasma T_4 in rat pituitary, liver, and kidney (25, 38). We have found that the nuclear ^{125}I-T_3/plasma ^{125}I-T_4 ratio is about 20% higher at 3 h than at 2 h after injection of ^{125}I-T_4 in both pituitary and liver suggesting that neither tissue has reached equilibrium at 3 h. Therefore, calculation of nuclear T_3 from T_4 at 3 h does not overestimate (but could underestimate) the contribution of this process to the nuclear T_3. That this is, in fact, an underestimate is suggested by our recent results in euthyroid rats indicating that the peak of the ratio of intracellularly derived nuclear ^{125}I-T_3 to plasma ^{125}I-T_4 is not attained until 16–18 h after ^{125}I-T_4 injection.[3]

We have demonstrated that nuclear binding of the T_3 derived from T_4 to T_3 conversion can be completely blocked by 20 μg T_3/100 g body wt (14). These results and those of the present study showing a decrease in the fraction of nuclear T_3 derived fromT_4 as plasma T_3 concentration increases (Table I) indicate that the nuclear pools of T_3 derived from both sources are miscible. We would speculate that the relative proportions of each source of T_3 in the nuclei of the various tissues would be determined by the plasma concentrations of T_4 and T_3, the transfer rates of these hormones into the cell, the rate of intracellular T_4 to T_3 conversion in each tissue, and the characteristic subcellular distribution of the T_3 derived from both sources in that particular tissue. The weight of each of these factors in determining the sources of nuclear T_3 is not known and is currently under investigation.[4] Hepatic and renal tissues have been demonstrated to be quite active in T_4 monodeiodination, and yet our studies indicate that relatively small amounts of this locally produced T_3 are bound to the nuclear T_3 receptor. This is certainly in part influenced by the fact that <10% of total tissue T_3 is found in the nucleus in these tissues, whereas more than 40% of pituitary T_3 is nuclear (21).

There is a striking correlation between plasma T_3 3 h after injection with the nuclear occupancy and with TSH suppression (Fig. 4). Three facts allow us to extend this observation to say that there is an inverse linear relationship between the nuclear occupancy by T_3 and the rate of TSH release. First, there is a short delay (<1 h) between nuclear occupancy by T_3 and changes of TSH release rate (14, 15). Second, because of the short half-life of TSH (30 min), decreases in TSH release rate are rapidly reflected in the plasma TSH concentration. Third, the nuclear receptor occupancy by T_3 at 3 h follows closely the time of the peak nuclear occupancy, and this time is not affected by increasing T_3 doses over the range used here (14, 28). Therefore, the integrated nuclear occupancy from 0–3 h is linearly related to the nuclear occupancy at 3 h after T_3 injection. These considerations provide strong support for a linear relationship between nuclear T_3 receptor occupancy and the rate of TSH release and suggest that the correlation apparent in Fig. 4 is more than fortuitous.

The slight deviation to the left observed in the TSH suppression curve (Fig. 4) ($[T_3]_{50}$ 0.62 ng/ml for TSH suppression as opposed to the $[T_3]_{50}$ of 0.92 ng/ml for nuclear occupancy) can be accounted for by the latency between changes in nuclear occupancy and in the rate of release of TSH (14). Thus, the plasma TSH concentration at 3 h reflects the nuclear occupancy ≅60 min previously. For the range of T_3 doses examined, this is ≅20% higher at 2 than at 3 h. These data add further support to the concept that T_3 effects on TSH release, like those on growth hormone stimulation and prolactin suppression in pituitary cell cul-

[3] Silva, J. E., T. E. Dick, and P. R. Larsen. Manuscript submitted for publication. These data showed that in euthyroid rats the ratio of nuclear T_3 from local T_4 monodeiodination to plasma T_4 had equilibrated in pituitary, liver, and kidney by 16–18 h. At that time, the relative contributions of local T_4 monodeiodination to nuclear T_3 showed the pattern observed in the present experiments.

[4] Joffe, J., J. E. Silva, and P. R. Larsen. Manuscript in preparation.

tures (5, 11), and α-GPD and malic enzyme induction in liver tissue (12, 13) are initiated by events occurring in the nucleus. As was demonstrated in our earlier report (14) and confirmed above, the acute suppression of TSH after T_4 can also be correlated with the quantity of pituitary nuclear T_3, further supporting this hypothesis. However, as with the data for αGPD and malic enzyme and growth hormone stimulation and prolactin suppression, the fact that two events are correlated in time and magnitude cannot be used as evidence of causality because the precise nature of the interrelationship between the two events is not known.

As mentioned, hepatic and renal tissues are thought to be the major sources of extrathyroidal T_3 production from T_4. In euthyroid rats or T_4-maintained hypothyroid rats, this deiodination process has been shown to be partly sensitive (~70%) to inhibition by PTU in doses similar to those used in these experiments (17, 39). However, we have repeatedly failed to demonstrate inhibition of acute local pituitary T_4 to T_3 conversion in PTU-treated animals. The explanation for this is not clear. It is possible that the mechanism for converting T_4 to T_3 in the hypothyroid pituitary is less sensitive to acute inhibition by PTU. Alternatively, PTU may not penetrate pituitary tissue, or may be inactivated therein. Whatever the reason, if our hypothesis that nuclear T_3 receptor binding is involved in suppression of TSH release is correct, then the contribution of T_4 to pituitary nuclear T_3 could not be depressed by PTU pretreatment, because this does not affect the acute TSH response to T_4 as we have shown previously (17) and confirmed in the present study. The lack of a significant decrease in hepatic nuclear T_3 due to local T_4 monodeiodination in acutely PTU-treated hypothyroid rats could be explained in a similar way and by the fact that the fraction of the T_3 derived from T_4 that binds to liver nuclei is so small. The often demonstrated inhibition of induction of hepatic αGPD associated with chronic T_4 + PTU administration to hypothyroid rats is better explained by a decrease in the nuclear T_3 fraction derived from plasma T_3 (substantially depressed under these circumstances) than by a decrease in the nuclear T_3 derived directly from intracellular T_4 monodeiodination. Likewise, the small increment in TSH or in thyroid releasing hormone responsiveness observed in PTU-treated euthyroid or hypothyroid rats and man seems better explained by the demonstrated decrease in serum T_3 than by inhibition of T_4 to T_3 conversion in the pituitary (17, 40–42). More data are needed in euthyroid rats to exclude completely a PTU effect on intrapituitary T_4 to T_3 conversion under these circumstances.

If local pituitary T_4 to T_3 conversion is an important source of pituitary nuclear T_3, if there is a linear relationship between nuclear T_3 receptor occupancy and the rate of TSH release, and if in the euthyroid rat the TSH secretion rate is only ≅10% of the maximal attainable in hypothyroidism (15), one must consider the possibility that in euthyroid rats, the pituitary nuclear receptor sites are saturated to a significantly greater extent than has been estimated previously (21). The latter estimate of 48% saturation of pituitary nuclear receptors was determined by saturation analysis using increasing doses of ^{125}I-T_3. This methodological approach is adequate to calculate the MBC because all the receptor sites are available to plasma T_3 as demonstrated by the full blockade of tracer T_3 binding (from plasma T_3 or tissue T_4) by an excess of cold T_3 (14). However, because the intrinsic in vivo affinity of these sites is unknown and the degree of the dilution of the T_3 entering the cell from plasma by the T_3 derived from tissue T_4 is also unknown, this method might considerably underestimate nuclear T_3 saturation in the pituitary, though probably only modestly underestimate that in the liver and kidney. An excellent example of this is seen in Fig. 2 where, using plasma T_3 concentrations and the N/P T_3 ratio alone, one would have estimated that pituitary nuclear receptors were only 34% saturated. The true pituitary nuclear saturation at this time was 58% due to the additional contribution of the T_3 derived locally from tissue T_4. This significant contribution was found in association with a plasma T_4 concentration in the physiological range for the rat (3 μg/dl). Using techniques similar to those of the present study, we have recently estimated that pituitary nuclear receptor saturation is ≅78% in euthyroid rats in agreement with this hypothesis. One-half of this nuclear T_3 was derived from intrapituitary T_4 monodeiodination.[3]

The above considerations have important physiological consequences. We have shown that a decrease in saturation of nuclear binding sites is associated with an increase in the acutely suppressed plasma TSH in hypothyroid animals (14). Presumably, a decrease in pituitary nuclear saturation due to a decrease in T_3 derived locally from T_4 would be similarly followed by an increased TSH release from the euthyroid pituitary. Thus, a decrease in plasma T_4 alone could lead to an increase in TSH release. Our present studies suggest that the contribution of T_4 to nuclear T_3 in the liver and kidney is so small that significant nuclear desaturation would not occur in these tissues as a result of a modest decrease in plasma T_4 alone. This could explain the circumstances in the iodine-deficient rat where an apparently euthyroid state is associated with a low plasma T_4, normal plasma T_3, and an elevated TSH (17, 43). Similar arguments can be applied to explain why patients with endemic goiter, a high plasma TSH, and normal plasma T_3 can appear euthyroid as well as why patients with early thyroid dysfunction with reduced plasma T_4, normal plasma T_3, and elevated TSH

are often asymptomatic in metabolic terms (44–47). It would appear that the presence of a system in the thyrotroph responsive to decreases in either plasma T_3 or T_4 would provide maximum protection against the onset of metabolic hypothyroidism in tissues such as the liver and kidney whether the threat to the euthyroid state is a result of primary thyroid disease or iodine deficiency.

APPENDIX

A sample calculation of nuclear T_3 in animals given ^{131}I-T_3 and ^{125}I-T_4 is given below. Suitable corrections for ^{131}I appearing in the ^{125}I spectrometer window and changes in geometry have been performed where indicated. The nuclear T_3 content was calculated based on the specific activity of injected iodothyronines as follows:

Because the nuclear ^{131}I was >95% ^{131}I-T_3, the nuclear T_3 from injected T_3 was:

$$\frac{\text{Nuclear } ^{131}\text{I counts}}{\text{Total injected } ^{131}\text{I}} \times \frac{1}{\text{mg DNA}} \times \text{dose } T_3 \text{ (ng)} = \text{ng } T_3\text{/mg DNA.} \quad (1)$$

The ^{125}I-T_3 in the nucleus was determined by paper chromatography using ^{131}I-T_3 in the nucleus as a recovery and localization standard (Methods).

Nuclear ^{125}I-T_3 (percent of ^{125}I-T_4 dose/mg DNA)

$$= \frac{^{125}\text{I-}T_3 \text{ counts selected area}}{^{131}\text{I-}T_3 \text{ counts selected area}} \times \text{total nuclear } ^{131}\text{I-}T_3 \text{ counts} \times \frac{1}{\text{mg DNA}} \times \frac{100}{\text{total injected } ^{125}\text{I}}.$$

To deduct from the above ^{125}I-T_3 that coming from the plasma, quantitation of the plasma ^{125}I-T_3 content was required. The T_3-Ab Sepharose conjugate (T_3-Ab-S) was used to reduce the ^{125}I-T_4 in plasma as described (14, 18). As an example in one rat:

	^{125}I	^{131}I
	counts/100 μl plasma	
Trichloroacetic acid precipitable	689,598 counts/min (100%)	10,242 counts/min (100%)
T_3-AB-S	7,240 counts/5 min (0.21%)	16,203 counts/5 min (31%)
T_3 area on paper	2,870 counts/10 min	28,370 counts/10 min (28%)

Plasma ^{125}I-T_3 (percent ^{125}I dose/ml)

$$\times \frac{2{,}870 \times 10}{0.28} \times \frac{1}{\text{Total } ^{125}\text{I counts injected}} \times 100 = 0.0039\% \ ^{125}\text{I/ml}$$

Since the N/P ^{131}I ratio was known, the contribution of plasma ^{125}I-T_3 to the nucleus could be determined. In the above rat,

$$\text{N/P } ^{131}\text{I-}T_3 = \frac{3{,}379 \text{ (counts/10 min per nucleus)}}{0.054 \text{ (mg DNA)}} \div 102{,}420 \text{ (counts/10 min per ml)} = 0.61.$$

The N/P for the 20 μg/100 g-injected rat was 0.016±0.003 (nonspecific binding). Therefore, the nuclear ^{125}I-T_3 from plasma ^{125}I-T_3 was:

$$(0.61 - 0.016) \times 0.0039\%\text{/ml} = 0.0023\% \text{ dose/mg DNA.}$$

In this example, the total nuclear ^{125}I-T_3 was 0.018% dose/mg DNA. Therefore the ^{125}I-T_3 derived from local T_4 monodeiodination was:

$$0.018 - 0.0023 = 0.0157\% \text{ dose/mg DNA.}$$

To convert to nanograms T_3:

$$0.0157 \times \frac{1}{100} \times 2 \times \frac{651}{777} \times 400 \text{ (ng) } T_4 \times \frac{315 \text{ (g)}}{100} = 0.33 \text{ (ng } T_3\text{/mg DNA).}$$

To allow estimates of the dispersion of the data, the detailed calculation of pituitary nuclear T_3 in the experiment in which 70 ng ^{131}I-T_3 plus 400 ng ^{125}I-T_4 was given is presented as follows: (results are the mean (±SD) of four rats): Injected ^{125}I counts, 61,688,400 cpm/100 g body wt; Injected ^{131}I counts, 657,800 cpm/100 g body wt; Animal weight, 340±17 g; Pituitary nuclear DNA, 0.047±0.009 mg; Total N ^{125}I-T_3, 11,063±2,210 (counts/10 min); Total N ^{131}I-T_3, 1,470±372 (counts/10 min); Total plasma trichloroacetic acid-precipitable counts at 3 h: ^{125}I, 45,199,090±1,852,365 counts/10 min per ml; ^{131}I, 61,326±9,015 counts/10 min per ml; Plasma ^{125}I-T_3: (*a*) from contamination, 63,446±10,881 counts/10 min per ml (1% of ^{125}I-T_4 was ^{125}I-T_3), (*b*) from $T_4 \rightarrow T_3$ in extrapituitary tissues, 98,578±31,992 counts/10 min per ml (0.15 ng/ml), Total, 161,500±39,905 counts/10 min per ml. ^{131}I-T_3 N/P ratio, 0.51±0.12; Total nuclear ^{125}I-T_3, 283,150 ±50,337 counts/10 min per mg DNA; From plasma, 82,847±26,996 counts/10 min per mg DNA; Local $T_4 \rightarrow T_3$ conversion, 201,350±41,948 counts/10 min per mg DNA, (9.6±2) × 10^{-3}% ^{125}I dose/mg DNA, 0.23±0.05 ng T_3/mg DNA; From injected T_3, 0.33±0.08 ng T_3/mg DNA.

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