Effect of Alterations in Thyroid Status on the Metabolism of Thyroxine and Triiodothyronine by Rat Pituitary Gland In Vitro

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A B S T R A C T   The metabolism of thyroxine (T_4) was studied in slices of rat pituitary gland and liver from the same animal incubated in vitro with [^{125}I]T_4 and 10 mM dithiothreitol. In the pituitary gland, generation of ^{125}I-labeled 3,5,3'-triiodothyronine (T_3), as well as overall T_4 degradation, increased significantly at 24 h after thyroidectomy and by 2 wk were approximately five times control values. Conversely, following a single injection of T_3 (1.5 μg/100 g body wt), values for both functions were significantly decreased at 4 h, and reached a nadir of ~20% of control values at 12 and 24 h. Net T_3-neogenesis accounted for ~70% of T_4 degradation in control pituitaries from intact rats. This proportion was increased by thyroidectomy and decreased by T_3 replacement. Indirect evidence indicated that thyroidectomy decreased, and T_3 administration increased, non-T_3 generating pathways of T_4 metabolism, probably 5-monodeiodination leading to formation of 3,3'5'-triiodothyronine (rT_3). As judged from studies by others, the prompt changes in T_4 metabolism that followed thyroidectomy or T_3 administration could not be explained by changes in pituitary cell type. Changes in T_3-neogenesis in liver were the converse of those in pituitary, and were much slower to occur.

In the thyroidectomized rat, administration of cycloheximide resulted in an ~60% inhibition of pituitary T_3-neogenesis and T_4-degradation at 4 h, a time-course of inhibition similar to that produced by T_3. Unlike T_3, cycloheximide did not alter the proportion of T_4 degradation that could be accounted for by T_3 neogenesis, and appeared, therefore, to inhibit both T_3 generating and non-T_3 generating pathways. The time-course of the inhibitory effect of cycloheximide

on the incorporation of [^{3}H]leucine into hemipituitaries in vitro was parallel to its effect on T_3-neogenesis. The inhibition of T_3-neogenesis that occurred when T_3 and cycloheximide were given together did not exceed the effect of T_3 alone, suggesting a common mechanism of action of the two agents.

From the foregoing information, the following tentative conclusions are drawn: (*a*) turnover of the 5'-monodeiodinase for T_4 in rat pituitary is rapid, substantially more so than in liver; (*b*) thyroidectomy enhances, and T_3 inhibits, the conversion of T_4 to T_3 in the pituitary; these manipulations have opposite effects on the non-T_3 generating pathways of T_4 metabolism, probably the 5-monodeiodination of T_4 that produces rT_3; (*c*) these changes are probably the result of parallel effects on the synthesis of the corresponding enzymes; (*d*) the changes in T_3-neogenesis described may permit an intrapituitary feedback mechanism that damps the changes in TSH secretion mediated by classical feedback regulatory control; (*e*) the effects of hypothyroidism and T_3-replacement on T_3-neogenesis and overall T_4 degradation in liver were opposite to those produced in the pituitary. Hence, among differing tissues, the same stimuli may produce greatly different responses in pathways of peripheral T_4 metabolism, thus making possible differing metabolic sequelae within each.

INTRODUCTION

Recent reports have emphasized the importance of 3,5,3'-triiodothyronine (T_3)[1] generated locally from thyroxine (T_4) within the pituitary in the regulation of TSH secretion (1–3). This would imply the existence of a critical interaction between the thyroid secretion

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[1] *Abbreviations used in this paper*: DTT, dithiothreitol; MMI, methimazole; rT_3, 3,3'5'-triiodothyronine; T_4, thyroxine; T_3, 3,5,3'-triiodothyronine; 3,3'-T_2, 3,3'-diiodothyronine.

of T_4 and the pituitary conversion of T_4 to T_3 (T_3-neogenesis) in the regulation of thyroid function.

Because of the potential importance of this relationship, we undertook studies of pituitary T_3-neogenesis, using the rat pituitary as an in vitro model. Bisected, rather than homogenized, pituitaries were mainly used in order to maintain conditions as near physiological as possible. We also compared the properties of the T_3-generating systems in the pituitary with those in the liver, the organ in which T_3-neogenesis has been most extensively studied.

METHODS

Animals and reagents. All experiments were performed in male Sprague-Dawley rats of the CD strain (Charles River Laboratories, Wilmington, MA), weighing 180–200 g. Animals were maintained on rat chow (Ralston Purina Co., St. Louis, MO) and tap water ad lib. [^{125}I]T_4 (sp act, 120–130 μCi/μg) and [^{125}I]T_3 (sp act, 70–80 μCi/μg) were obtained from Abbott Laboratories, North Chicago, IL. [^{125}I]rT_3 (sp act, 1,000–2,000 μCi/μg) and [^{3}H]leucine (sp act, 110 Ci/mmol) were obtained from New England Nuclear, Boston, MA. L-T_4, L-T_3, DL-Dithiothreitol (DTT), cycloheximide, and methimazole (MMI) were purchased from Sigma Chemical Co., St. Louis, MO.

Preparation of tissues and incubation procedures. At the end of each treatment regimen described below, the rats were killed by cervical subluxation; the pituitary and liver were then removed immediately. Each pituitary was then bisected and the two hemipituitaries were placed together in an incubation vessel. In some experiments, in which the effect of an in vitro additive was to be tested, each hemipituitary was placed into a separate vessel, one to serve as a control for the other. Liver slices of uniform thickness were prepared with the aid of a Stadie-Riggs microtome. After an initial weighing, liver slices were closely matched in weight ($\sim$300 mg/vessel) by trimming. In 10 specimens of each tissue, protein/wet wt ratio for liver averaged 177.0±6.0 μg/mg (mean±SEM) and for pituitary 90.4±6.1 μg/mg.

Bisected pituitaries and liver slices from the same animals were incubated in individual vessels (12 × 75 or 16 × 120-mm tubes) containing 1.5 or 2.5 μCi ^{125}I-T_4, respectively, in Krebs-Ringer-phosphate buffer, pH 7.4, for 3 h at 37°C in room air. Final volumes of the buffer in which hemipituitaries and a single slice of liver were suspended were 200 μl and 2 ml, respectively. Thus, incubation media contained $\sim$6 μg T_4/dl in the case of pituitaries and 1 μg/dl in the case of liver. In all experiments, 10 mM DTT was added to all media, since, as others have reported (4), T_4 metabolism by the limited quantities of pituitary tissue available was too small to be accurately assessed in the absence of this stimulatory factor. To make results comparable, DTT was also added to the media used with slices of liver. Tissues from four to five animals were studied in each experiment.

At the end of incubation, vessels were placed on ice and the tissues were then homogenized in their own medium. An aliquot of homogenate was taken for the measurement of protein concentration by the method of Lowry and co-workers (5), and a volume of methanol-2 N ammonia (99:1, vol/vol) equal to that of the remaining homogenate was then added.

Further experiments were performed to assess the extent to which the results obtained concerning the metabolism of [^{125}I]T_4 might have been influenced by the degradation of the [^{125}I]T_3 and any [^{125}I] 3,3'5'-triiodothyronine (rT_3) derived

therefrom. Metabolism of [^{125}I]T_3 and [^{125}I]rT_3 was studied using hemipituitaries and pituitary homogenates. Tissues were obtained both from thyroidectomized rats and rats given a single subcutaneous injection of T_3 (1.5 μg/100 g body wt) 24 h earlier. Methods employed in the case of hemipituitaries were the same as those used in experiments with [^{125}I]T_4, except that [^{125}I]T_3 (1–2 μCi/ml) or [^{125}I]rT_3 (1–2 μCi/ml) served as substrate. In experiments with homogenates, pituitaries were homogenized (6%, wt/vol) in standard incubation medium containing a final concentration of 10 mM DTT. All specimens were incubated at 37°C in air, for 3 h in studies with [^{125}I]T_3, and for 15 min with [^{125}I]rT_3. Reactions in homogenates were terminated by the addition of methanol-ammonia, and the samples were processed and analyzed as in experiments with [^{125}I]T_4.

Two types of control vessels were employed in each experiment. One contained tissue slices or homogenates, but was incubated for 0 min (zero-time control); the other contained no tissue and was incubated for 3 h at 37°C (tissue-free control).

Analysis of reaction products. After methanol-ammonia had been added, reaction mixtures were analyzed by paper chromatography. 10 μl of uniformly dispersed homogenate were applied to Whatman 1 MM chromatography strips (Fisher Scientific Co., Pittsburgh, PA) together with MMI (2 mg/ml), carrier iodide, T_4, and T_3 dissolved in methanol-ammonia. Chromatography was carried out in descent for 20–24 h, using a tertiary amyl alcohol:hexane:2 N NH$_4$OH (10:1:11) solvent system. Iodothyronines were then localized by fluorescent light, and iodide by staining with 0.1% palladium chloride. ^{125}I-labeled compounds were localized by autoradiography, and all labeled bands excised and counted in a well-type scintillation counter. Autoradiographs were exposed for a sufficient period to permit detection of any band that contained at least 1–2% of added ^{125}I. Together, discrete bands accounted for 95–98% of the total ^{125}I applied to the strip.

Calculation of results. From the resulting data, the percentage degradation of [^{125}I]T_4 and percentage generation of each labeled product was calculated. From the analysis of control samples, corrections were then made for contamination of the original [^{125}I]T_4 by [^{125}I]T_3 and [^{125}I]iodide, and for the percentage of the total ^{125}I in the original substrate contributed by [^{125}I]T_4. Preparations of [^{125}I]T_4 were $\sim$95% pure, detectable contaminants being [^{125}I]T_3 $\sim$1% and [^{125}I]iodide ($\sim$4%).

Values for the percentage degradation of [^{125}I]T_4 and the percentage generation of each labeled product were corrected for these factors and were then expressed in relation to unit protein content of the tissue incubated.

Two derived functions were also calculated. The first was the fraction of [^{125}I]T_4 degradation that could be accounted for by the net generation of [^{125}I]T_3. This was calculated as [2 × (%[^{125}I]T_3 generated)/(%[^{125}I]T_4 degraded)]. The factor of 2 is employed because, for each molecule of [^{125}I]T_3 formed, two atoms of ^{125}I must have been lost from the original [^{125}I]T_4 substrate, regardless of whether one or both outer ring iodine atoms were labeled. Values for the fractional contribution of 5'-monodeiodination to overall T_4 degradation, calculated in this manner, are in fact minimum estimates, since they would be lowered by any degradation of newly generated [^{125}I]T_3 that had occurred during the incubation period.

A second derived function is one that we have previously termed excess iodide generation (6). This was calculated as [(%[^{125}I]iodide generated) − (%[^{125}I]T_3 generated)]. The basis of this calculation is that for each molecule of labeled T_3 generated from outer ring-labeled T_4, one atom of labeled

iodide must also be formed. Excess iodide generation thus reflects the activity of pathways for T_4 degradation other than 5'-monodeiodination, just as the net percent generation of T_3 reflects the activity of the 5'-monodeiodinating pathway of T_4 metabolism. To the extent that newly-formed $[^{125}I]T_3$ is deiodinated during the incubation period, excess iodide generation will be overestimated. However, the extent of this overestimation will be slight when deiodination of T_3, relative to the activity of non-T_3 generating pathways, is slow, as proved to be the case.

Statistical analyses. The statistical significance of differences between the results obtained in various experimental groups was assessed by means of the *t* test when experiments involved only two groups and analysis of variance followed by the Newman-Keuls test when results in more than two groups were being compared among one another (7).

RESULTS

In many of the experiments, the time-course of the effect of a particular experimental manipulation was assessed. In experiments of this type, pituitaries from both control and experimental animals were studied at each time point. Because of substantial variation from experiment to experiment in the control values for the several aspects of $[^{125}I]T_4$ metabolism studied, results were calculated on an absolute basis and, in addition, results in experimental groups were calculated as a percentage of the mean value obtained in simultaneously studied controls. This was done to permit more accurate conclusions concerning the proportionate changes with time in the various functions studied. However, the statistical significance of differences between concomitantly measured experimental and control values was calculated on the basis of absolute, rather than percentage, values.

Effect of tissue concentration. As judged from studies with liver, the rate of T_4 degradation and T_3 formation varied directly with the weight of the tissue employed. No effort was made to demonstrate a similar relationship in the case of the pituitary, owing to the limited weight of the pituitary gland. Only small and insignificant differences in pituitary wet weight or protein content per unit wet weight were observed among any of the experimental groups studied. Nonetheless, all results were related to protein content in an effort to compensate for the very small variations in total pituitary weight that were observed among the individual specimens.

Effect of incubation time. The products of T_4 metabolism increased in proportion to the incubation time up to 2 h, and only slowly thereafter. In view of evidence that T_3 is degraded only slowly by liver and pituitary, incubation periods of 3 h were employed in almost all experiments in order to maximize the quantities of T_3 formed by the small amounts of pituitary tissue available.

Reaction products. In all experiments involving 3-h incubations of $[^{125}I]T_4$, only T_3, iodide, and origin material appeared as labeled products of $[^{125}I]T_4$ metabolism in pituitary, regardless of the experimental manipulation performed. Detectable amounts of $[^{125}I]rT_3$ and $[^{125}I]3,3'$-diiodothyronine $(3,3'\text{-}T_2)$ were not present, though small proportions of both were formed during short-term (15 min) incubations. The same products were present or absent in liver, except that a trace of ^{125}I migrating in the region of $3,5,3'$-triiodothyroacetic acid was occasionally seen. Since this compound and ^{125}I-labeled origin material were only minor products, they will not be discussed further.

Relative rates of T_4 metabolism in pituitary and liver. Since the standard conditions under which pituitary and liver were incubated were not the same with respect to T_4 concentration or incubation volume per unit tissue weight, experiments were performed in which small pieces of liver, equivalent in size and weight to hemipituitaries, were incubated under the standard conditions used for hemipituitary. Patterns of T_4 metabolism by pituitaries and livers from five intact rats were studied in this manner. Both T_3 formation and overall T_4 degradation were much greater in pituitary than in liver. The former activity (% added $[^{125}I]T_4$/mg protein) averaged 4.0 ± 0.5 in pituitary and 0.093 ± 0.004 in liver $(P < 0.01)$. Values for T_4 degradation (% added $[^{125}I]T_4$/mg protein) were 10.8 ± 1.3 and 0.384 ± 0.011, respectively.

Effect of thyroidectomy. Rats surgically thyroidectomized at the Charles River Breeding Laboratories were promptly delivered to the laboratory. The time-course of changes in pituitary and hepatic T_4 metabolism in vitro was studied in animals killed 4 and 6 h and 1, 2, 3, 7, and 14 d after operation.

In control pituitaries from intact rats $(n = 28)$, T_3-neogenesis averaged $4.1\pm0.4\%$ of added $[^{125}I]T_4$/mg protein (mean$\pm$SEM) (Table I). Compared with values in simultaneously studied controls, values in thyroidectomized rats were unchanged at 4 and 6 h, but had doubled by 24 h $(P < 0.01)$, and were greater still at 2, 3, 7, and 14 d (Fig. 1). Values for overall T_4 degradation by pituitary averaged $10.9\pm0.9\%$ of added $[^{125}I]T_4$/mg protein in specimens from intact rats, and increased progressively in pituitaries obtained after thyroidectomy, so that 14 d postoperative they were nearly five times those found in simultaneously studied controls $(P < 0.01)$. Values for the percent of T_4 degradation that could be accounted for by T_3 formation increased from $71.1\pm3.7\%$ in pituitaries of intact rats to $95.8\pm4.0\%$ $(P < 0.01)$ in specimens from thyroidectomized animals. Excess iodide generation decreased moderately $(P < 0.05)$.

The pattern of T_4 metabolism and the effect thereon of thyroidectomy were quite different in liver. Values for T_3 formation in control livers were far lower than those seen in the pituitary. After thyroidectomy, T_3-

TABLE I

TABLE I

Effect of Various Manipulations on the Metabolism of [^{125}I]T$_4$ in Slices of Rat Pituitary and Liver[*]

Tissue	Time	n	(A) [^{125}I]T$_4$ Degradation	(B) [^{125}I]T$_3$ Generation	(C) [^{125}I]Iodide Generation	T$_3$ Generation/ T$_4$ degradation	Excess iodide generation
			% added T$_4$ per mg protein	% added T$_4$ per mg protein	% added T$_4$ per mg protein	%, 100 × 2B/A	% added T$_4$ per mg protein, C-B
Intact rat: effect of thyroidectomy							
Pituitary	Control	28	10.9±0.9	4.1±0.4	6.9±0.6	71.1±3.7	2.5±0.4
Pituitary	14 d	4	48.8±2.4‡	23.7±1.3‡	25.0±1.1‡	95.8±4.0†	1.2±0.3§
Liver	Control	28	0.309±0.016	0.089±0.006	0.189±0.010	56.9±2.0	0.096±0.013
Liver	14 d	4	0.147±0.010‡	0.013±0.003‡	0.078±0.006‡	17.4±3.9‡	0.061±0.007§
Thyroidectomized rat: effect of T$_3$, 1.5 μg/100 g body wt							
Pituitary	Control	23	23.8±1.9	11.3±0.9	12.6±1.1	94.7±1.4	0.9±0.3
Pituitary	24 h	4	6.4±0.1‡	1.6±0.3‡	3.4±0.1‡	49.7±9.7‡	1.8±0.2§
Liver	Control	23	0.146±0.009	0.019±0.002	0.096±0.008	28.1±3.6	0.062±0.014
Liver	48 h	4	0.321±0.011‡	0.074±0.005‡	0.195±0.009‡	46.2±0.3‡	0.121±0.004‡
Thyroidectomized rat: effect of cycloheximide, 1.0 μg/g body wt							
Pituitary	Control	4	33.9±5.2	15.4±2.5	17.2±2.9	94.1±0.6	1.7±0.8
Pituitary	4 h	4	12.2±2.8§	5.7±1.8§	6.3±1.5§	93.6±1.4	0.6±0.2

[*] Values shown are mean±SEM of those obtained in the number of specimens indicated by n. For each of the experimental manipulations, data are shown only for the time of maximum effect. Values for control specimens are pooled values from specimens obtained at all time points.

‡ P < 0.01 vs. values in corresponding control group.

§ P < 0.05 vs. values in corresponding control group.

neogenesis decreased, rather than increased, and this effect was relatively slow to appear, a significant decrease first being seen at 3 d (Fig. 1). Values for T$_4$
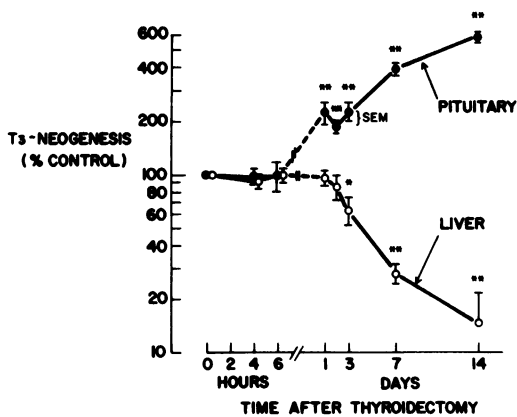

FIGURE 1 Time-course of changes in pituitary (●) and hepatic (O) T$_3$-neogenesis in vitro after thyroidectomy in the rat. Values for T$_3$-neogenesis are corrected to unit tissue protein content and then expressed as a percentage of the mean value obtained in tissues from unoperated controls, simultaneously studied. Data are expressed as the mean±SEM of results obtained in four to five separate specimens. Statistically significant differences from control values are indicated by * or ** for P < 0.05 or P < 0.01, respectively, and are based on absolute values, rather than percentages.

degradation in control specimens were also far lower than in pituitary (P < 0.01), and decreased progressively following thyroidectomy, so that by 14 d they averaged about half of those found in simultaneously studied controls (P < 0.01). Net T$_3$ generation accounted for 56.9±2.0% of T$_4$ degradation in control livers, a value lower than that seen in pituitary, and decreased markedly (P < 0.01) in animals 14 d postoperative. Values for excess iodide generation also decreased significantly (P < 0.05).

Effect of T$_3$ in vivo. Experiments were performed to determine the effects of exogenous T$_3$ on the metabolism of [^{125}I]T$_4$ in slices of pituitary and liver from animals thyroidectomized 2 wk earlier. In initial studies, experimental animals were given a dose of 0.25, 1.5, or 7.5 μg/100 g body wt in 0.01 N NaOH s.c. daily for 2 d. Control animals received NaOH alone. 24 h after the last injection, animals were killed and pituitaries were studied. Compared to values in pituitaries of thyroidectomized controls, T$_3$-neogenesis was decreased in a dose-related manner by the administration of T$_3$ itself (Fig. 2). Values in animals given 0.25 and 1.5 μg daily bracketed those seen in other experiments with normal animals, while values in animals given 7.5 μg daily were far lower. Overall T$_4$ degradation (% added [^{125}I]T$_4$/mg protein) decreased in a parallel fashion, from values of 40.2±2.1 in thyroidectomized
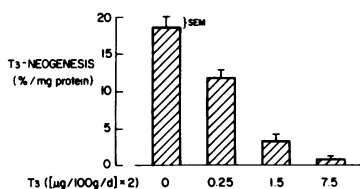
FIGURE 2 Effect of graded doses of T_3 in vivo on in vitro T_3-neogenesis in the pituitary of the thyroidectomized rat. Animals were given two daily doses of T_3 subcutaneously, and were killed 24 h later. Values for T_3-neogenesis are corrected to unit tissue protein content. Data shown are the mean±SEM of those obtained in four specimens in each group.

controls to 26.4±1.2 in rats given 0.25 μg doses of T_3, to 9.7±0.9 in animals given 1.5 μg and to 6.4±0.3 in animals given 7.5 μg T_3 daily.

Other experiments were performed to determine the time-course of the effect of a single dose of T_3 on the pituitary and hepatic metabolism of T_4. Rats thyroidectomized 2 wk earlier were given an injection of T_3 subcutaneously (1.5 μg/100 g body wt) and were killed at varying intervals thereafter (Fig. 3, Table I). In the pituitaries from 23 thyroidectomized rats that served as control specimens in this group of experiments, T_3-neogenesis averaged 11.3±0.9% of added [^{125}I]T_4/mg protein. After administration of T_3, compared with simultaneously studied controls, values decreased slightly, but not significantly, at 2 h. By 4 h, however, a significant decrease in T_3-neogenesis to about half of control values was seen ($P < 0.01$). T_3-neogenesis was further depressed to about one-fifth of control values at 12 and 24 h, respectively, but had returned to control values by 48 h.

Changes in other aspects of pituitary T_4 metabolism induced by T_3 were also the converse of those that followed thyroidectomy. At 24 h and even before, T_4 degradation was greatly decreased, and the percentage of [^{125}I]T_4 degradation that could be accounted for by the formation of [^{125}I]T_3 was approximately halved. Calculated values for excess iodide generation approximately doubled.

Once again, in this experiment, the pattern of T_4 metabolism and the effect thereon of exogenous T_3 were quite different in liver. After T_3 treatment, the liver responded more slowly than the pituitary, and in an opposite direction (Fig. 3). Hepatic T_3 neogenesis in the hypothyroid rats was unchanged at 4 h, when pituitary T_3-neogenesis had already decreased to half of the initial values. T_3-neogenesis in liver was increased significantly ($P < 0.01$) at 24 h, and was still greater at 48 h, when activity in the pituitary had already returned to control values.

Changes in other aspects of hepatic T_4 metabolism induced by T_3 were again the converse of those induced by thyroidectomy (Table I). At 48 h, overall T_4
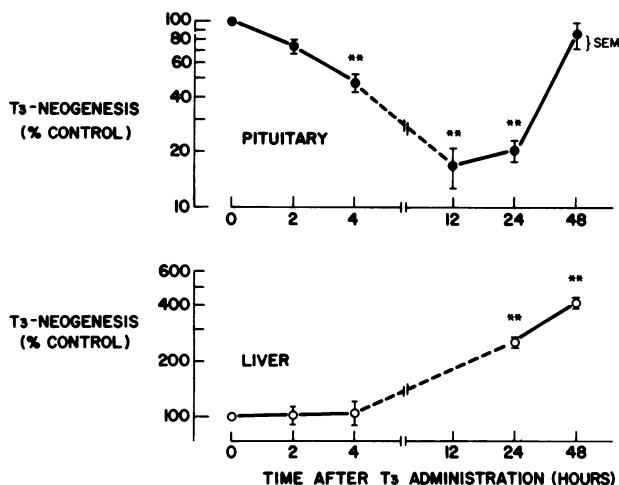

FIGURE 3 Time-course of changes in pituitary (●) and hepatic (○) T_3-neogenesis in vitro after T_3 administration (1.5 μg/100 g body wt) in the thyroidectomized rat. Values for T_3-neogenesis after T_3 treatment are corrected to unit tissue protein content and then expressed as a percentage of the mean value obtained in tissues from untreated controls simultaneously studied. Data are expressed as the mean±SEM of results obtained in four separate specimens. Statistically significant differences from control values are indicated by ●● for $P < 0.01$, and are based on absolute values, rather than percentages.

degradation, the fraction of T_4 degradation accounted for by T_3 generation, and excess iodide generation had all increased significantly.

Effect of T_3 in vitro. The effect of T_3 added in vitro on T_3-neogenesis in the pituitary of the thyroidectomized rat was also studied. Paired hemipituitaries from hypothyroid rats were incubated for 3 h in media to which either no T_3 or concentrations of T_3 ranging between 10 and 800 ng/ml had been added. These changes in the extracellular T_3 concentration had no apparent effect on T_3-neogenesis or on other aspects of [^{125}I]T_4 metabolism (data not shown).

Effect of T_4 in vivo. To determine whether exogenous T_4, like T_3, would decrease pituitary T_3-neogenesis in vitro, rats thyroidectomized 2 wk earlier were given a single injection of T_4 (10 μg/100 g body wt, s.c.). Pituitaries were obtained 3.5 h later and their metabolism of [^{125}I]T_4 compared with that of pituitaries from untreated controls. T_4 administration induced a profound decrease in the rate of T_3-neogenesis, values decreasing from 12.7±0.5 to 1.3±0.2% of added [^{125}I]T_4/ mg protein ($P < 0.01$). T_4 degradation was also decreased by the administration of T_4, from 25.8±0.9 to 4.9±0.4% of added [^{125}I]T_4/mg protein ($P < 0.01$). Changes induced by T_4 in the derived values for various aspects of T_4 metabolism were similar to those that followed the administration of T_3.

Effect of cycloheximide. Because of its very prompt onset, we wondered whether the inhibition of T_3-neo-

genesis induced by T_3 in the pituitary of the hypothyroid rat could be due to inhibition of the synthesis of the 5'-monodeiodinase for T_4. As a consequence, we undertook studies of the effects of the inhibitor of protein synthesis, cycloheximide. Thyroidectomized rats were given a single injection of cycloheximide (1.0 μg/g body wt s.c.), a dose twice that shown in previous studies to inhibit greatly the in vivo incorporation of amino acids into protein in multiple tissues and to be negligibly toxic (8). Control rats received saline alone. Rats were killed at 2, 4, 6, and 20 h following the administration of cycloheximide, and the time-course of changes in pituitary T_3-neogenesis were studied. Values were expressed as a percentage of those in simultaneously studied untreated controls.

Cycloheximide had no significant effect on the pituitary protein/wet weight ratio. It did, however, produce a prompt, though transient, inhibition of pituitary T_3-neogenesis. Values were reduced significantly ($P < 0.05$) to 36.0±5.8% of those seen in control pituitaries at 4 h (Table I), but values had returned to near normal (90% of control) in pituitaries obtained 6 h after administration of cycloheximide and thereafter (Fig. 4). The effects of cycloheximide on [^{125}I]T_4 degradation paralleled its effects on T_3-neogenesis. Almost identical results were obtained in three additional experiments in which the effects of cycloheximide were evaluated.

Values for the percent of [^{125}I]T_4 degradation that could be accounted for by the formation of [^{125}I]T_3 ($\sim$95%) were unchanged after cycloheximide administration. Calculated values for excess iodide generation were lower than control values at 4 h after cycloheximide, but not significantly so.

Combined effects of T_3 and cycloheximide. These experiments were performed in order to elucidate whether or not T_3 and cycloheximide act on pituitary
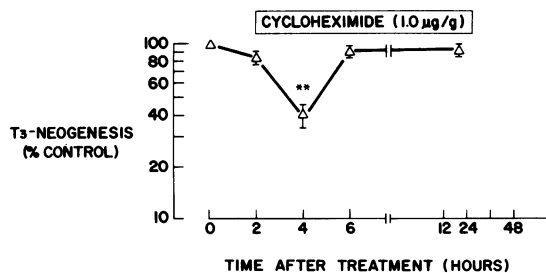
T_3-neogenesis through the same mechanism. To study the separate and combined effects of T_3 and cycloheximide on T_3-neogenesis in the pituitary of the hypothyroid rat, T_3 (1.5 μg/100 g body wt s.c.) and cycloheximide (1.0 μg/g body wt s.c.) were administered, singly or together, and pituitaries were obtained at the time of the maximum cycloheximide effect, 4 h.

In pituitaries obtained at that time, generation of T_3 from T_4 was inhibited both by cycloheximide and by T_3, and to about the same extent. When the two agents were given together, however, no evidence that their effects were additive could be obtained (Fig. 5). Almost identical results were obtained in two additional experiments in which the combined and separate effects of T_3 and cycloheximide were studied.

Effects of cycloheximide on amino acid incorporation. To examine the correlation between the effects of cycloheximide on T_3-neogenesis and its effects on protein synthesis, the experiments with cycloheximide described above were repeated, in part, except that pituitaries were incubated with [^{3}H]leucine rather than [^{125}I]T_4. Rats thyroidectomized 2 wk earlier were given a single injection of cycloheximide (1.0 μg/g body wt s.c.) and were killed 4 or 6 h later; controls received saline alone. Bisected pituitaries were then incubated individually for 3 h at 37°C in room air in a medium of Krebs-Ringer phosphate, pH 7.4, containing [^{3}H]leucine (1 μCi/ml). At the end of the incubations, pituitaries were homogenized in their own medium. Aliquots were treated with an equal volume of 12% cold TCA. After the resulting precipitates had been washed with 6% TCA they were transferred to scintillation vials containing Toluene-Triton X-100 counting fluid and their content of ^{3}H was measured. A separate aliquot of each homogenate was added directly to the counting fluid so that total ^{3}H counts in each incubation vessel could be determined. Percentage incorporation of total [^{3}H]leucine into TCA-precipitable moieties was then calculated.
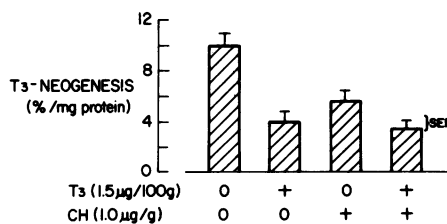

FIGURE 4   Effect of a single injection of cycloheximide (1.0 μg/g body wt s.c.) on in vitro T_3-neogenesis in the pituitary of the thyroidectomized rat. Values shown represent the mean±SEM of those obtained in four specimens in each group. Asterisks indicate value significantly different from control value ($P < 0.01$). Almost identical results were obtained in three other similar experiments.

FIGURE 5   Separate and combined effects of 1.5 μg T_3/100 g body wt and 1.0 μg cycloheximide/g body wt on in vitro T_3-neogenesis in the pituitary of the hypothyroid rat. Animals were killed 4 h after injection. Values for T_3-neogenesis are corrected to unit tissue protein content. Data are expressed as the mean±SEM of values obtained in four specimens in each group. Almost identical results were obtained in two other comparable experiments.

In control pituitaries $(n = 7)$, incorporation of [³H]leucine averaged 14.8±1.2% of the total. In pituitaries from animals killed 4 h after cycloheximide $(n = 5)$, values decreased significantly $(P < 0.01)$ to 7.2±0.4%. A return toward control values was observed in the pituitaries of animals given cycloheximide 6 h before death $(n = 5)$ The mean of 10.6±0.5% in this group was significantly greater $(P < 0.01)$ than values in the pituitaries of 4-h animals, but significantly less than the mean values in controls $(P < 0.01)$.

Degradation of T_3 and rT_3. During 3-h incubations, only a very small proportion (1–2%) of [¹²⁵I]T_3 was degraded by pituitary homogenates or slices, and this was true in tissues obtained from both thyroidectomized rats and comparable rats treated with T_3 (Table II). In contrast, metabolism of rT_3 was exceedingly rapid, somewhat more so in homogenates than in slices, and was not influenced by the thyroid status of the animal.

DISCUSSION

The present studies have served to clarify several properties of the enzymatic mechanisms by which T_4 undergoes metabolism in the rat pituitary, at least as judged from the results of incubations of [¹²⁵I]T_4 with hemipituitaries. The major findings of these studies relate to the activity of the 5'-monodeiodinase that converts T_4 to T_3 (and by inference the 5-monodeiodinase that converts T_4 to rT_3), and to the rapidity and direction of changes in the activity of this enzyme that follow alterations in the thyroid hormone status of the animal.

In earlier studies, Cheron and associates (9) and Kaplan (10) have examined the effects of chronic alterations in thyroid hormone status on the metabolism of T_4 by pituitary slices or homogenates, respectively. Hypothyroidism of 2–3-mo duration was shown to be

TABLE II
*Degradation of [¹²⁵I]T_3 and [¹²⁵I]rT_3 by Slices and Homogenates of Pituitary from Thyroidectomized Rats: Effect of T_3 Administration**

| ¹²⁵I-Substrate | ¹²⁵I-Substrate Degradation (% added substrate/mg protein) | | | |
| | Thyroidectomized (T_x) | | T_x, T_3-Treated | |
	Homogenates	Slice	Homogenates	Slice
T_3	1.0±0.1	2.0±0.1	1.2±0.1	1.6±0.3
rT_3	47.7±1.5	32.2±3.0	45.4±5.7	29.4±4.2

* Studies conducted in homogenates and slices from pituitary glands of thyroidectomized rats and thyroidectomized rats given T_3 (1.5 μg/100 g body wt) 24 h earlier. Values shown are mean±SEM of those obtained in four separate specimens.

associated with enhanced conversion of T_4 to T_3. Conversely, formation of T_3 from T_4 was depressed in animals made thyrotoxic by the administration of T_4 (10 μg/100 g body wt) daily for 5 d. Our findings complement these observations by demonstrating that alterations in the rate of T_3 formation from T_4 do not require chronic changes in thyroid hormone status, but occur with extreme rapidity. Thus, in vitro T_3-neogenesis by hemipituitaries was more than doubled by 24 h after thyroidectomy. Conversely, when a single, relatively small, dose of T_3 (1.5 μg/100 g body wt) was given to thyroidectomized animals, in vitro T_3-neogenesis by hemipituitaries was significantly decreased by 4 h, and was greatly reduced by 24 h. Addition of T_3 to incubation media, even in high concentrations, did not cause a similar inhibition of T_3-neogenesis; however, this may have been due to an insufficient period of exposure to T_3 (3 h) or to insufficient penetration of the added T_3 into the pituitary cells.

Several tentative conclusions can be drawn concerning the mechanism whereby these rapid effects of in vivo T_3 on pituitary T_4 metabolism were brought about. With the small quantities of pituitary tissue available for study, the rate of metabolism of T_4 was very limited in the absence of cofactor. Consequently, all studies were conducted in the presence of DTT, an agent that stabilizes reduced sulfhydryl groups and is thought to uncover thereby the maximum activity of the deiodinating enzymes (11). This would suggest that the changes in T_3-generating activity that we observed were the result of alterations in enzyme activity per se, rather than changes in the availability of sulfhydryl cofactors.

It is possible that some of the changes in overall T_4 metabolism and in T_3-neogenesis that we observed were the result of variations in the rate of tissue uptake of T_4 under the various conditions of study, rather than the result of differences in the rate and pathways of intracellular T_4 metabolism. Operation of this mechanism cannot be entirely excluded, since tissue uptake of T_4 was not studied. Nonetheless, it cannot account for all of the changes observed, since the alterations in the rate and direction of T_4 metabolism induced by varying thyroid states, as reported here for hemipituitaries, were also observed by us (data not shown) and others (10) in pituitary homogenates.

The second conclusion relates to the possible role of changes in pituitary cell type on the patterns of T_4 metabolism observed. Kaplan has suggested that the 5'-monodeiodinating activity of thyrotrophs might exceed that of other pituitary cells, and that an increase in the proportion of thyrotrophs would explain, therefore, the increased T_3-neogenesis seen in the pituitary of the hypothyroid rat (10). One might then infer that the decreased 5'-monodeiodination of T_4, as seen in

pituitaries of thyrotoxic rats by previous workers (9, 10), is the result of a decrease in the proportion of thyrotrophs. Our findings, considered in relation to the data of De Fesi et al. (12), suggest that this is not the case. Their studies revealed that the percentage of thyrotropin (TSH) cells in the rat pituitary is increased only slightly by 5 d after thyroidectomy (from 7.5 to 9.8% of the total), whereas we find that T_3-neogenesis is doubled by 24 h after thyroidectomy and is four times that in control pituitaries by 7 d. Conversely, in the same studies (12), the percentage of TSH-cells in the pituitaries of thyroidectomized rats was not reduced by 2 d of treatment with T_3, 10 $\mu g/100$ g body wt daily. In contrast, we found the generation of T_3 from T_4 to be reduced to ~50% of control levels at 4 h, and to 20% of control levels at 24 h, after only one injection of 1.5 μg $T_3/100$ g body wt. We conclude, therefore, that the changes in pituitary T_3-neogenesis that follow alterations in thyroid hormone status are not primarily the result of a change in the relative abundance of TSH cells within the pituitary, but reflect, instead, an alteration in the T_4 metabolizing enzymes within one or more varieties of pituitary cell. Whether this change occurs solely in the thyrotroph is uncertain, since, even in animals thyroidectomized 2 wk earlier, TSH-cells comprised only 20–30% of the cells within the pituitary (12).

The present studies with the inhibitor of protein synthesis, cycloheximide, have apparently provided the first information concerning the rate of turnover of the pituitary 5'-monodeiodinase for T_4, and suggest that it is very rapid. Within 4 h after the administration of cycloheximide to the hypothyroid rat, in vitro T_3-neogenesis by the pituitary was greatly decreased, presumably owing to an inhibition of the synthesis of the 5'-monodeiodinase for T_4. The likelihood that cycloheximide decreased pituitary T_3-neogenesis by inhibiting synthesis of the 5'-monodeiodinase is enhanced by the parallelism between the time-course of the effects of cycloheximide on pituitary T_3 formation and on [^{3}H]leucine incorporation, when these functions were studied under comparable conditions. Hence, the similarity between the rates of decline in T_3-neogenesis produced by T_3 and by cycloheximide would suggest, in turn, that T_3 also acts by inhibiting the synthesis of the 5'-monodeiodinating enzyme. This conclusion was supported by the results of experiments in which the two agents were given together, since their combined effect on T_3-neogenesis was not significantly different from their individual effects when given alone. If the action of T_3, like that of cycloheximide, is to abolish enzyme synthesis abruptly, then no additivity of the effects of the two agents when given together would have been expected, and none was found. The prompt-

ness of this apparent inhibitory effect of T_3 on synthesis of the 5'-monodeiodinase for T_4 in the pituitary of the hypothyroid rat is reminiscent of its ability to inhibit the synthesis of TSH within 3 h in comparable preparations (13, 14).

Substantial interest and importance attaches to the relative roles of intrapituitary T_4 itself, T_3 generated from T_4 within the pituitary, as well as T_3 reaching the pituitary via the bloodstream, in the regulation of TSH secretion (1, 3). Similar questions can be raised regarding the role of these factors in the regulation of pituitary 5'-monodeiodinase activity. In the present study, T_4 administered in vivo, like T_3, produced a prompt decrease in pituitary T_3-neogenesis. The significance of this finding with regard to the questions raised above is uncertain, however, since the effect of T_4 could have resulted from several possible mechanisms: accumulation of stable T_4 within the pituitary, with a resulting dilution of the [^{125}I]T_4 used as a marker for T_3-neogenesis; a direct effect of T_4 itself, analogous to that of T_3; an effect of T_3 generated locally within the pituitary from the T_4 administered; and an effect of T_3 that was generated peripherally from the administered T_4 and that reached the pituitary via the circulation.

Additional light on the metabolism of T_4 in rat pituitary, and the influence of thyroid hormones thereon, is also shed by the two derived functions that we have calculated. Thus, in the pituitary of the thyroidectomized rat, almost all (>95%) of T_4 metabolism could be accounted for by T_3 generated from it. Much lower values for this function were seen in pituitaries of intact animals and of thyroidectomized animals given T_3. These variations in the relative contribution of T_3 generation to overall T_4 degradation were not merely the result of a decrease in the rate of 5'-monodeiodination, but appeared to be due, in addition, to an increase in the activity of non-T_3 generating pathways of T_4 deiodination. This is evidenced by the decrease in calculated values for excess iodide generation that followed thyroidectomy and the increase that followed T_3 administration. These changes in excess iodide generation probably cannot be ascribed to alterations in the rate of deiodination of [^{125}I]T_3 generated from [^{125}I]T_4 during the period of incubation, since studies of exogenous [^{125}I]T_3 metabolism in both homogenates of pituitary and in pituitary slices revealed it to be both slow relative to the metabolism of T_4 and unchanged by variations in thyroid status. We would conclude, therefore, that lack of thyroid hormone leads to enhancement of the 5'-monodeiodination of T_4 in the pituitary and a lesser activity of alternative pathways of T_4 degradation, while converse changes are induced by thyroid hormone replacement or excess.

The nature of the non-T_3 generating pathway for T_4 metabolism in rat pituitary whose activity varies directly with thyroid hormone status is not clearly proven. We have been unable to detect a pathway for the oxidative cleavage of the ether-link of the T_4 molecule with loss of outer ring iodine (15), since no labeled iodotyrosines were detected after incubation of hemipituitaries with biosynthetically derived, uniformly labeled T_4. On the otherhand, we have demonstrated generation of $[^{125}I]rT_3$ from $[^{125}I]T_4$ during short term (15 min) incubations. Our inability to detect $[^{125}I]rT_3$ after long term incubations is likely due to rapid degradation of the $[^{125}I]rT_3$, since studies in pituitary homogenates revealed that exogenous $[^{125}I]rT_3$ was deiodinated with great rapidity. We would suggest, therefore, that the pathway of T_4 metabolism in rat pituitary whose activity is decreased by thyroid hormone insufficiency and increased by hormone replacement is the 5-monodeiodination of T_4 that leads to the formation of rT_3.

It seems clear from the present findings that there exists within the pituitary itself a feedback mechanism that regulates T_3-neogenesis, in which increased pituitary T_3 (and perhaps T_4) inhibits further local T_3-neogenesis, while decreases in T_3 and T_4 produce the opposite effects. This mechanism should interact with classical feedback control of TSH secretion, which is negatively correlated with intrapituitary T_3 concentration (1–3). Thus, as decreased quantities of T_3 and T_4 were available to the pituitary, generation of T_3 from T_4 would become more efficient, and this would tend to limit the increase in TSH secretion that would otherwise occur. Opposite consequences would follow an increase in the availability of T_4 and T_3 to the TSH-secreting cell. In this light, the feedback mechanism for the regulation of intrapituitary T_3-neogenesis would serve to minimize any oscillations in TSH secretion that result from classical regulatory control.

A final aspect of the present findings is the manner in which the effects of thyroidectomy and of T_3 administration to the hypothyroid rat differed in the pituitary and liver. One major difference was in the rate at which changes in the pattern of T_4 metabolism emerged following changes in thyroid status, those in the pituitary occurring much more promptly than those in the liver. Major qualitative differences were also observed. In confirmation of earlier findings (16–20), hypothyroidism decreased, and T_3 administration restored to normal, both T_4 degradation and T_3-neogenesis in liver, effects opposite to those in the pituitary. These findings highlight what may prove to be an extremely important aspect of peripheral T_4 metabolism, i.e., the extent to which patterns of thyroid hormone metabolism differ among various tissues, both under normal circumstances and in response to various stimuli.

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