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Research Article

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ABSTRACT Studies were undertaken in hypothyroid rats in an effort to define the kinetics of growth hormone (GH) accumulation in response to i.v. pulse injections of triiodothyronine (T_3) and to calculate the relationship between nuclear occupancy by T_3 and the instantaneous rate of accumulation of pituitary GH. Results were contrasted to the findings in previous studies of the induction of hepatic mitochondrial α-glycerophosphate dehydrogenase (α-GPD) and malic enzyme (ME) by T_3. The dose of T_3 required to achieve half-maximal accumulation of GH in 24 h was 0.6 μg/100 g body wt, a value 15-fold less than the half-maximal dose for α-GPD and ME induction at a comparable time after injection. Although significant increases in pituitary GH were evident as early as 3 h after injection of maximally effective doses of T_3, the rate of increase became linear only 12 h after injection. After achievement of peak values, the pituitary content of GH decayed with a similar terminal $t_{1/2}$ of 3.9 days and 4.1 days in two groups of animals injected with a single dose of 1.0 and 50 μg T_3/100 g body wt, respectively. In vivo isotopic displacement studies carried out at the equilibrium time point indicated that the pituitary nuclear binding capacity was 5.5 ng T_3/g tissue and that the plasma concentration at which one-half of the nuclear sites are occupied is 1.0 ng/ml. Nuclear occupancy as a function of time was calculated from the estimated plasma T_3 concentration after injection of the dose and the half-occupancy plasma concentration. These data were then analyzed by application of the mathematical model previously developed to ascertain the relationship between nuclear occupancy and the rate of hepatic enzyme induction. Results indicated that the pituitary nuclear occupancy-response relationship was generally linear, in marked contrast to the highly amplified relationship between nuclear occupancy and the response of ME and α-GPD to T_3 in the liver. In supplementary experiments, euthyroid rats received daily injections of 200 μg of T_3 for 7 days to keep nuclear sites nearly saturated for the duration of the experiment. No significant increase in the pituitary GH content above euthyroid base-line levels was noted. This also contrasts with the marked increase above euthyroid levels in α-GPD and ME observed in previous studies. Our findings suggest the existence of major differences between the specific mechanisms which lead to the induction of pituitary GH and the hepatic enzymes by T_3.

INTRODUCTION

The fundamental role of thyroid hormones in growth and development of the mammalian organism is well-recognized (1). In part, thyroid hormone may exert these effects by stimulating the formation of growth hormone (GH).[1] Thus, the levels of radioimmunoassayable pituitary and circulating GH are extremely low in hypothyroid animals (2), and studies of hypothyroid children indicate that plasma GH response to insulin administration is blunted. The serum GH value, however, does not appear to be as uniformly depressed in hypothyroid patients as in hypothyroid rats (3). Recently, Hervas et al. (4) have shown by use of a sensitive radioimmunoassay that the circulating levels of pituitary GH

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[1] *Abbreviations used in this paper*: GH, growth hormone; α-GPD, α-glycerophosphate dehydrogenase; *k*, plasma concentration yielding half-nuclear occupancy; M, binding capacity; ME, malic enzyme; *q*, fractional nuclear occupancy; R, responsivity or rate of induction; T_3, triiodothyronine.

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in rats fell to almost undetectable values within 24 days after thyroidectomy and that these levels could be restored to normal by the injection of triiodothyronine (T_3). Thyroid hormone appears to stimulate pituitary synthesis of GH directly as Samuels and Shapiro (5) have shown in tissue culture studies that the addition of T_3 to the incubation medium results in a fourfold stimulation in the rate of GH synthesis by GH_1 cells, a cell line derived from a rat pituitary tumor. Such stimulation appears to be mediated by an increase in the rate of formation of specific messenger RNA coding for GH (6, 7), a finding which is compatible with the prevailing view that thyroid hormone initiates its action by altering nuclear processes (8). Nevertheless, the growth-promoting and developmental functions of thyroid hormone are not mediated exclusively through the generation of GH by the pituitary. Administration of maximal doses of GH both to hypothyroid rats (9) and to hypothyroid children (10) fail to rectify completely deficiencies in growth and differentiation associated with thyroid hormone deficiency.

Samuels et al. have analyzed the relationship between nuclear T_3 occupancy and the rate of synthesis of pituitary GH in their tissue-culture system (11). A linear relationship was found between the occupancy of a subset of T_3-depletable receptor sites and the rate of synthesis of pituitary GH. This relationship, however, differed markedly from what we had observed in studies of the induction by T_3 of hepatic mitochondrial α-glycerophosphate dehydrogenase (α-GPD) and cytosolic malic enzyme (decarboxylating) (ME) in the intact rat. In our studies, the number of detectable T_3 nuclear-binding sites appeared to be independent of the level of T_3 occupation (12), and the rate of enzyme induction was shown to become increasingly amplified with progressive nuclear occupancy by hormone (13). Thus, with full saturation of the nuclear sites, the rate of induction of α-GPD and ME was 10–18 times that observed under physiological conditions at which approximately one-half of the sites are normally occupied. The relationship between hepatic nuclear occupancy and responsivity was calculated by application of a mathematical model in which the assumption was made that the rate of formation of a T_3-inducible protein could be related to the level of T_3 nuclear occupancy attained (13).

Because of the marked discrepancy between the occupancy-response relationships reported by Samuels et al. (11) and by us (13), and because of the intrinsic biologic importance of the induction of pituitary GH by T_3, we undertook a series of studies to analyze the kinetics of accumulation of pituitary GH in the intact hypothyroid rat. The mathematical model used in our analysis of hepatic enzyme induction was applied to the pituitary GH data generated. We now report the results of our studies. These indicate that there are, in fact, major differences in the relationship between the nuclear occupancy by T_3 and the rate of pituitary GH induction on the one hand and the induction of hepatic ME and α-GPD by T_3 on the other. A generally linear relationship between nuclear occupancy and the rate of induction of pituitary GH was observed in the intact animal, in overall agreement with the results of the cell culture studies by Samuels et al. (11).

METHODS

Male Sprague-Dawley rats (150–225 g) were obtained from Charles River Breeding Laboratories, Wilmington, Mass. Tap water and food (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.) containing 1.0 μg I/g were freely available. Surgically thyroidectomized rats were obtained from the breeder at a weight of 100–125 g. Upon arrival, each rat was placed on a low-iodine diet (<0.05 μg I/g) for 7 days and then injected i.p. with 100 μCi of ^{131}I-Na (Mallinckrodt Inc., St. Louis, Mo.). The animals were weighed twice weekly and used experimentally when their weight had stabilized, generally 4–5 wk after radioiodine administration. The weight of the rats at that time ranged between 180 and 210 g and the pituitary GH content was less than 2.0 μg GH as measured by radioimmunoassay.

Pituitary GH was measured by double antibody radioimmunoassay with the reagents kindly provided by the National Institutes of Health (Rat Pituitary Hormone Distribution Program). Rats were killed by exsanguination through the abdominal aorta after ether anesthesia. The anterior pituitary was rapidly removed and homogenized in 1.0 ml of phosphosaline buffer (0.05 M phosphate, 0.15 M NaCl, 0.025 M EDTA, 1% bovine serum albumin, pH 7.6) and frozen at −20°C until the assay. Pituitary GH content was measured in duplicate at four different dilutions. The intra- and interassay coefficient of variation were 8 and 15%, respectively. To reduce error, pituitary samples from a given experiment were therefore measured in the same assay. In euthyroid rats that weighed 210–250 g, we observed a pituitary GH content between 476 and 990 μg. In hypothyroid rats, the range for pituitary GH content was 0.1–2.0 μg, values less than 0.4% of the normal content observed in the euthyroid rats.

The maximal nuclear binding capacity of hypothyroid pituitaries was determined by the application of in vivo displacement techniques (14, 15). Plasma concentrations of T_3 were measured by the radioimmunoassay procedure of Surks et al. (16). The dose-response relationship was analyzed by the four parameter logistic equation described by Rodbard (17) with a computer program (APL) written in our laboratory (CYBER 74, University of Minnesota Computer Center, Minneapolis, Minn.). Nuclear occupancy was determined as previously described (18). Thus, the fraction of the nuclear sites occupied was calculated from the plasma concentration p by a rearrangement of the law of mass action: $q = P/p + k$, where k is the apparent dissociation constant of the nuclear-T_3 complex in relationship to plasma T_3 (i.e., the plasma concentration of T_3 when the nuclear sites are half saturated); p, the plasma concentration of T_3; and q, the fraction of nuclear sites occupied by T_3. We have estimated the value of k as 1.0 ng/ml (Results).

Analysis. To ascertain the relationship between nuclear occupancy and responsivity, the following equation was solved by numerical methods as previously described (13). Briefly,

$$\frac{dE}{dt} = R(q) - \lambda E, \qquad (1)$$

where E = pituitary content of growth hormone; R = the re-

sponsivity, i.e., the rate of instantaneous GH accumulation (μg/day); λ = the fractional rate of disappearance of GH (day^{-1}); t = time in days after the initial lag period (*vide infra*); and q = fractional nuclear occupancy.

To determine R(q), Eq. 1 was solved by numerical methods as previously outlined (13). The following relationships and parameters require definition: (*a*) the general empirical relationship between the dose of T_3 injected and the pituitary GH content 24 h after the injection; (*b*) the terminal fractional rate of pituitary GH depletion after a pulse injection of T_3; (*c*) the average lag time between the injection of T_3 and the time of maximal GH induction; and (*d*) the relationship between the instantaneous plasma concentration of T_3, the nuclear content of specifically bound T_3, and the fraction of specific nuclear sites occupied. Studies were undertaken to define these parameters and interrelationships. In addition, solution of Eq. 1 requires knowledge of the relationship between the dose injected and the concentration of T_3 in plasma as a function of time in hypothyroid animals. This information is provided in a previous communication (13).

RESULTS

Determination of basic parameters

Dose response relationship at 24 h. Hypothyroid rats were injected i.v. with graded doses of T_3 (0.3, 0.6, 1.5, 5.0, 10.0, 50.0, and 100.0 μg/100 g body wt) and pituitary GH content was measured 24 h after the administration of the hormone. Four experiments were carried out. The mean increase in GH content at each dose level, expressed as a percentage of the maximal response, is represented in Fig. 1. The solid line represents the curve fitted by nonlinear regression (17) whereas the dashed line illustrates, for purposes of comparison, the dose-response relationship for α-GPD determined in our previous study. The dose required for half-maximal response calculated from the nonlinear regression analysis for the pituitary GH content was 0.63 μg T_3/100 g body wt, a value 15.2-fold less than the half-maximal dose observed for α-GPD (9.6 μg/100 g body wt).

Kinetics of appearance of pituitary GH: Determination of lag time. To determine the time course of onset of appearance of newly synthesized GH in the pituitary of hypothyroid rats, two groups of rats were injected i.v.; one with 50 μg T_3/100 g body wt and the other with 100 μg T_3/100 g body wt. These doses were selected because they are known to saturate the nuclear sites for the period of the experiment. At designated intervals (3, 6, 12, 16, 20, and 24 h) after the injection, rats were killed and pituitary GH content was measured. As shown in Fig. 2, an increase in GH content was observed as early as 3 h after the injection ($P < 0.001$) of both doses of T_3 but a maximal rate of accumulation did not occur until shortly after 12 h after injection. The observed splay in the accumulation curve can be considered to be the result of variation in the

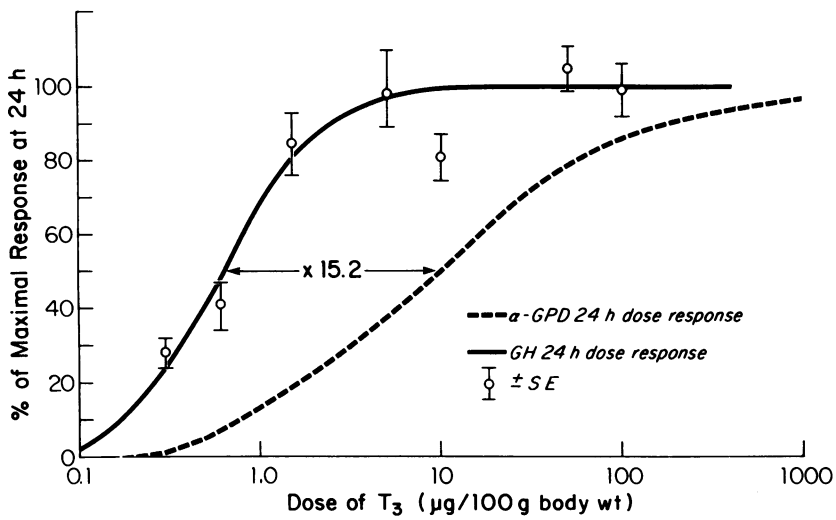

FIGURE 1 Hypothyroid rats were injected i.v. with graded doses of T_3 (0.3, 0.6, 1.5, 5.0, 10, 50, and 100 μg/100 g body wt). Animals were killed 24 h later and the pituitary GH content was measured. Results are represented here by the mean of four separate experiments in which five animals were used for every dosage level injected. Results are expressed as the percent of the maximal growth hormone content obtained in each experiment which was taken as the mean of the GH content in 50 and 100 μg dose. Individual percentage values were averaged. Continuous line represents the function obtained by application of the equations for nonlinear regression cited in Methods (17). Half-maximal effects for GH accumulation were achieved at a dose of 0.63 μg T_3/100 g body wt. In contrast, dose-response relationships for the accumulation of α-GPD 24 h after T_3 injection previously reported (13) and now analyzed by nonlinear regression showed a half-maximal dose 15.2-fold greater than the comparable half-maximal dose for the accumulation of GH in the pituitary. Bars, ±1 SE.

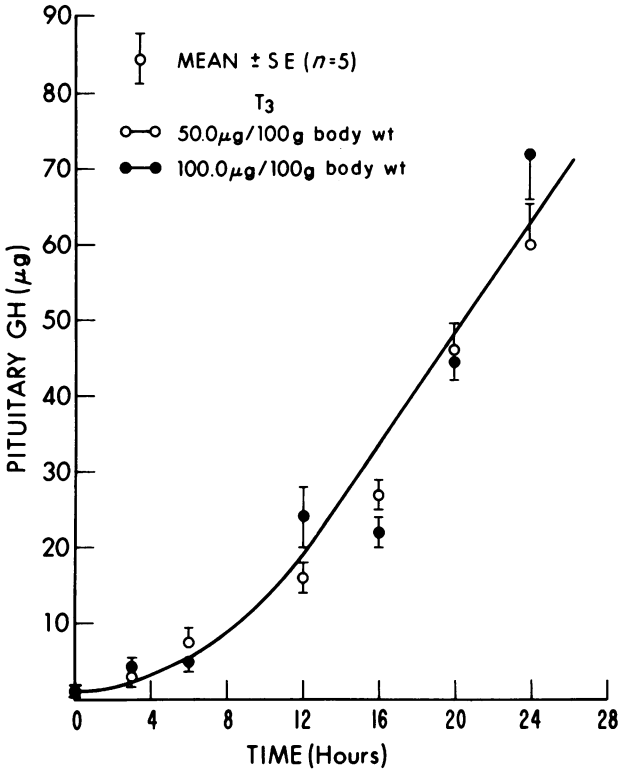

FIGURE 2 The kinetics of appearance of pituitary GH is illustrated in serial determinations after the injection of a 50- and a 100-μg dose/100 g body wt. These doses were selected because they can be calculated to saturate the nuclear sites for the period of the experiment. Animals were killed at the times indicated, each point represents the mean of four animals (±SE). No statistical differences were noted between the two groups and a composite line through all points was drawn. Although maximal rates of accumulation did not appear to have been established until 12 h after the injection of T_3, a statistical increase above base-line level was detected in the first determination obtained, at 3 h ($P < 0.001$). The initial splay can be considered to represent a reflection of varying times required for individual members of the population of hypothetical subcellular metabolic units to become activated by T_3 in the induction of GH. The average time for such activation was calculated by application of the Stewart-Hamilton theorems (19, 20). For the 50 μg/100 g body wt dose this was 5.5 h and 5.7 h for the 100 μg/100 g body wt dose. Statistically, these values were not significantly different.

delay time of individual molecular units in the full activation of GH production. Thus some units are active already at 3 h and essentially all are active by 12 h. To determine the average delay time (τ), calculations were made on the basis of the Stewart-Hamilton theorem (19, 20). Thus, the fraction of molecular units not activated at any time (t) can be represented by the following equation:

$$y = 1 - \frac{(\dot{c})_t}{(\dot{c})_{max}}, \qquad (2)$$

where $(\dot{c})_t$ is the instantaneous rate of change of pituitary GH content with respect to time and $(\dot{c})_{max}$ the maximal rate of change of GH content. From the Stewart-Hamilton theorem therefore,

$$\tau = \frac{\int_0^\infty ytdt}{\int_0^\infty ydt} \qquad (3)$$

Graphic analysis yielded essentially identical values for τ for the 50- and 100-μg/100 g body wt doses, 5.5 and 5.7 h, respectively.

Kinetics of pituitary growth hormone decay. Studies were carried out to evaluate λ in Eq. 1. This term refers to the fractional removal rate of GH from the pituitary and is the sum of secretory processes and the *in situ* removal of immunoreactive GH. Hypothyroid rats were injected i.v. with a single dose of T_3. One group of rats received a relatively low dose (1.0 μg T_3/100 g body wt) whereas the other group received a larger dose (50 μg T_3/100 g body wt). The appearance and disappearance of T_3-induced pituitary GH was studied (Fig. 3).

Maximal values for the 1-μg dose were reached at about the third day, whereas maximal values were reached at about 4 days with the 50-μg dose. There-

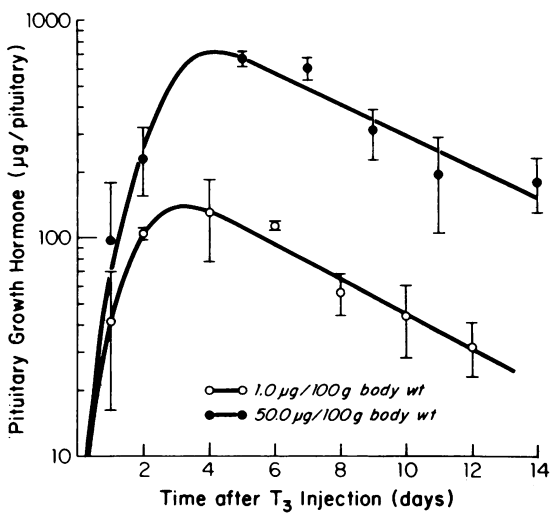

FIGURE 3 Two groups of hypothyroid rats were injected with 1 μg T_3/100 g body wt (○ —— ○) and 50 μg T_3/100 g body wt (● —— ●). Pituitary content of GH was measured on the days indicated. The decline in GH content for both doses appeared to follow an exponential decay. The $t_{1/2}$ of the 50 μg dose was 4.1 days, whereas the corresponding $t_{1/2}$ for the 1 μg/100 g body wt dose was 3.9 days. Statistically these values were not significantly different from each other. Thus as a first approximation, after single pulse injection of T_3, decay characteristics of decline appeared to be independent of the dose injected. Bars, ±SE ($n = 3$).

after, the decline in pituitary GH content proceeded in an approximately exponential fashion, with a $t_{1/2}$ of 4.1 days for the 50-μg dose and a $t_{1/2}$ of 3.9 days for the 1-μg dose. Because these slopes were not significantly different, the fractional removal rate of pituitary GH appears to be independent of dose administered, at least for single injections in the range examined.

Plasma: nuclear relationships. In vivo isotopic displacement studies were carried out to assess the binding characteristics of hypothyroid pituitary nuclei. These were carried out at the "equilibrium point". This is defined as the time after the injection of tracer ^{125}I-T_3 when the rate of change of pituitary radioactive T_3 is zero. A broad plateau in radioactive T_3 content was observed between 1 and 3 h both in euthyroid and hypothyroid animals, and the equilibrium time point was therefore assumed to occur midway (2 h) after the injection. The nuclear binding capacity of the pituitaries of hypothyroid rats (M) and the plasma concentration of T_3 yielding half-nuclear occupancy (k) were determined in experiments in which graded doses of unlabeled T_3 were injected together with tracer (Fig. 4). The plasma:nuclear ^{125}I-T_3 ratio was plotted as a function of the plasma T_3 concentration which was determined by dividing the plasma TCA-precipitable radioactivity by the specific activity of the injected T_3. As previously pointed out (12), the slope of the resulting curve is equal to the binding capacity and the intercept on the abscissa is the reciprocal of kM. Results of these experiments yielded a binding capacity of 5.5 ng T_3/g tissue for hypothyroid animals, which is somewhat less than the value previously reported by us for euthyroid rats (6.6 ng/g (8). The value for k was estimated as 1.0 ng/ml.

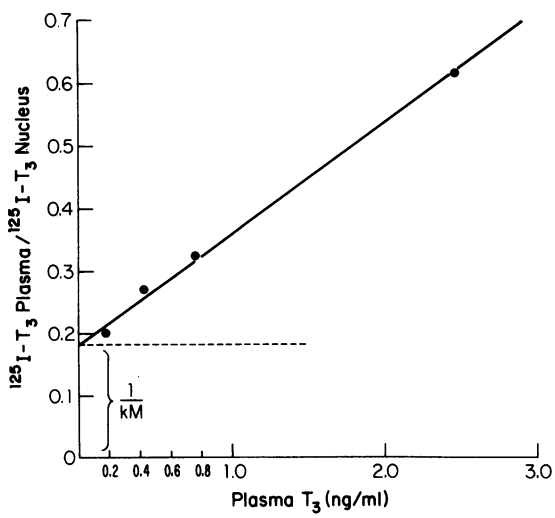

FIGURE 4 Pituitary nuclear binding capacity and affinity were determined by in vivo displacement techniques (14, 15). Two pituitaries were used for each point. The nuclear: plasma ratio of ^{125}I-T_3 was corrected for nonspecific binding by injection of 10,000 ng T_3/100 g body wt. As previously pointed out, the corrected plasma:nuclear ratio of ^{125}I-T_3 is linearly related to concomitant plasma T_3 concentration (12). The slope of the function is equal to the M, and the intercept on the ordinate is equal to the reciprocal of the production of M and k, the apparent dissociation constant. The latter is that concentration of T_3 required to occupy one-half of the nuclear sites. Results of these studies indicate a k of 1.0 ng/ml and M of 5.5 ng/g tissue.

Relationship between the rate of GH induction (R) and the fraction of nuclear sites occupied by T_3 (q)

The mathematical framework previously developed for analyzing the kinetics of hepatic enzyme induction by T_3 (11) was applied to the data generated in this study to determine the function R(q). Each of four dose response experiments was separately analyzed. Fig. 5 illustrates the two curves representing the extremes of the family of curves generated. Both sets of results differed markedly from the occupancy-responsivity relationships previously calculated for α-GPD and ME (13). With GH, the rate of response appeared to be linear from 30 to 70% occupation. The amplified relationship of α-GPD and T_3 is illustrated for the purposes of comparison. The ratio of the responsivity of GH at full occupancy to that at 50% occupancy in the two curves for GH illustrated was 2.0 and 3.6. In contrast, the value of the comparable ratio for α-GPD was $\cong$18. An ideal linear response would yield a value of $\cong$2.

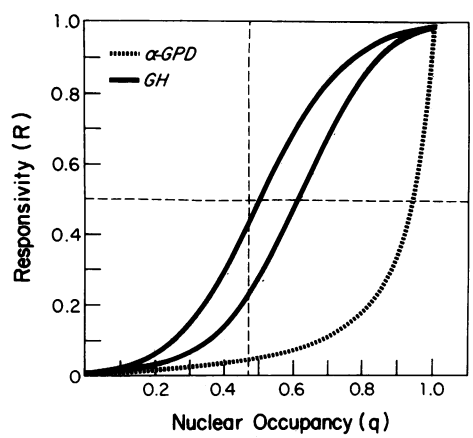

FIGURE 5 The relationship between R and q is illustrated for two sets of dose-response experiments. The data base and calculations are described in the text. For the sake of comparison, the relationship between R and q for α-GPD induction by T_3 previously calculated (13) is illustrated by the broken line. Note that the relationship between nuclear occupancy and response of GH accumulation in the pituitary is substantially more linear than the corresponding relationship for α-GPD content in liver.

Comparison of dose-response relationships at 24 and 36 h

The following experiment was performed to test the validity of the model used. One group of hypothyroid rats was injected with increasing doses of T_3 (0.3, 0.6, 1.5, 5.0, 50, and 100 μg/100 g body wt) and killed 24 h later. A second group of hypothyroid animals was injected with doses which were five times higher than those used in the first group (1.5, 3.0, 7.5, 25.0, 250, and 500 μg/100 g body wt). Animals were killed at 36 h. The pituitary GH content was measured in both groups. R(q) was determined in the first group as described above. Because the plasma disappearance curve after a given dose of T_3 could be estimated for 36 h, it was possible by application of the basic model to compute the expected 36-h dose-response relationship (Fig. 6). The predicted ratio of the maximal GH content at 36 h to that at 24 h was 1.61. The experimentally observed ratio of the maximal value at 36 h to that at 24 h was 1.85, some 15% higher than the predicted ratio. Although the basis of this discrepancy is not clear, the results support the use of this model as a first approximation of the pituitary GH response system in the hypothyroid rat.

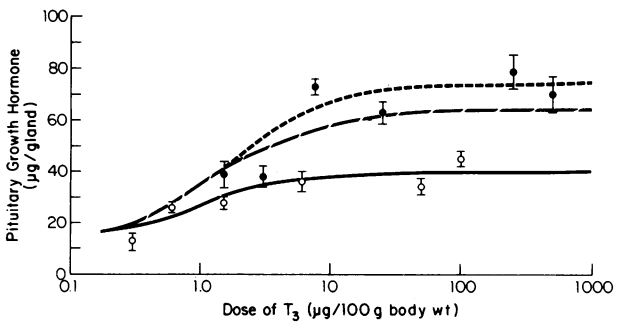

FIGURE 6 Two groups of hypothyroid rats were injected i.v. with graded doses of T_3. Group A received 0.3, 0.6, 1.5, 5.0, 50.0, and 100.0 μg T_3/100 g body wt and were killed 24 h after the injection. Group B was injected with 1.5, 3.0, 7.5, 25.0, 250.0, and 500.0 μg T_3/100 g body wt and were killed 36 h afterwards. Pituitary GH content was measured. Dose-response relationship of Group A was fitted by the nonlinear regression described in the text (○ —— ○). R as a function of q was determined as described in the text and, together with the known relationship between the dose of T_3 injected and the plasma T_3 concentration as a function of time (13), it was possible to estimate the expected dose-response relationship at 36 h (●——●). This computer-generated curve can be compared to the actual values observed in Group B (●----●). The predicted ratio of maximal response at 36 h to that at 24 h is 1.60, some 14% less than the actual ratio observed, 1.85. Whether this deviation is a result of experimental error or is a function of inherent limitations in the theoretical model has not been determined, but the results appear to justify application of the model at least as a first approximation of the dose-response relationship within the first 2 days after the injection of T_3. Bars, ±SE (n = 4).

Response of euthyroid rats to T_3 administration

The effect of full occupancy of nuclear sites in euthyroid rats was evaluated in animals receiving daily i.p. injections of 200 μg T_3/100 g body wt for a period of 1–7 days. This dosage regimen can be shown to result in full occupation of nuclear sites for the period of the experiment, and has previously been used to evaluate maximal response in α-GPD and ME response (13). Rats were killed at the time periods indicated (1–5, and 7 days) and the pituitary GH content was measured by radioimmunoassay. Results illustrated in Fig. 7 differ markedly from those previously observed for α-GPD and ME (13). Even after 4 days of injection, the level of pituitary GH did not appear to be significantly above the base-line levels (analysis of variance). In fact, animals treated for the full 7 days appeared to have a significant 45% decrease in pituitary GH content (P < 0.01) as compared to the 4-day value, although the decrease at 7 days was not statistically significant when compared to the base-line control values.

DISCUSSION

Our findings indicate a marked difference between the induction of pituitary GH on one hand and the induc-

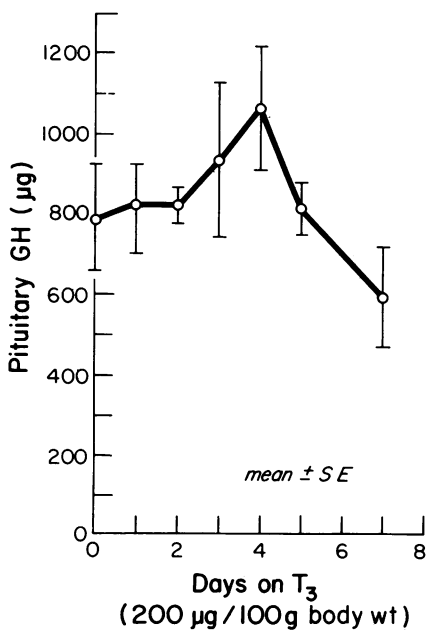

FIGURE 7 Euthyroid rats received daily i.p. injections of 200.0 μg of T_3/100 g body wt and were killed at the indicated time intervals. The dose of T_3 injected can be shown to occupy over 95% of the receptor sites for the period of the experiment. Analysis of variance of the results indicates that this treatment did not induce a significant change in pituitary GH content any time as compared to the base-line euthyroid controls. Nevertheless, there was a significant fall at 7 days in comparison to the 4-day value (P < 0.01). Bars, ±SE (n = 5).

tion of α-GPD and ME by T_3 on the other. These differences are immediately apparent when the dose required to attain one-half maximal induction at 24 h is considered. For α-GPD this was about 10.0 μg/100 g body wt (13), whereas the corresponding dose for GH was determined to be 0.6 μg/100 g body wt in the present experiments. When the relationship between nuclear occupancy and responsivity was analyzed by application of the mathematical model developed for hepatic enzyme induction, it became clear that the relationship between nuclear occupancy and the rate of induction is substantially more linear for GH accumulation than for the induction of the two hepatic enzymes which are strongly amplified as nuclear occupancy by T_3 is increased. Although the absolute increment in growth hormone response varied widely from one group of rats to another, the similarities in the nature of the dose-response relationship are apparent when the induction of GH is expressed as a fraction of the maximal response at 24 h.

For α-GPD, a single injection of a dose of T_3, designed to keep nuclear sites occupied for the duration of the experiment, could restore the enzyme value to above euthyroid values one day after the injection. A comparable dose of T_3 injected into hypothyroid rats, however, failed to restore the pituitary GH to euthyroid levels at this time. On the average, the levels of pituitary GH after a maximally effective dose on the 1st day were only ≅one-fifth of the euthyroid level. Additional injections were required to achieve longer periods of saturation and the attainment of a euthyroid pituitary GH content. These differences probably reflect the amplification of the T_3 signal in the α-GPD response and an absence of such amplification for pituitary GH. With the nuclear sites fully occupied, we have estimated that the rate of induction of hepatic enzymes is 10- to 20-fold greater than the rate of induction at euthyroid levels of occupancy. In the case of the pituitary, the comparable ratio is only ≅2.0–3.6.

The nonamplified relationship between nuclear occupancy and GH induction is in general agreement with the results reported by Samuels et al. who have examined this problem with GH_1 cells grown in culture (11). These investigators have postulated two pools of receptor sites, one of which is depletable by T_3, and the other, nondepletable. An almost perfect linear relationship was reported between the rate of GH synthesis and nuclear occupancy of the depletable sites. In our studies with the intact animals, we have not been able to establish that T_3 induces a steady-state alteration in the concentration of hepatic nuclear receptor sites (12). The nuclear pituitary-binding capacity of hypothyroid animals determined in the present study, 5.5 ng/g, is actually somewhat less than we had previously reported for euthyroid animals, 6.6 ng/g. One would have anticipated the opposite if "down regulation" had occurred in all constituent cell types.[2] Any comparison between the characteristics inferred from studies of a cell line in vitro and those derived from studies in the intact animals, however, is obviously difficult. Clearly, the possibility must be recognized that secondary biochemical and physiological factors could have influenced the effects studied in the intact animal. Potential deviations from normal function in rat pituitary tumor cells grown in tissue culture are equally well recognized. Nevertheless, the results of studies at both levels of organization indicate that the strong signal amplification of T_3, which characterizes hepatic induction of hepatic enzymes by T_3, does not describe the induction of GH by the rat somatotroph. Of interest in this connection is the linear relationship between the fractional decrement in pituitary thyroid-stimulating hormone and the estimated nuclear occupancy reported by Silva and Larson 3 h after the injection of varying doses of T_3 (22). As pointed out by these authors, the findings raise the possibility that thyroid-stimulating hormone inhibition, in general, may be a linear function of nuclear occupancy of T_3.

Another striking difference between the induction of GH and the hepatic enzymes in vivo is the discrepancy in the response of euthyroid animals to prolonged saturation of the nuclear receptors. In previous experiments we have shown that daily doses of 200 μg T_3/100 g body wt administered for 7 consecutive days in a dose regimen designed to keep these sites nearly occupied for this period of time results in a 9- to 10-fold increase in the increments of α-GPD and malic enzyme activity above hypothyroid base-line values. In contrast, the same dosage regimen in the present set of experiments failed to result in a significant increase in GH above euthyroid levels. This was surprising because one would have anticipated that with an estimated 50% nuclear occupancy in the euthyroid state (15) the level of GH would increase by a factor of 2 in an ideal linear system. A number of possible explanations can be advanced to account for our findings: (*a*) some other factor may become rate-limiting in the synthesis of GH, (*b*) tissue hyperthyroidism per se may have resulted in an increased fractional removal rate of intrapituitary growth hormone, thus resulting in the maintenance of relatively unchanged levels of pituitary GH in the presence of enhanced formation of GH, (*c*) hyperthyroidism could have resulted in a

[2] On the basis of data provided by Surks and DeFesi (21) it is possible to calculate that the binding capacity per mg DNA in the hypothyroid pituitary is 0.94 ng T_3/mg DNA, and in the euthyroid pituitary 1.0 ng T_3/mg DNA. Because the DNA content of individual cell types does not vary greatly (21) "down regulation" cannot be a general phenomenon governing the receptor content of all pituitary cell types in the steady state.

reduction of specific receptors, (*d*) alterations could have occurred in the rate of transcription or processing of nuclear RNA, or (*e*) increased turnover of cytoplasmic messenger RNA. Lastly, (*f*) Larsen et al. (23) have suggested that pituitary nuclear receptors may be saturated to a substantially higher degree than previously believed. Further studies are clearly required to resolve this issue. It should be noted, however, that other effects of thyroid hormone apparently are also limited by an apparent "ceiling" set at the euthyroid level. Thus, in previous experiments (24) we have found that the levels of a hepatic cytosolic androgen and T_3-dependent protein, probably identical to α_{2U} globulin within the hepatocyte (25), do not exceed normal, despite induction of hyperthyroidism. Thus, the differences between the response characteristics of pituitary GH and hepatic α-GPD and ME are not a reflection of tissue-specific characteristics.

Even though the mechanistic basis for the observed differences between the induction of GH and the hepatic enzymes is unclear, such differences may have important physiological and pathophysiological consequences. Despite the finding that GH is an exceedingly "sensitive" index of T_3 response in hypothyroid rats, significant increases in pituitary content above the normal could not be elicited. In unpublished experiments we have also been unable to demonstrate increases over euthyroid values in the plasma GH of these animals. These findings imply that the response typified by GH does not contribute to the symptomatology of hyperthyroidism.

Both pituitary GH and hepatic enzyme induction by T_3 are characterized by a significant delay between the time of injection of T_3 and the maximal rate of product accumulation. We have previously estimated that in euthyroid animals the delay in α-GPD and ME formation was $\cong$13.4 and 8.2 h, respectively. In the present experiments, response of pituitary GH can be detected within 3 h, but maximal rates of increase were not apparent until sometime after 12 h. The mean delay time was estimated to be $\cong$5.6 h. Because the nuclear sites are occupied within minutes after the injection of large doses of T_3, and because translational processes proceed in a matter of minutes, it is highly likely that the delay in the appearance of GH and the hepatic enzymes is a result of some undefined pretranslational process. The kinetics of accumulation of GH and the delay time, however, did not appear to be a function of the dose as long as all the sites remained saturated. This constitutes further evidence which indicates that the initiation of thyroid hormone action is constrained by the occupation of specific saturable receptors.

The terminal $t_{1/2}$ of GH decay was found to be $\cong$4 days. This is very long in comparison to the $t_{1/2}$ of T_3 which is $\cong$6 h, and is reminiscent of the long $t_{1/2}$ characteristic of the induction of α-GPD and ME, 2.7 days. Unfortunately, because neither the intrinsic $t_{1/2}$ of intrapituitary GH nor the $t_{1/2}$ of messenger RNA for GH is known, the rate-limiting factor cannot be identified. It appears possible, however, that the slow decay is related to the formation of a long-lived T_3 "imprint" at the nuclear level which we have postulated for hepatic effects of T_3 (26, 27).

Throughout the study we have examined the appearance and disappearance of an immunoreactive species of GH and not a uniquely defined chemical substance. The biochemical heterogeneity of pituitary GH (28–30) has been established, and alterations in the proportion of different forms of GH as a function of time after the injection of T_3 would complicate our analysis. Additional studies designed to examine this question appear in order. Moreover, experiments with standard isotopic labeling procedures would also be helpful in quantitating synthesis and degradation. Further, it should be emphasized that in these studies no effort was made to distinguish between intrapituitary degradation of GH and export of GH into the circulation. The disappearance of intrapituitary GH is clearly the sum of both processes. Moreover, these studies did not distinguish between the effect of T_3 on cellular replication of GH-producing pituitary cells. The effect on DNA replication in the time frame of these experiments probably is minimal because DeFesi and Surks[3] have recently shown that the rate of decrease in pituitary GH cells takes place at a rate of about 1% per day.

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