In Vivo Stimulation of Sugar Uptake in Rat Thymocytes

An Extranuclear Action of 3,5,3′-Triiodothyronine

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Abstract

In previous studies we have demonstrated that 3,5,3′-triiodothyronine (T_3) in vitro produces a prompt increase in the uptake of the sugar analogue 2-deoxyglucose (2-DG) by freshly isolated rat thymocytes. This effect is prompt, being evident at 20 min after addition of T_3, is independent of new protein synthesis, and can be elicited by physiologic concentrations of the hormone. In the present studies, we have sought to determine whether physiologic doses of T_3 are capable of inducing an increase in 2-DG uptake in the thymocytes of the living animal. Therefore, 26–28-d-old female rats were injected with increasing doses of i.v. T_3, followed 60 min later by ^{3}H-labeled 2-DG. 30 min later, animals were killed, thymocytes were isolated, and their ^{3}H content determined. Uptake of [^{3}H]2-DG was increased by T_3 in a dose-dependent manner. The lowest effective dose was 10 ng/100 g of body weight (30% above control) and the maximally effective dose 1 μg/100 g of body weight (116% above control). The effect of T_3 was independent of new protein synthesis in that it was not blocked by a dose of cycloheximide that inhibited the incorporation of [^{3}H]leucine into thymocyte protein by 92–95%. Comparable studies with various thyronine analogues revealed the following rank order of potency: L-T_3 > L-3,5,3′5′-tetraiodothyronine (L-T_4) > D-T_3 ≥ D-T_4 > L-3,3′5′-triiodothyronine > 3′-isopropyl-3,5-L-diiodothyronine (T_2) = 3,5-L-T_2. DL-thyronine was without effect.

These studies indicate that T_3 in physiologic doses acts in vivo to increase the uptake of sugar by rat thymocytes by a mechanism that is extranuclear in origin, in that it is independent of new protein synthesis. The findings support the conclusion that the previously demonstrated effects of T_3 on thymocyte sugar uptake in vitro, which seem clearly to be mediated at the level of the plasma membrane, have physiologic relevance.

Introduction

We have previously demonstrated that 3,5,3′-triiodothyronine (T_3)[1] increases the uptake of the glucose analogue 2-deoxyglucose (2-DG) in isolated rat thymocytes in vitro (1–5). This effect of T_3 is evident within 20 min after the addition of the hormones; it is independent of new protein synthesis, is calcium-dependent, and is mediated by an increase in cellular cyclic AMP (cAMP) concentration secondary to an increase in adenylate cyclase activity. On the basis of these and other findings, we have suggested that this action of T_3 is mediated at the level of the thymocyte plasma membrane, and consonant with this is our demonstration of the presence of specific, saturable binding sites for T_3 in highly purified thymocyte plasma membrane preparations (6).

Although supraphysiologic concentrations of T_3 are required to produce these effects when T_3 is tested alone, in the presence of adequate concentrations of insulin and epinephrine, the in vitro effect of T_3 on sugar uptake in the intact thymocyte is elicited by physiologic concentrations of T_3 as low as 5 pM (2). Nonetheless, in respect to these actions of T_3, as with other in vitro metabolic responses to a hormonal agent, ultimate proof of their physiologic relevance depends on the demonstration that they can be reproduced by physiologic doses in the living animal. In the present studies, we have shown that very small doses of T_3 (10 ng/100 g of body weight [bw]) increase the uptake of 2-DG by thymocytes in the living rat, a response that, like its in vitro counterpart, is dose-dependent and independent of new protein synthesis.

Methods

Animals and reagents. Animals used were 26–28-d-old female rats of the Sprague-Dawley (CD) strain purchased from Charles River Laboratories, Wilmington, MA, and weighing ~65 g at the time of study.[2] Rats were maintained for up to 5 d on tap water and a standard diet (OVAL, Charles River RMH 3000) supplied by Agways, Inc., Syracuse, NY.

Other materials used in these studies were purchased from the following sources: ^{125}I-T_3 (sp act 1,200 μCi/μg), [^{3}H]2-DG (sp act 5–8 Ci/mmol), [^{3}H]mannitol (sp act 17 Ci/mmol), and [^{3}H]leucine (sp act 141 Ci/mmol) from New England Nuclear, Boston, MA; cycloheximide and DL-thyronine from Sigma Chemical Co., St. Louis, MO; L-T_3, L-3,5,3′5′-tetraiodothyronine (L-T_4), D-T_3, D-T_4, and L-3,5-diiodothyronine (T_2) from Henning GmbH, Berlin, Federal Republic of Germany; L-3′5′-triiodothyronine (rT_3) from Calbiochem-Behring, San Diego, CA. 3′-isopropyl-T_2 was a gift from Henning GmbH.

Unless otherwise stated, the various materials administered were dissolved in saline or mildly alkaline saline solution and injected into the tail vein in a volume of 0.1 ml/100 g of bw.

Measurement of tissue ^{125}I-T_3 accumulation. Initial experiments were conducted to determine the time course of accumulation of administered T_3 in several organs, including the thymus gland. Animals were injected with 250 nCi of ^{125}I-T_3/100 g of bw (0.21 ng of T_3/100 g of bw)[3] and

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1. *Abbreviations used in this paper:* bw, body weight; 2-DG, 2-deoxy-D-glucose; T_2, diiodothyronine; T_3, triiodothyronine; T_4, tetraiodothyronine.

2. Animals used in this study were maintained in accord with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. (NIH) 78-32, revised 1978).

3. Purity of ^{125}I-T_3, measured by descending paper chromatography (7), was >98%.

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were killed by means of a guillotine at various times. Blood was collected and the thymus gland, liver, and kidneys were quickly removed. The ^{125}I-T_3 concentration in serum and in these organs was then measured in a gamma-scintillation counter.

Measurement of [^{3}H]2-DG uptake by thymocytes. Additional experiments examined the time course of the uptake of [^{3}H]2-DG in thymocytes. Here, animals were injected with 30 μCi of [^{3}H]2-DG/100 g of bw (0.8 μg of 2-DG/100 g of bw) and were killed by means of a guillotine at various times thereafter. Blood was collected, from which serum was obtained, and thymus glands were quickly removed and placed into ice-cold Tris–Krebs–Ringer buffer, pH 7.4. Thymocytes were then isolated by methods previously described (1). The isolated cells were resuspended in ice-cold buffer, and 200-μl aliquots (~30 × 10^6 cells/ml) were transferred to microtubes and centrifuged in a Beckman Microfuge (Beckman Instruments, Inc., Palo Alto, CA) for 30 s. The supernatant was aspirated, and the pelleted cells were transferred to vials containing liquid scintillation cocktail, as were aliquots of serum. ^{3}H content of the samples was measured in a liquid scintillation counter.[4]

In each experimental group, the extracellular [^{3}H]2-DG included in the pellet of isolated thymocytes was determined. Prior to centrifugation, [^{3}H]mannitol was added to cell suspensions obtained from animals that had not been injected with [^{3}H]2-DG in each of the experimental groups. Cells were pelletted, and the [^{3}H]mannitol contents of the pellets determined. These values were used to correct values of the [^{3}H]2-DG uptake for the labeled sugar occluded in the cell pellet. Values for occluded ^{3}H did not exceed 0.1% of the total added and did not differ among various experimental groups.

In each animal injected with [^{3}H]2-DG, blood was obtained at the time of sacrifice and serum derived therefrom was analyzed for ^{3}H concentration. To determine the chemical nature of the ^{3}H-labeled compounds within the thymocyte, thin-layer chromatographic analysis for sugars was performed in cellulose, using ethyl acetate, pyridine, water, and glacial acetic acid (36:36:21:7) as the running solvent (8).

Measurement of [^{3}H]leucine incorporation in thymocytes. Animals were injected with 40 μCi of [^{3}H]leucine/100 g of bw into the tail vein. 15 min later, animals were killed by means of a guillotine and thymocytes were immediately isolated. 150-μl aliquots of thymocyte suspension were transferred into small plastic tubes containing 150 μl of ice-cold 12% trichloroacetic acid (TCA), mixed well and put on ice for 20 min. Tube contents were transferred onto 0.45-μm Millipore filters (Millipore Corp., Medford, MA) under vacuum. Tubes and filters were washed three times with 2 ml of ice-cold 6% TCA, filters were dried, transferred to vials containing liquid scintillation cocktail, and their content of ^{3}H was measured with a beta-scintillation counter.

Cell viability. Cell viability was assessed by means of the trypan blue technique (1), in which a viable cell is one that excludes the dye.

Statistical analysis. Where appropriate, statistical analyses of the differences between experimental groups were performed using the analysis of variance followed by the Newman–Keuls multiple range test for comparison of multiple groups with one another (9).

Results

Tissue accumulation of ^{125}I-T_3 in vivo. Serum ^{125}I-T_3 concentration declined rapidly, in an apparent exponential fashion, following i.v. injection of the labeled hormone (Fig. 1). Concentrations of ^{125}I in liver and kidney rose rapidly, reaching a peak at ~20 min, decreasing rapidly thereafter, and ultimately

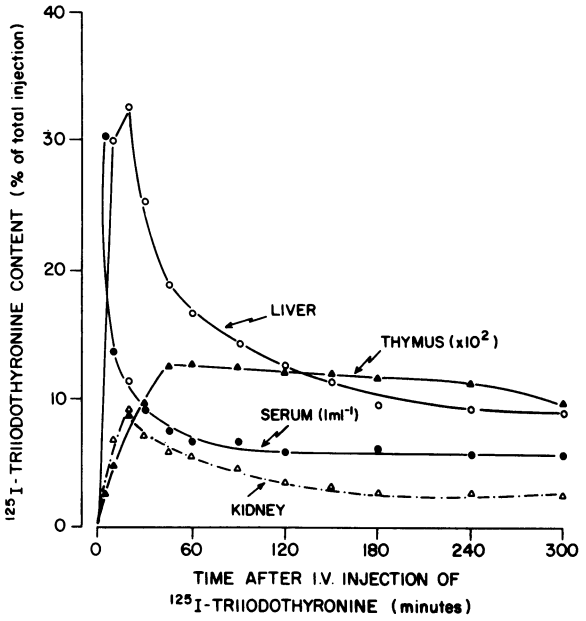

Figure 1. Time course of ^{125}I-triiodothyronine accumulation in several tissues in the rat. Animals were injected with 250 nCi of ^{125}I-T_3/100 g of bw. Animals were killed at the time periods indicated, and serum, thymus, liver, and kidneys were collected and their content of ^{125}I was measured. Values shown are the results obtained in a single experiment involving one animal per time point, but are typical of those obtained in two additional experiments.

declining in parallel with the decrease in serum ^{125}I concentration. In contrast, the concentration of ^{125}I in the whole thymus gland reached a peak at ~40 min, but remained relatively constant thereafter.

[^{3}H]2-deoxyglucose uptake. After the i.v. injection of [^{3}H]2-DG, the serum concentration of [^{3}H]2-DG displayed a rapid, apparently exponential, rate of decline. This was accompanied by a progressive increase in the thymocyte uptake of the labeled sugar, which appeared to represent an exponential approach to a maximum, reflecting in a mirror-image fashion the decline in serum [^{3}H]2-DG concentration (Fig. 2). This behavior is consonant with the known metabolism of 2-DG, i.e., uptake into the cell, phosphorylation by hexokinase, and the retention of 2-DG-6-phosphate within the cell without further metabolism (10, 11). In subsequent experiments, animals were killed for measurement of thymocyte [^{3}H]2-DG uptake 30 min after administration of [^{3}H]2-DG. This was an interval at which serum [^{3}H]2-DG concentration was declining more slowly than it would have been at earlier time periods, making the precision of the time of killing animals less critical.

Effect of T_3 on thymocyte 2-DG uptake. Animals were given i.v. T_3 in doses ranging from 2 ng to 100 μg/100 g of bw, followed in 60 min by [^{3}H]2-DG (30 μCi/100 g of bw). 30 min later, animals were killed, thymocytes were isolated, and their content of [^{3}H]2-DG was measured.

T_3 produced a dose-related increase in the uptake of [^{3}H]2-DG by thymocytes (Fig. 3). The lowest effective dose was 10 ng/100 g of bw (130% of control values, $P < 0.05$), and the maximal effect was reached at a dose of 1 μg/100 g of bw (216% of control values, $P < 0.01$). From the dose-response curve, the calculated half-maximal effect was obtained with a dose of 50–100 ng/100 g of bw.

4. The thymocyte content of [^{3}H]2-DG was also measured by a second method in which 200-μl aliquots of the suspended cells were transferred onto GF/A glass-fiber filters (Whatman Chemical Separation Inc., Clifton, NJ), and washed three times with 2 ml of ice-cold buffer. Filters were then dried and transferred to vials containing liquid scintillation cocktail, and their content of ^{3}H was measured. Similar results were obtained with both procedures. Because the procedure utilizing the microtubes was more convenient, it was employed in ensuing experiments.

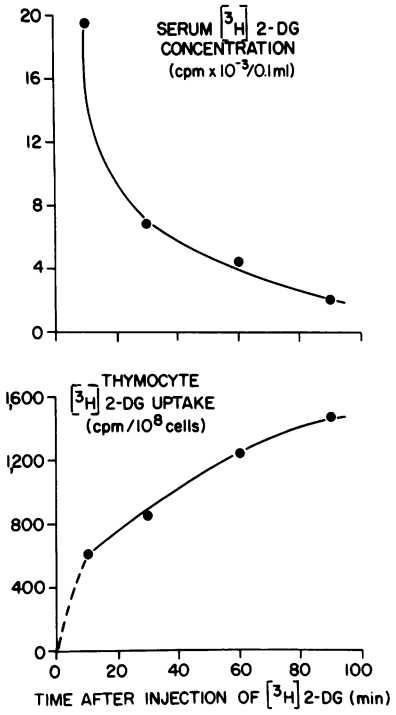

Figure 2. Time course of the uptake of [^{3}H]2-DG by rat thymocytes. Animals were injected with 30 μCi of [^{3}H]2-DG/100 g of bw into the tail vein. Thereafter, at the periods of time indicated, animals were killed, and the content of ^{3}H in serum (*upper panel*) or thymocytes (*lower panel*) was measured. Values shown are the results obtained in a single experiment involving one animal per time point, but are typical of those obtained in two additional experiments.

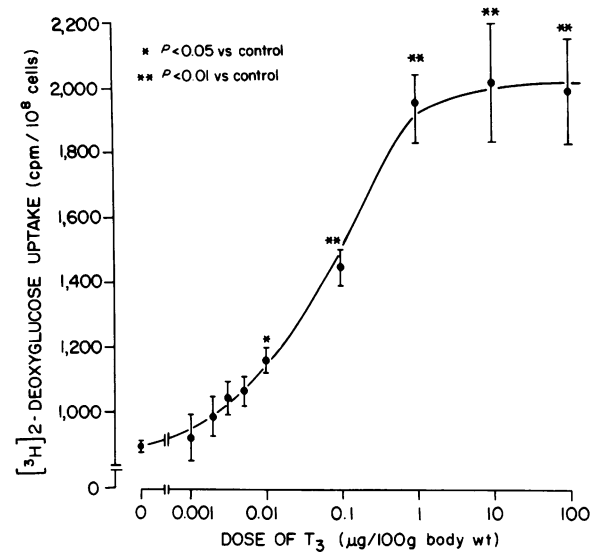

Figure 3. Dose-related effect of 3,5,3'-T_3 on [^{3}H]2-DG uptake by rat thymocytes in vivo. Animals were given the indicated doses of T_3 by a single injection into the tail vein. 60 min later, [^{3}H]2-DG (30 μCi/100 g of bw) was injected into the tail vein and its 30-min uptake by thymocytes was then measured. Values shown are the mean±standard deviation of pooled data obtained in two (0.001 and 0.002 μg of T_3), three (0.005, 0.01, 2, and 100 μg of T_3), four (0.003, 0.1, and 10 μg of T_3), or seven (control and 1 μg T_3) separate experiments, each involving a single animal.

Effects of T_3 on thymocyte 2-DG uptake could not be explained by a reduction in uptake of the sugar by other tissues leading to a higher serum [^{3}H]2-DG concentration, since the concentration of ^{3}H in the serum of animals given the maximally effective dose of T_3 (1 μg/100 g of bw) was 93.0±16.2% (mean±SD) of that found in control animals. Additional experiments related to this point are described below.

As judged from thin-layer chromatographic analysis, >90% of the ^{3}H in thymocytes from control and T_3-treated animals was [^{3}H]2-DG-6-phosphate.

Inhibition of thymocyte protein synthesis by cycloheximide. Rats were given a single i.p. injection of cycloheximide in various doses, and 30 min later [^{3}H]leucine (40 μCi/100 g of bw) was injected into the tail vein. Rats were killed 15 min later, their thymocytes were harvested, and [^{3}H]leucine incorporation into thymocyte protein was measured. Cycloheximide produced a dose-related decrease in [^{3}H]leucine incorporation; 0.1 mg and 1.0 mg/100 g of bw inhibited [^{3}H]leucine incorporation by 65% and 95%, respectively. In additional experiments, cycloheximide (1 mg/100 g of bw) was injected i.p., and 30, 60, 120, and 180 min later animals were injected with [^{3}H]leucine. After 15 min, animals were killed and incorporation of [^{3}H]leucine into thymocyte protein was measured. In all the four time periods studied, cycloheximide inhibited [^{3}H]leucine incorporation by not <92%. Hence, in subsequent experiments, animals were injected with i.p. cycloheximide (1 mg/100 g of bw) 30 min prior to the injection of T_3.

T_3 effect and cycloheximide. To examine whether the stimulatory effect of T_3 on thymocyte 2-DG uptake requires new protein synthesis, animals were first injected with i.p. cycloheximide (1 mg/100 g of bw). 30 min later, T_3 in doses ranging from 0.1 to 1 μg/100 g of bw was injected into the tail vein, followed in 60 min by [^{3}H]2-DG. Animals were killed 30 min later and both the [^{3}H]2-DG concentration in serum and uptake by thymocytes were measured.

Cycloheximide reduced basal (control) thymocyte 2-DG uptake by 60%, an effect similar to that produced by both puromycin and cycloheximide in thymocytes in vitro (1). However, the proportionate increase in thymocyte 2-DG uptake that T_3 produced was generally the same in animals that were treated with cycloheximide as in those that were not (Fig. 4).

Effect of T_3 and cycloheximide on serum ^{3}H concentration. Because the uptake of [^{3}H]2-DG by tissues over time is in part a function of the integrated plasma [^{3}H]2-DG concentration to which they are exposed during this period, additional experiments were undertaken to verify that the changes in thymocyte 2-DG uptake seen in response to T_3, cycloheximide, or both were not merely the result of a change in overall [^{3}H]2-DG disposal. In these experiments, animals were injected with T_3 (1 μg/100 g of bw), cycloheximide (1 mg/100 g of bw), or both, followed by [^{3}H]2-DG according to the protocols described above. Animals in each of the groups were killed at 5, 10, 20, and 30 min after injection of [^{3}H]2-DG and the ^{3}H concentration in specimens of serum obtained at those times was measured.

In animals given T_3 alone, serum ^{3}H concentration was consistently ~10% lower than in controls at all time points. The converse was true in animals treated with cycloheximide, the average increase being ~50%. Serum ^{3}H concentrations in animals given both cycloheximide and T_3 were ~10–20% lower than those in animals given cycloheximide alone (Table I).

Thyronine analogues. The effect of various thyronine analogues on the in vivo uptake of [^{3}H]2-DG by rat thymocytes

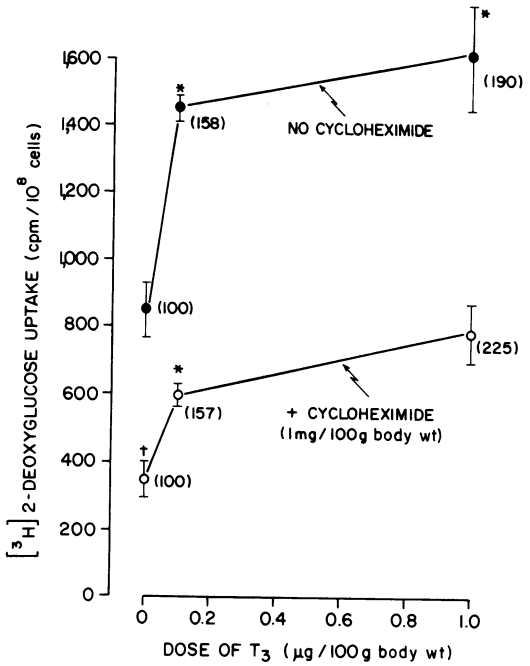

Figure 4. The effect of cycloheximide on the T_3-induced increase in 2-DG uptake by rat thymocytes in vivo. Animals were first injected i.p. with 1 mg of cycloheximide/100 g of bw. 30 min later, T_3 in various doses was injected into the tail vein, and 60 min later the thymocyte uptake of [^{3}H]2-DG was measured. Values shown are the mean±standard deviation of those obtained in four separate experiments, each involving a single animal. *, significantly greater than the corresponding value in the absence of T_3 administration ($P < 0.01$). +, significantly lower than the corresponding value in the absence of cycloheximide and T_3 ($P < 0.01$). The numbers in parentheses are the values in T_3-treated animals expressed as a percent of those in corresponding animals given no T_3.

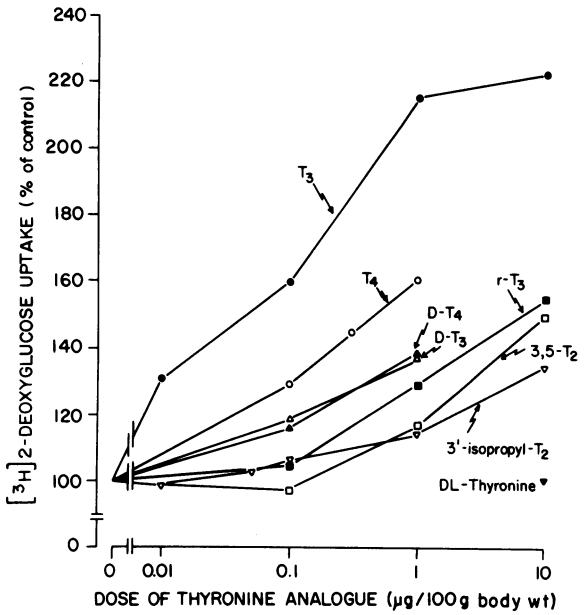

Figure 5. The effect of several thyronine analogues on 2-DG uptake by rat thymocytes in vivo. The experimental procedure was the same as that described in the legend to Fig. 3. Values shown are the mean of those obtained in eight (L-T_3) or three (other analogues) separate experiments, each involving a single animal per point. Values of standard deviations were 5–15% of their respective mean values.

was studied, using the same experimental procedure as that used for T_3. Relative potencies were estimated from dose-response curves (Fig. 5), and the following rank-order of potency was observed: L-T_3 > L-T_4 > D-T_3 = D-T_4 > rT_3 > 3′-isopropyl-T_2 = L-3,5-T_2. DL-thyronine was without effect.

Table I. Effects of T_3 and Cycloheximide, Alone and Together, on the Disappearance of ^{3}H from the Serum of Animals Injected with [^{3}H]2-DG

Time after [^{3}H]2-DG injection	Serum ^{3}H concentration			
	Control	T_3	CH	CH + T_3
min	*cpm × 10^{-3}/0.1 ml*			
5	33.3±11.4	28.2±6.6	58.7±21.1	47.6±11.9
10	26.4±11.8	18.5±5.7	35.1±15.4	32.0±13.1
20	10.7±3.1	10.1±7.4	19.2±4.7	15.9±6.0
30	8.0±2.4	7.2±2.2	13.3±4.7	9.5±1.6

Animals were injected with T_3 (1 μg/100 g of bw) and/or cycloheximide (CH) (1 mg/100 g of bw) as described in Methods. [^{3}H]2-DG was injected i.v. and animals were killed at the indicated intervals thereafter. Values shown are mean±standard deviation of those obtained in three separate experiments involving one animal for each data point. As judged from Dunnett's test (9), results in the three experimental groups are not significantly different from those in the corresponding control group at any time point.

Discussion

In the present studies, we have shown that administration of T_3 leads to an increase in the uptake of the glucose analogue 2-DG by thymocytes in the living female weanling rat. The effect of T_3 was relatively prompt in onset, being evident 90 min after T_3 administration, the earliest time point studied. This response to T_3 was independent of new protein synthesis, because it was not blocked by a dose of cycloheximide that profoundly inhibited thymocyte protein synthesis throughout the entire 90 min period of T_3 action. These effects of T_3 and cycloheximide, alone and together, on thymocyte 2-DG uptake could not be explained by unrelated effects of these agents on the clearance of [^{3}H]2-DG from the circulation. Thus over the entire 30 min period during which [^{3}H]2-DG uptake was measured, ^{3}H concentrations in serum tended to be lower than in controls in animals that received T_3, higher than in controls in animals that received cycloheximide, and lowered by T_3 in animals of the latter group. Indeed, the apparent lowering of serum ^{3}H concentration would be consistent with an increased uptake of [^{3}H]2-DG by tissues other than thymocytes, in that the increase in [^{3}H]2-DG uptake by thymocytes alone could not have accounted for the lowering of serum ^{3}H concentrations that was observed.

Studies with various thyronine analogues revealed that, except in the case of 3′-isopropyl-T_2, which was much less potent than T_3, their rank order of potency in stimulating 2-DG uptake in vivo generally accorded well with that of their classical thyromimetic effects (12–15). Thus, L-T_3 was more potent than L-T_4, the L-isomers of the two hormones were more potent than their D-isomers, and rT_3, 3,5-T_2, and DL-thyronine were much less effective or not effective at all.

Over a range of T_3 doses from 10 ng/100 g of bw to 1 μg/100 g of bw, the response to T_3 was dose-related. Doses within this range can clearly be considered physiologic, and the response thereto to be physiologically relevant, in that the daily replacement dose of T_3 in the rat is ~0.5 μg/100 g of bw (16).

The major features of the in vivo increase in thymocyte sugar uptake induced by T_3 are similar to those of the increase in thymocyte sugar uptake produced by T_3 in vitro (1). This is true of the promptness of the effect, its independence of new protein synthesis, and the rank order of potency of various thyronine analogues, including the relative lack of potency of 3′-isopropyl-T_2. Further, as with the very small doses of T_3 required in vivo, responses to T_3 in vitro were elicited by physiologic concentrations of T_3, in the presence of insulin and epinephrine (2). These similarities suggest that the mechanism by which T_3 increases thymocyte 2-DG uptake in vivo is the same as that by which it does so in the in vitro system, a mechanism that seems clearly to be initiated at the level of the plasma membrane.

If this is so, then from the findings obtained in vitro, we would suggest that T_3 initiates its action in vivo by binding to specific receptors on the thymocyte plasma membrane (6). This is followed by an increase in cytoplasmic free calcium concentration resulting from an influx of extracellular Ca^{2+} (5, 17). This, in turn, leads to a calmodulin-mediated increase in adenylate cyclase activity (18), and the resulting increase in cellular cAMP concentration in some manner leads to an increase in the activity of sugar transport carriers within the thymocyte plasma membrane (4).

Iqbal and co-workers (19) have demonstrated that T_3 in vitro in near physiologic concentrations promptly increases the uptake of 2-DG, as well as amino acids, into slices of cerebral cortex from the hypothyroid mouse, findings that seem to accord with those in the thymocyte. It remains to be determined in what other tissues T_3 may influence sugar uptake in this way, and studies on that point are in progress, but the present findings lend credence to the concept that thyroid hormones exert their diverse metabolic effects in various tissues by more than a single mechanism and at more than a single primary site. In some cases, these actions may be complementary to one another, as in the case of the prompt, plasma membrane-mediated effect of T_3 to increase sugar transport into the chick embryo myocardial cell, an action that is followed by a delayed, nucleus-mediated increase in hexokinase activity (20–22).

An unusual aspect of the current findings is the low potency of 3′-isopropyl-T_2 in stimulating thymocyte 2-DG uptake in vivo, in that this analogue is more potent than T_3 and indeed is the most potent analogue known with respect to classic thyromimetic effects, such as oxygen consumption and goiter prevention (12, 13, 15). On the other hand, the present in vivo findings correlate very well with results obtained in vitro, since 3′-isopropyl-T_2 was far less potent than T_3 in stimulating the in vitro thymocyte uptake of 2-DG and in enhancing adenylate cyclase activity in thymocyte membrane preparations (23). Seemingly analogous are the observations of Will-Shahab and co-workers (24), who found 3′-isopropyl-T_2 much less potent than T_3 in stimulating adenylate cyclase activity in cat and rat myocardium. These and numerous other findings suggest that responses to thyroid hormones, and the relative potencies of thyroid hormone analogues in eliciting them, are much more heterogenous than has generally been recognized, varying with the nature of the response and perhaps its locus of initiation, and differing in some cases from tissue to tissue (14, 24–39).

Acknowledgments

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References

1. Segal, J., and S. H. Ingbar. 1979. Stimulation by triiodothyronine of the in vitro uptake of sugars by rat thymocytes. *J. Clin. Invest.* 63: 507–515.

2. Segal, J., and S. H. Ingbar. 1980. Stimulation of 2-deoxy-D-glucose uptake in rat thymocytes in vitro by physiological concentrations of triiodothyronine, insulin, or epinephrine. *Endocrinology.* 107:1354–1358.

3. Segal, J., and S. H. Ingbar. 1980. Direct and synergistic interactions of 3,5,3′-triiodothyronine and the adrenergic system in stimulating sugar transport by rat thymocytes. *J. Clin. Invest.* 65:958–966.

4. Segal, J., and S. H. Ingbar. 1981. Studies of the mechanism by which 3,5,3′-triiodothyronine stimulates 2-deoxyglucose uptake in rat thymocytes in vitro. Role of calcium and adenosine 3′:5′-monophosphate. *J. Clin. Invest.* 68:103–110.

5. Segal, J., and S. H. Ingbar. 1985. Cytosolic calcium: the first messenger in the plasma membrane-mediated responses to 3′,5,3′-triiodothyronine (T_3) in rat thymocytes. *Clin. Res.* 33:537A. (Abstr.)

6. Segal, J., and S. H. Ingbar. 1982. Specific binding sites for triiodothyronine in the plasma membrane of rat thymocytes. Correlation with biochemical responses. *J. Clin. Invest.* 70:919–926.

7. Borges, M., Z. Eizenstein, A. G. Burger, and S. H. Ingbar. 1981. Immunosequestration: a new technique for studying peripheral iodothyronine metabolism in vitro. *Endocrinology.* 108:1665–1671.

8. Zweig, G., and J. Sherma. 1978. Thin layer chromatography. *In* CRC Handbook Series in Chromatography. Sect. A, Vol. II. G. Zweig and J. Sherma, editors. CRC Press, Boca Raton, FL. 89–173.

9. Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, NJ. 151–162.

10. Kipnis, D. M., and C. F. Cori. 1959. Studies of tissue permeability. *J. Biol. Chem.* 234:171–177.

11. Smith, D. E., and J. Gorski. 1968. Estrogen control of uterine glucose metabolism. An analysis based on the transport and phosphorylation of 2-deoxyglucose. *J. Biol. Chem.* 243:4169–4174.

12. Pittman, C. S., and J. A. Pittman. 1974. Relation of chemical structure to the action and metabolism of thyroactive substances. *Handb. Physiol.* (Sect. 7. Endocrinol.)3:233–253.

13. Koerner, D., H. L. Schwartz, M. I. Surks, J. H. Oppenheimer, and E. C. Jorgensen. 1975. Binding of selected iodothyronine analogues to receptor sites of isolated rat hepatic nuclei. *J. Biol. Chem.* 250:6417–6423.

14. Goldfine, I. D., G. J. Smith, C. G. Simons, S. H. Ingbar, and E. C. Jorgensen. 1976. Activities of thyroid hormones and related compounds in an in vitro thymocyte assay. *J. Biol. Chem.* 251:4233–4238.

15. Jorgensen, E. C. 1978. Thyroid hormone structure-function relationships. *In* The Thyroid. 4th edition. S. C. Werner and S. H. Ingbar, editors. Harper and Row, New York. 125–137.

16. Duran-Garcia, S., J. Gomez-Nieto, M. Fouchereau-Peron, V. F. Padron, M. J. Obregon, G. Morreale De Escobar, and F. Escobar Del Rey. 1979. Effects of thyroid hormone on liver binding sites for human growth hormone, as studied in the rat. *Clin. Endocrinol.* 11:275–289.

17. Segal, J., and S. H. Ingbar. 1984. An immediate increase in calcium accumulation by rat thymocytes induced by triiodothyronine (T_3): its role in the subsequent metabolic responses. *Endocrinology.* 115:160–166.

18. Segal, J., M.-C. Rehder, and S. H. Ingbar. 1984. Calmodulin mediates the stimulatory effect of 3,5,3′-triiodothyronine (T_3) on adenylate cyclase activity in rat thymocytes. *Clin. Res.* 32:548A. (Abstr.)

19. Iqbal, Z., H. Koenig, and J. J. Trout. 1984. Triiodothyronine (T_3) regulates membrane transport processes in mature neurons and nerve endings. *Neurology.* 34(Suppl. 1):195.

20. Segal, J., H. Schwartz, and A. Gordon. 1977. The effect of triio-

dothyronine on 2-deoxy-D-[1-^{3}H] glucose uptake in cultured chick embryo heart cells. *Endocrinology.* 101:143–149.

21. Segal, J., and A. Gordon. 1977. The effects of actinomycin D, puromycin, cycloheximide and hydroxyurea on 3,5,3′-triiodo-L-thyronine stimulated 2-deoxy-D-glucose uptake in chick embryo heart cells in vitro. *Endocrinology.* 101:150–156.

22. Segal, J., and A. Gordon. 1977. The effect of 3,5,3′-triiodo-L-thyronine on the kinetic parameters of sugar transport in cultured chick embryo heart cells. *Endocrinology.* 101:1468–1474.

23. Segal, J., C. Buckley, and S. H. Ingbar. 1985. Stimulation of adenylate cyclase activity in rat thymocytes in vitro by 3,5,3′ triiodothyronine. *Endocrinology.* 116:2036–2043.

24. Will-Shahab, L., A. Wollenberger, and I. Kuttner. 1976. Stimulation of rat and cat heart adenylate cyclase by triiodothyronine in the presence of 5′-quanylylimidodiphosphate. 1976. *Acta Biol. Med. Ger.* 35:829–835.

25. McNiel, J. H., and T. M. Brody. 1968. The effect of triiodothyronine pretreatment on amine-induced rat cardiac phosphorylase activation. *J. Pharmacol. Exp. Ther.* 161:40–46.

26. Tsai, J. S., and A. Chen. 1978. Effect of L-triiodothyronine on (−) ^{3}H-dihydroalprenolol binding and cyclic AMP response to (−) adrenaline in cultured heart cells. *Nature (Lond.).* 275:138–140.

27. Brodde, O.-E., H.-J. Schumann, and J. Wagner. 1980. Decreased responsiveness of the adenylate cyclase system on left atria from hypothyroid rats. *Mol. Pharmacol.* 17:180–186.

28. Ishac, E. J. N., J. N. Pennefather, and G. M. Handberg. 1983. Effect of changes in thyroid state on atrial α- and β-adrenoceptors, adenylate cyclase activity, and catecholamine levels in the rat. *J. Cardiovasc. Pharmacol.* 5:396–405.

29. Vaughan, M. 1967. An in vitro effect of triiodothyronine on rat adipose tissue. *J. Clin. Invest.* 46:1482–1491.

30. Challoner, D. R., and D. O. Allen. 1970. An in vitro effect of triiodothyronine on lipolysis, cyclic AMP-C^{14} accumulation and oxygen consumption in isolated fat cells. *Metab. Clin. Exp.* 19:480–487.

31. Malbon, C. C., F. J. Moreno, R. J. Cabelli, and J. N. Fain. 1978. Fat Cell adenylate cyclase and -adrenergic receptors in altered thyroid states. *J. Biol. Chem.* 253:671–678.

32. Malbon, C. C., M. P. Graziano, and G. L. Johnson. 1984. Fat cell -adrenergic receptor in the hypothyroid rat. Impaired interaction with the stimulatory regulatory component of adenylate cyclase. *J. Biol. Chem.* 259:3254–3260.

33. Malbon, C. C., S.-Y. Li, and J. N. Fain. 1978. Hormonal activation of glycogen phosphorylase in hepatocytes from hypothyroid rats. *J. Biol. Chem.* 253:8820–8825.

34. Malbon, C. C. 1980. Liver cell adenylate cyclase and β-adrenergic receptors. Increased β-adrenergic receptor number and responsiveness in the hypothyroid rat. *J. Biol. Chem.* 255:8692–8699.

35. Segal, J., A. Coppens, and S. H. Ingbar. 1985. The effect of thyroid status on the calmodulin content of several tissues in the rat. *Endocrinology.* 116:1707–1711.

36. Golde, D. W., N. Bersh, I. J. Chopra, and M. J. Cline. 1977. Thyroid hormones stimulate erythropoiesis in vitro. *Br. J. Haematol.* 37:173–177.

37. Popovic, W. J., J. E. Brown, and J. W. Adamson. 1977. The influence of thyroid hormones on in vitro erythropoiesis. Mediation by a receptor with beta adrenergic properties. *J. Clin. Invest.* 60:907–913.

38. Lawrence, W. D., P. J. Davis, S. D. Blas, and M. Schoenl. 1984. Interaction of thyroid hormone and sex steroids at the rabbit reticulocyte membrane in vitro: control by 17 β-estradiol and testosterone of thyroid hormone responsive Ca^{2+}-ATPase activity. *Arch. Biochem. Biophys.* 235: 78–85.

39. Davis, P. J., and S. D. Blas. 1981. In vitro stimulation of human red blood cell Ca^{2+}-ATPase by thyroid hormone. *Biochem. Biophys. Res. Commun.* 99:1073–1080.