Inhibition of Intrapituitary Thyroxine to 3,5,3′-Triiodothyronine Conversion Prevents the Acute Suppression of Thyrotropin Release by Thyroxine in Hypothyroid Rats

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ABSTRACT Iopanoic acid has been shown to block thyroxine (T_4)-5′-monodeiodination in rat anterior pituitary in vitro. To test the hypothesis that the acute decrease in thyrotropin (TSH) after infusion of T_4 into hypothyroid rats requires intrapituitary T_4 to 3,5,3′-triiodothyronine (T_3) conversion, the effect of iopanoic acid treatment on the generation of nuclear T_3 from intrapituitary conversion and the response to TSH were compared in control and iopanoic acid-treated animals. 5 mg/100 g body weight iopanoic acid given 24, 16, and 1.5 h before administration of ^{125}I-T_4 reduced the quantity of pituitary nuclear ^{125}I-T_3 from local (intrapituitary) T_4 to T_3 conversion by 60–100%. In association with inhibition of intrapituitary T_4 to T_3 conversion, there was an increase in the binding of ^{125}I-T_4 to the nuclear receptor of the pituitary but the total iodothyronine content of the nuclei was still less than half of the nuclear iodothyronine in control animals. Iopanoic acid did not affect the nuclear/plasma ratio of injected ^{131}I-T_3 in the same animals, but did appear to impair ^{131}I-T_3 clearance or reduce its distribution volume. Treatment with iopanoic acid did not reduce the quantity of nuclear ^{125}I-T_3 in the liver, kidney, or heart of the same animals more than expected from the changes in serum ^{125}I-T_3. In control hypothyroid animals, 800 ng/100 g body weight T_4 caused a reduction in TSH to 41% of its initial value 3 h after injection. In animals pretreated with iopanoic acid, the mean TSH was not significantly decreased from the initial value by T_4 injection. Iopanoic acid pretreatment did not interfere with the acute TSH response of chronically hypothyroid rats to 70 ng of T_3/100 g body weight. These results establish that intrapituitary generation of T_3 from T_4 is required for the acute decrease in TSH which occurs after T_4 infusion. The data also are consistent with the concept that it is the nuclear binding of the T_3 generated from T_4 which initiates the inhibition of TSH release.

Received for publication 11 February 1978 and in revised form 12 March 1979.

INTRODUCTION

We have previously demonstrated a chronological and quantitative relationship between the acute inhibition of thyrotropin (TSH)[1] release by injected 3,5,3′-triiodothyronine (T_3) in chronically hypothyroid rats and the nuclear T_3 specifically bound in the anterior pituitary (1, 2). Although about 10-fold larger amounts are required, thyroxine (T_4) also acutely inhibits thyrotropin (TSH) release. The degree to which TSH release is inhibited after T_4 also parallels the nuclear T_3 content (1, 2). In these acute experiments, the bulk (>70%) of the nuclear T_3 after T_4 injection is derived from intrapituitary T_4-5′-monodeiodination. We have also reported that neither the TSH inhibition nor the increase in nuclear T_3 after T_4 injection were blocked by pretreatment of rats with 6-*n*-propyl-2-thiouracil (PTU) (2) despite the fact that this has been shown to block ≅70% of peripheral T_4-5′-monodeiodination in rats (3, 4). Because pituitary T_4-5′-monodeiodination was not inhibited by PTU, the hypothesis that T_4 to T_3 conversion was required for the effect of this dose of T_4 on pituitary TSH release could not be tested using this agent.

[1] *Abbreviations used in this paper*: PTU, 6-*n*-propyl-2-thiouracil; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; TSH, thyrotropin.

Several years ago, Bürgi et al. (5) reported that normal patients receiving iopanoic acid (Telepaque; Winthrop Laboratories, New York) for oral cholecystography had decreases in serum T_3 associated with increases in serum T_4 and TSH. Similar changes in serum T_4 and T_3 were recently reported for another oral cholecystographic agent, ipodate (Oragrafin; E. R. Squibb & Sons, Princeton, N. J.) by Wu et al. (6) in euthyroid and hyperthyroid subjects. Kaplan and Utiger (7) have also demonstrated that iopanoic acid blocks 5′-monodeiodination of both T_4 and 3,3′5′-triiodothyronine (reverse T_3) in rat liver homogenates (7) and similar effects have recently been observed in renal tissue homogenates as well.[2] These reports led us to compare the effects of iopanoic acid and PTU on T_4-5′-monodeiodination by rat anterior pituitary tissue with an in vitro system which we have recently described (8). In confirmation of our in vivo results, incubation of anterior pituitary tissue with PTU did not inhibit T_4 to T_3 conversion (9). However, in both tissue fragments and anterior pituitary homogenates, iopanoic acid blocked T_4-5′-monodeiodination (9, 10). The capacity of this agent to block both peripheral and intrapituitary T_4 to T_3 conversion suggested that it might be an appropriate tool to substantiate our hypothesis that intrapituitary T_4 to T_3 conversion is required for the acute response of TSH to T_4 in the hypothyroid rat.

METHODS

Materials. T_3 and T_4 (free acid) were obtained from Sigma Chemical Co., St. Louis, Mo. Iopanoic acid (Telepaque) was a gift of Dr. F. C. Nachod, Winthrop Laboratories, Sterling Drug Co., New York. ^{131}I-T_3 and ^{125}I-T_4 were labeled in the laboratory by previously described methods (11). Rats were obtained from Zivic-Miller Laboratories, Allison Park, Pa., and were thyroidectomized by the supplier before shipment. They were held for 2–3 mo until growth plateaued before use in these experiments. Materials for immunoassay of rat TSH were kindly provided by the Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases.

Iopanoic acid injections. For a typical experiment, 125 mg of iopanoic acid was dissolved in 2.1 ml of 0.1 N NaOH. 2.5 ml of propylene glycol and 0.4 ml of 0.25 N NCl were added subsequently. The final solution was 25 mg/ml with respect to iopanoic acid in ≅0.03 N NaOH. Experimental rats were given 5 mg/100 g body weight i.p. 24, 16, and 1.5 h before injection of the labeled or unlabeled iodothyronines. Control animals were given injections of vehicle alone.

Injection of iodothyronines. Before injection of iodothyronines, serum was obtained for TSH, T_3, and T_4 measurements in all animals to confirm the hypothyroid state. In some experiments, TSH measurements were also obtained before iopanoic acid injections. Labeled or unlabeled iodothyronines were given in ≅0.5 ml of 10% hypothyroid rat serum in phosphate-buffered saline (pH 7.4) by jugular injection. In two experiments (A and B), 800 ng ^{125}I-T_4/100 g body weight was given 3 h and tracer ^{131}I-T_3 90 min before killing the rats. In a third experiment (C), the ^{125}I-T_4 and ^{131}I-T_3 were given simultaneously 3 h before quantitation of serum and nuclear iodothyronines. Because of the requirement for large quantities of ^{125}I-T_4 (rats received about 100 μCi ^{125}I-T_4/100 g body weight), the numbers of rats were limited to six or eight for each experiment.

Calculation of nuclear and plasma ^{125}I-T_3, ^{131}I-T_3, and ^{125}I-T_4. These determinations were made by methods previously described in detail with one major modification (1, 2, 12, 13). Previously, we have used TCA precipitation of serum to estimate the quantity of serum ^{131}I-T_3. Our recent unpublished studies have indicated that this approach is not optimal. Affinity chromatographic isolation of T_3 with specific T_3 antibody-Sepharose conjugates has shown that only 50–70% of the TCA-precipitable ^{131}I 1.5 h after ^{131}I injection was actually ^{131}I-T_3. This method has been previously described (13). With longer intervals subsequent to injection, the fraction of TCA-precipitable ^{131}I that was T_3 was even lower. These results have been confirmed by chromatography of butanol extracts of serum. The TCA-precipitable, but non-T_3, material is presumably T_4 as well as the other unidentifiable degradation products of ^{131}I-T_3, such as 3,3′-diiodothyronine. The difference between TCA-precipitable serum ^{131}I and ^{131}I-T_3 becomes appreciable 1–1.5 h after injection of the T_3 isotope. Therefore, the following approach was developed. To establish that the recovery of T_3 in the experimental rat serum samples was identical to that obtained in the pooled rat serum, ^{131}I-T_3 (≅100,000 cpm) was added to 0.1-ml aliquots of each rat's sera. These sera already contained 1,000–3,000 cpm ^{131}I-T_3 from the experimental injection. ^{131}I-T_3 recovery through the affinity and paper chromatographic procedure was determined and found to be identical to that for the same ^{131}I-T_3 added to pooled serum from normal rats. Accordingly, the following method was used to determine serum ^{131}I-T_3 and ^{125}I-T_3 concentrations. 0.1 ml of each rat serum (in duplicate) was incubated overnight with 0.25–0.5 ml anti-T_3-Sepharose conjugate at 4°C as we have previously described (13). Fresh ^{131}I-T_3 was added to pooled serum from other rats and subjected to the identical procedure at the same time. The Sepharose conjugate was washed, iodothyronines were extracted with methanol-2-N-NH_4OH (99:1, vol/vol, and were chromatographed with added T_3, T_4, and iodide in tertiary amyl alcohol:hexane:2 N NH_4OH, 5:1:6 (2, 13). The T_3 spot was located by chemical staining, and counted with appropriate corrections for paper background and cross-over of ^{131}I into the ^{125}I spectrophotometer channel. The recovery of ^{131}I-T_3 added to normal rat serum varied from 17 to 35% using different Sepharose conjugates. This recovery factor was then applied to both the ^{131}I-T_3 and ^{125}I-T_3 in the experimental rat serum samples. This procedure was not modified from the earlier description (2). ^{131}I-T_3 accounted for 66–92% of the serum TCA-precipitable radioactivity in experiments A and B (1.5 h after ^{131}I-T_3 injection) and 28–61% of the TCA precipitable radioactivity in experiment C (3 h after ^{131}I-T_3 injection).

Miscellaneous. ^{125}I-T_3 contamination of ^{125}I-T_4 was measured as previously described (2, 13). ^{125}I-T_3 was 0.9, 1.1, and 1.1% of the ^{125}I-T_4 isotopes used in experiments A, B, and C, respectively. ^{131}I-T_4 in ^{131}I-T_3 varied from 0.5 to 1%, but the use of the affinity method for estimation of serum ^{131}I-T_3 obviated the need for correction of the TCA-precipitable ^{131}I-T_3 for the 10-fold relative increase in ^{131}I-T_4 concentration which occurs during the initial distribution phases of the two isotopes after injection (12). Assays for serum TSH were performed as previously described (14).

Statistical calculations. The significance of differences between means was determined by unpaired Student's *t* test.

[2] Kaplan, M. M. Unpublished observations.

RESULTS

Effect of iopanoic acid on T_3 and T_4 metabolism

The effects of pretreatment of rats with iopanoic acid on T_3 and T_4 metabolism were studied with two protocols. In experiments A and B, ^{125}I-T_4 was given 3 h before, and ^{131}I-T_3 1.5 h before analysis of serum and nuclear radioactivity. This time interval for T_3 injection was selected because 90 min appears to be the approximate time of maximal nuclear occupancy by T_3 in hypothyroid rat anterior pituitary nuclei (1). Because we wished to determine the contribution of serum ^{125}I-T_3 to nuclear ^{125}I-T_3 3 h after ^{125}I-T_4 injection, it was necessary that the pituitary nuclear/serum T_3 ratio be measured at a time when the specific activity of T_3 in the nuclear and the plasma-cytoplasm compartment were identical. This situation is only present at the time after injection that nuclear tracer T_3 is maximal (12, 15–17). In experiment C, both ^{131}I-T_3 and ^{125}I-T_4 were given simultaneously 3 h before killing the rats. Because ^{125}I-T_3 contamination of the ^{125}I-T_4 was known, this protocol allowed determination of the fate of the ^{125}I-T_3 contaminant. Amounts of serum or nuclear ^{125}I-T_3 in excess of that due to contaminant in these animals could then be attributed to in vivo T_4-5′-monodeiodination.

RESULTS OF EXPERIMENTS A AND B

Radioactivity in plasma. The data presented in Table I indicate that pretreatment of rats with 5 mg/100 g body weight iopanoic acid 24, 16, and 1.5 h previously had no statistically significant effect on the acute metabolism of ^{131}I-T_3 and ^{125}I-T_4. However, in both experiments, serum ^{131}I-T_3 was higher in rats receiving iopanoic acid than in controls. In the last column of Table I is shown the concentration of serum ^{125}I-T_3 3 h after injection. As is indicated in the table, serum ^{125}I-T_3 is either that injected as a contaminant or derived from in vivo 5′-monodeiodination of ^{125}I-T_4 in the liver and kidney, and then appearing in plasma. In both experiments, ^{125}I-T_3 was higher in the vehicle-injected groups, but again the difference was too small to be statistically significant. The effects of iopanoic acid pretreatment on these parameters is discussed again under experiment C.

Nuclear ^{131}I-T_3 and ^{125}I-T_3. Results of the analyses of nuclear iodothyronine in anterior pituitary, liver, kidney, and heart from experiments A and B are shown in Table II. Nuclear ^{131}I-T_3 was slightly higher in the anterior pituitary and liver of iopanoic acid-treated rats in experiment A and in the anterior pituitary of experiment B, but the serum ^{131}I-T_3

TABLE I

*Effect of Iopanoic Acid Pretreatment on Serum ^{131}I-T_3, ^{125}I-T_4, and ^{125}I-T_3 in Hypothyroid Rats Given ^{131}I-T_3 90 min Previously and ^{125}I-T_4 3 h Previously**

Experiment	Treatment	Wt	^{131}I-T_3	^{125}I-T_4	^{125}I-T_3‡
		g	% dose T_3/ml	% dose T_4/ml	
A					
	1 Vehicle	306	0.20	2.7	0.0032
	2 Vehicle	213	0.26	2.5	0.0043
	3 Vehicle	245	0.32	2.6	0.0044
	Mean±SE		0.26±0.03	2.6±0.06	0.0040±0.0004
	4 Iopanoic acid	265	0.48	3.0	0.0030
	5 Iopanoic acid	231	0.33	3.4	0.0032
	6 Iopanoic acid	251	0.41	3.1	0.0032
	Mean±SE		0.41±0.03	3.2±0.1	0.0031±0.001
B					
	1 Vehicle	244	0.46	3.2	0.0052
	2 Vehicle	255	0.44	3.2	0.0064
	3 Vehicle	205	0.61	2.9	0.0104
	Mean±SE		0.50±0.05	3.1±0.1	0.0073±0.0016
	4 Iopanoic acid	214	0.50	2.3	0.0041
	5 Iopanoic acid	230	0.61	2.9	0.0044
	6 Iopanoic acid	228	0.54	2.8	0.0040
	Mean±SE		0.55±0.03	2.7±0.2	0.0042±0.001

* Iopanoic acid, 5 mg/100 g body weight, was given 24, 16, and 1.5 h before ^{125}I-T_4.

‡ ^{125}I-T_3 either injected as a contaminant or derived from in vivo 5′-monodeiodination of ^{125}I-T_4.

TABLE II

*Effect of Pretreatment of Hypothyroid Rats with Iopanoic Acid on Nuclear ^{131}I-T_3 and ^{125}I-T_3 after ^{131}I-T_3 and ^{125}I-T_4 Injections**

	Nuclear ^{131}I-T_3		^{131}I-T_3 N/S ratio		Nuclear ^{125}I-T_3‡	
	Vehicle	Iopanoic acid	Vehicle	Iopanoic acid	Vehicle	Iopanoic acid
	% dose T_3/mg DNA		*% dose T_3/mg DNA/% dose/ml serum*		*% dose T_4/mg DNA ($\times 10^{-4}$)*	
Experiment A						
Anterior pituitary	0.23±0.01	0.32±0.06	0.90±0.11	0.79±0.09	60±3	35±3§
Liver	0.13±0.01	0.18±0.01	0.51±0.05	0.43±0.06	15±2	12±2
Heart	0.16±0.01	0.17±0.01	0.64±0.11	0.43±0.04	21±1	18±1
Kidney	0.06±0.01	0.07±0.01	0.24±0.02	0.18±0.01	7.2±0.1	6.7±0.6
Experiment B						
Anterior pituitary	0.22±0.02	0.26±0.02	0.44±0.02	0.47±0.02	80±3	22±1§
Liver	0.13±0.01	0.14±0.2	0.26±0.02	0.27±0.05	13±2	8.4±1
Heart	0.11±0.01	0.11±0.01	0.22±0.02	0.20±0.02	14±3	8.4±0.6
Kidney	0.07±0.01	0.08±0.02	0.14±0.02	0.14±0.02	7.2±0.6	5.3±0.6

Mean±SE.

* Animals were given iopanoic acid, 5 mg/100 g body weight i.p., 24, 16, and 1.5 h before ^{125}I-T_4 injection. Controls received equivalent vehicle. ^{131}I-T_3 was given 1.5 h after ^{125}I-T_4 and rats were killed 1.5 h later. There were three animals in each group (vehicle and iopanoic acid) in each experiment.

‡ Results are not corrected for the contribution of serum ^{125}I-T_3 to nuclear ^{125}I-T_3.

§ $P < 0.005$ for difference from vehicle. There were no other effects of iopanoic acid pretreatment which were statistically significant.

was also higher in these animals (Table I). Therefore, the nuclear/serum ratio for ^{131}I-T_3 (middle portion of Table II) was not significantly different for either experiment in any of the four tissues examined. These results indicate there is no substantial effect of iopanoic acid on the distribution of T_3 between cell nuclei and serum in these organs. The data for nuclear ^{125}I-T_3 are shown in the right hand portion of Table II. These figures include both ^{125}I-T_3 from plasma and that generated in the tissue. In both experiments, anterior pituitary nuclear ^{125}I-T_3 was significantly lower in the iopanoic acid-treated animals ($P < 0.005$). Nuclear ^{125}I-T_3 was slightly less in the liver, heart, and kidney of iopanoic acid-treated rats than in controls. However, the small differences were not sufficient to be statistically different. It should also be noted that in experiment B particularly, serum ^{125}I-T_3 was somewhat lower in the iopanoic acid-treated animals (Table I).

As there was no significant difference in nuclear ^{125}I-T_3 in the iopanoic acid-treated rats in any other tissues, analysis of the specific sources of nuclear ^{125}I-T_3 is presented only for the anterior pituitary (Table III). As was apparent from Table II, there was no significant difference in nuclear ^{131}I-T_3 as a result of iopanoic acid treatment in either experiment A or B, while the ^{125}I-T_3 was reduced in the treated animals. Because plasma ^{125}I-T_3 contributes to nuclear ^{125}I-T_3, it is necessary to estimate and deduct this contribution to evaluate the effect of iopanoic acid on intrapituitary T_4 to T_3 conversion. The contribution of plasma ^{125}I-T_3 to nuclear ^{125}I-T_3 was determined by multiplication of the serum ^{125}I-T_3 by the nuclear/serum ratio for ^{131}I-T_3 as we have previously described (1, 2, 12). In experiment A, serum ^{125}I-T_3 accounted for over one-half of the nuclear ^{125}I-T_3 in both groups of animals. In experiment B, serum ^{125}I-T_3 accounted for about 40% of nuclear ^{125}I-T_3 in the control animals, and for virtually all of the nuclear ^{125}I-T_3 in the iopanoic acid-treated group. The estimated nuclear ^{125}I-T_3 derived from intrapituitary 5′-monodeiodination was reduced by 60% in experiment A and by 90% in experiment B ($P < 0.01$). Because the specific activity of ^{125}I-T_3 derived from distal ring-labeled ^{125}I-T_4 is only one-half that of the precursor, calculation of the T_3 generated requires multiplication of the net ^{125}I-T_3 by a factor of 2. The results of this calculation are presented in the last column of Table III. These results show there is substantial inhibition of local T_4 to T_3 conversion in pituitary tissues by iopanoic acid in both experiments.

EFFECT OF IOPANOIC ACID ON PLASMA AND NUCLEAR IODOTHYRONINES IN ANIMALS GIVEN ^{131}I-T_3 AND ^{125}I-T_4 SIMULTANEOUSLY (EXPERIMENT C)

Although there is an obvious decrease in the nonplasma component of pituitary nuclear ^{125}I-T_3

TABLE III

*Sources of Pituitary Nuclear ^{125}I-T_3 in Hypothyroid Rats Receiving Pretreatment with Iopanoic Acid before ^{125}I-T_4 and ^{131}I-T_3 Injections**

Experiment	Treatment	Pituitary nuclear ^{131}I-T_3	Pituitary nuclear ^{125}I-T_3			
			Total	Plasma contribution	From local T_4 to T_3 conversion	Net local T_4 to T_3 conversion‡
		% dose T_3/mg DNA	*% dose T_4/mg DNA ($\times 10^{-4}$)*			
A	1 Vehicle	0.23	58	36	22	44
	2 Vehicle	0.21	57	35	22	44
	3 Vehicle	0.25	65	34	31	62
	Mean±SE	0.23±0.01	60±3	35±1	25±3	50±6
	4 Iopanoic acid	0.44	37	28	9	18
	5 Iopanoic acid	0.28	38	27	11	22
	6 Iopanoic acid	0.25	30	19	10	20
	Mean±SE	0.32±0.06	35±3	25±3	10±1	20±1
	P value§	NS	<0.005	<0.025	<0.01	<0.01
B	1 Vehicle	0.19	74	22	52	104
	2 Vehicle	0.21	85	30	55	110
	3 Vehicle	0.26	81	44	37	74
	Mean±SE	0.22±0.02	80±3	32±6	48±6	96±11
	4 Iopanoic acid	0.22	20	18	2	4
	5 Iopanoic acid	0.29	23	21	2	4
	6 Iopanoic acid	0.27	23	19	4	8
	Mean±SE	0.26±0.002	22±1	19±1	3±1	5±1
	P value	NS	<0.001	NS	<0.005	<0.001

* Rats received 5 mg/100 g iopanoic acid 24, 16, and 1.5 h before ^{125}I-T_4 injection. ^{131}I-T_3 was given 1.5 h after ^{125}I-T_4, and rats were killed 1.5 h later.

‡ Corrected for the twofold decrease in specific activity resulting from 5′-monodeiodination of T_4 labeled in the distal ring.

§ Comparison of iopanoic acid with control rats by unpaired Student's *t* test.

in the above experiments, it is not clear from these data what fraction of the plasma contribution to nuclear ^{125}I-T_3 is the result of contaminating ^{125}I-T_3 and what portion is the result of newly generated ^{125}I-T_3. Because it is likely that in vivo hepatic and renal T_4-5′-deiodination would be inhibited by iopanoic acid pretreatment, the question arises as to what fraction of total ^{125}I-T_3 in the plasma originates via this pathway. The results of this experiment (C) are shown in Table IV. Iopanoic acid (or vehicle) was given 24, 16, and 1.5 h before injection of ^{131}I-T_3 and ^{125}I-T_4 to groups of four hypothyroid rats. The percentage dose of ^{131}I-T_3 in the sera of iopanoic acid-treated rats, 0.32±0.02 (mean±SE) was about twice that in control animals ($P < 0.01$). The serum ^{125}I-T_4 concentration was also statistically significantly higher in these iopanoic acid-treated animals but the serum ^{125}I-T_3 was again not significantly lower in the treated group. This is true despite the fact that the ^{125}I-T_3 concentration in controls is roughly twice that of the iopanoic acid-treated group. Because the two isotopes were given simultaneously, and because the ^{125}I-T_3 contamination was known, it was possible to calculate the amount of ^{125}I-T_3 contaminant still present in each animal by use of the ^{131}I-T_3 distribution. The contribution of contaminant ^{125}I-T_3 to serum ^{125}I-T_3 is given in the last column of Table IV. In control animals it was 1.6×10^{-3}% of the dose/ml. In the iopanoic acid-treated group, the contaminant accounted for all of the serum ^{125}I-T_3; that is, there was no new plasma ^{125}I-T_3 in these rats. In the vehicle-treated animals, the difference between the ^{125}I-T_3 contaminant and the total ^{125}I-T_3 must arise from peripheral T_4 to T_3 conversion. This newly formed ^{125}I-T_3 constitutes about 75% of the ^{125}I-T_3 present 3 h after injection.

The analysis of nuclear T_3 and nuclear/serum T_3 ratios in these rats is presented in the lower portion of Table IV. Anterior pituitary nuclear ^{131}I-T_3 was higher in iopanoic acid-treated rats (0.28±0.04 vs. 0.20±0.03) but the difference was not statistically significant. The nuclear/serum T_3 ratio was lower than in control animals, but again not significantly due to the broad

TABLE IV

*Effect of Iopanoic Acid Pretreatment on Serum and Nuclear Iodothyronines in Hypothyroid Rats Given ^{131}I-T_3 and ^{125}I-T_4 Simultaneously 3 h Previously (Experiment C)**

	Serum			
			^{125}I-T_3	
			Total	From contamination‡
	% dose ^{131}I-T_3/ml	% dose ^{125}I-T_4/ml	% dose T_4/ml	
Vehicle (n = 4)	0.15±0.04	2.4±0.31	0.0066±0.0014	0.0016±0.0004
Iopanoic acid (n = 4)	0.34±0.02 (<0.01)§	3.3±0.15 (<0.05)	0.0032±0.0003	0.0037±0.0002 (<0.01)

	Nuclear T_3 and nuclear/serum T_3 ratios			
	^{131}I-T_3		^{125}I-T_3‖	
		Nuclear/serum T_3 ratio		Nuclear/serum T_3 ratio
	% dose/mg DNA		% dose T_4/mg DNA ($\times 10^{-4}$)	
Vehicle				
Anterior pituitary	0.20±0.003	1.7±0.5	112±17	2.0±0.5
Liver	0.04±0.01	0.34±0.07	15±4	0.24±0.14
Heart	0.06±0.01	0.43±0.05	10±3	0.16±0.05
Kidney	0.04±0.01	0.27±0.04	9±2	0.14±0.02
Iopanoic acid				
Anterior pituitary	0.28±0.04	0.85±0.17	25±8 (<0.005)	0.82±0.27
Liver	0.10±0.01 (<0.01)	0.28±0.03	8.0±0.8	0.25±0.03
Heart	0.11±0.02 (<0.05)	0.33±0.05	7.4±0.8	0.24±0.04
Kidney	0.06±0.01 (<0.05)	0.18±0.02	6.2±1.0	0.20±0.04

* Four rats were given iopanoic acid (or appropriate vehicle) 5 mg/100 g body weight, 24, 16, and 1.5 h before ^{125}I-T_4 and ^{131}I-T_3 injection.

‡ Calculated from % dose ^{131}I-T_3 per ml × ^{125}I-T_3 contaminant in injected ^{125}I-T_4 (1.1%).

§ Numbers in parentheses are P values for comparison of results in iopanoic acid-treated rats with those receiving vehicle.

‖ ^{125}I-T_3 from both contamination and in vivo T_4-5′-monodeiodination.

range of the results. Nuclear ^{131}I-T_3 in liver, heart, and kidney were all statistically significantly higher in iopanoic acid-treated animals, although the nuclear/serum ratios were not different. Therefore, these differences in nuclear ^{131}I-T_3 can be attributed to the higher concentrations of ^{131}I-T_3 present in the sera of the iopanoic acid-treated rats. The pattern of results for nuclear ^{125}I-T_3 and the nuclear/serum ^{125}I-T_3 ratios is quite similar to that obtained in experiments A and B. In anterior pituitary, the nuclear T_3 was only $25\pm8 \times 10^{-4}$% of the dose/mg DNA in iopanoic acid-treated rats while controls had $112\pm17 \times 10^{-4}$% of the dose/mg DNA ($P < 0.005$). Neither the nuclear ^{125}I-T_3 nor the nuclear/serum ^{125}I-T_3 ratio were different in any of the other tissues studied. The results in table IV indicate that there was an apparent effect of iopanoic acid on either the volume of T_3 distribution or the metabolic clearance of T_3 in these animals and this is discussed further below.

The results of the analyses of the sources of pituitary nuclear T_3 in experiment C is shown in Table V. Because ^{131}I-T_3 was given 3 h before killing the rats, the nuclear/serum ^{131}I-T_3 ratio obtained is not appropriate for estimating the contribution of serum ^{125}I-T_3 to nuclear ^{125}I-T_3 in the pituitary. The pituitary nuclear/serum T_3 ratio increases with time after the time of maximal nuclear occupancy as we have shown previously in euthyroid rats (12). In addition, some 12–18 h is required for serum ^{125}I-T_3 to reach a peak after injection of ^{125}I-T_4 to euthyroid rats (12). A similar, or perhaps even longer, period would presumably be required in the hypothyroid animal. Therefore, the newly formed ^{125}I-T_3 is rising steadily and that amount generated in the hour or so previous to killing the rat has not achieved complete equilibration with the pituitary nuclear T_3 receptors. For this analysis, we have used the nuclear/serum ^{131}I-T_3 ratio obtained in experiments A and B, recognizing that this is probably an overestimate of the appropriate nuclear/serum T_3 ratio to be applied to the serum ^{125}I-T_3 in these animals.

In the first two columns of Table V are shown the weights of the animals studied and the total pituitary nuclear ^{125}I-T_3. In the vehicle-injected animals, the plasma contribution can be divided into two portions: that derived from the injected contaminant and that derived from peripheral T_4 to T_3 conversion. The in-

TABLE V

Analysis of the Sources of Pituitary Nuclear ^{125}I-T_3 in Experiment C

		Pituitary nuclear ^{125}I-T_3				
		Plasma contribution			Contribution of intrapituitary T_4 to T_3 conversion§	Fraction of nuclear ^{125}I-T_3 from intrapituitary T_4 to T_3 conversion (excluding contaminant)
	Wt	Total	Injected contaminant*	Peripheral T_4 to T_3 conversion‡		
	g	% dose T_4/mg DNA ($\times 10^{-4}$)				%
Vehicle						
1	205	65	17	52	0	0
2	250	112	17	25	70	74
3	215	130	25	36	69	66
4	205	140	27	21	92	81
Mean	221	112	22	34	58	55 (74)‖
SE	14	17	3	7	20	19 (4)
Iopanoic acid						
5	220	18	29	—	—	—
6	210	7	27	—	—	—
7	195	37	22	—	—	—
8	260	39	42	—	—	—
Mean	218	25	30	0	0	0
SE	12	8	4			

* Fraction dose ^{131}I-T_3/mg DNA × contaminating ^{125}I-T_3 (1.1% of ^{125}I-T_4 dose).

‡ Serum ^{125}I-T_3 from peripheral T_4 to T_3 conversion × nuclear/serum ratio for ^{131}I-T_3 in hypothyroid control rats (0.67±0.12; SE) from Table II.

§ Total ^{125}I-T_3 less ^{125}I-T_3 from both contaminant and peripheral T_4 to T_3 conversion.

‖ Excluding animal 1.

jected contaminant was determined by multiplying the ^{125}I-T_3 contamination (1.1% of the ^{125}I-T_4 dose) by the fraction of the ^{131}I dose present per milligram DNA in the pituitary. The mean contaminant in the pituitary nuclei was $22\pm3 \times 10^{-4}$% of the T_4 dose/mg DNA. The contribution of newly formed serum ^{125}I-T_3 can be estimated by multiplication of the de novo-generated ^{125}I-T_3 in the serum (5×10^{-3}% T_4 dose/ml, Table IV) by the mean nuclear/serum ratio for control rats calculated from Table II in experiments A and B (0.67±0.12). The mean contribution to pituitary nuclear ^{125}I-T_3 from peripheral T_4 to T_3 conversion is $34\pm7 \times 10^{-4}$% of the T_4 dose per milligram DNA. The contribution of intrapituitary T_4 to T_3 conversion is the total nuclear ^{125}I-T_3 less that contributed by serum ^{125}I-T_3 regardless of its source. The mean was $58\pm20 \times 10^{-4}$% of the dose of T_4 per milligram DNA. There was no apparent contribution of intrapituitary conversion to nuclear ^{125}I-T_3 in animal one and we have no explanation for this discrepant result. With these data, it is possible to estimate the fraction of nuclear ^{125}I-T_3 that is derived from peripheral T_4 to T_3 conversion and from intrapituitary T_4 to T_3 conversion (excluding the ^{125}I-T_3 contaminant). This value is given in the last column in Table V. Including animal one, local intrapituitary T_4 to T_3 conversion accounts for 55±19% of the total nuclear ^{125}I-T_3. Excluding this animal, the mean contribution of local T_4 to T_3 conversion was 74±4%. The results in the iopanoic acid-treated rats are also presented in Table V. The data indicate that all of the nuclear ^{125}I-T_3 present 3 h after injection in the iopanoic acid-treated rats is the result of contaminant. Therefore, in these rats, iopanoic acid caused complete inhibition of intrapituitary, hepatic, and renal T_4 to T_3 conversion.

EVALUATION OF THE EFFECT OF IOPANOIC ACID PRETREATMENT ON NUCLEAR IODOTHYRONINES IN THE ANTERIOR PITUITARY

Because there was significant inhibition of pituitary T_4-5′-monodeiodination in iopanoic acid-treated animals, it was of interest to determine whether increased quantities of ^{125}I-T_4 might be bound to the nuclear receptors in place of the ^{125}I-T_3. Data relative to this point are shown in Table VI, in which the counts of nuclear ^{125}I found in the T_4 and T_3 spots (corrected for appropriate paper background and ^{131}I crossover) are presented. Unfortunately, one sample in experiment A was partially lost, which obviated statistical analysis of the T_4 data in this experiment, because there was no recovery standard for T_4. In all three experiments, the quantity of nuclear ^{125}I-T_4 was increased in iopanoic acid-treated rats. However, this

TABLE VI

Chromatographic Analysis of Nuclear ^{125}I Iodothyronines in Hypothyroid Rats 3 h after ^{125}I-T_4 with or without Iopanoic Acid Pretreatment (5 mg/100 g body weight, 24, 16, and 1.5 h before ^{125}I-T_4)

Treatment	Counts nuclear ^{125}I/10 min		
	T_4	T_3*	$(T_3/T_3 + T_4) \times 100$
Experiment A			
1 Vehicle	1,902	4,359	70
2 Vehicle	1,960	4,210	68
3 Vehicle	933‡	2,661	74
Mean±SE	1,931	4,284	71±2
4 Iopanoic Acid	2,132	2,024	49
5 Iopanoic Acid	2,444	1,531	39
6 Iopanoic Acid	3,588	2,476	41
Mean±SE	2,721±442	2,010±273	43±3‖
Experiment B			
1 Vehicle	1,400	7,687	85
2 Vehicle	2,458	9,121	79
3 Vehicle	1,800	8,797	83
Mean±SE	1,886±308	8,535±434	82±2
4 Iopanoic Acid	3,019	1,527	34
5 Iopanoic Acid	3,600	2,417	40
6 Iopanoic Acid	3,401	2,343	41
Mean±SE	3,340±170§	2,096±285¶	38±2¶
Experiment C			
1 Vehicle	1,500	3,595	71
2 Vehicle	250	2,002	89
3 Vehicle	1,100	3,185	74
4 Vehicle	2,470	5,830	71
Mean±SE	1,330±461	3,653±801	76±4
5 Iopanoic Acid	1,100	1,008	48
6 Iopanoic Acid	2,003	1,025	34
7 Iopanoic Acid	2,780	933	25
8 Iopanoic Acid	1,330	243	15
Mean±SE	1,803±378	802±187§	31±7‖

* Includes both locally derived and plasma ^{125}I-T_3.

‡ Portion of sample lost; not included in mean.

§ $P < 0.025$.

‖ $P < 0.005$.

¶ $P < 0.001$ for difference from vehicle-injected rats by unpaired t test.

was statistically significant only for the rats in Experiment B. Nevertheless, there was a decrease in the fraction of nuclear iodothyronine that was T_3 from a mean of 76% in the three control groups to a mean of 37% in the iopanoic acid-treated groups. This difference was highly significant in each experiment. However, because the increment in nuclear ^{125}I-T_4 was substantially less than the decrement of nuclear T_3, especially considering the fact that the specific activity of the ^{125}I-T_3 is only one-half that of ^{125}I-T_4, the total nuclear iodothyronines were still reduced in iopanoic acid-treated animals compared with controls.

Effect of iopanoic acid administration on TSH response to injected iodothyronines

Results of injection of 800 ng T_4/100 g body weight to chronically hypothyroid rats with and without iopanoic acid pretreatment are shown in Fig. 1. In animals given vehicle alone, TSH was suppressed to 41% of its control

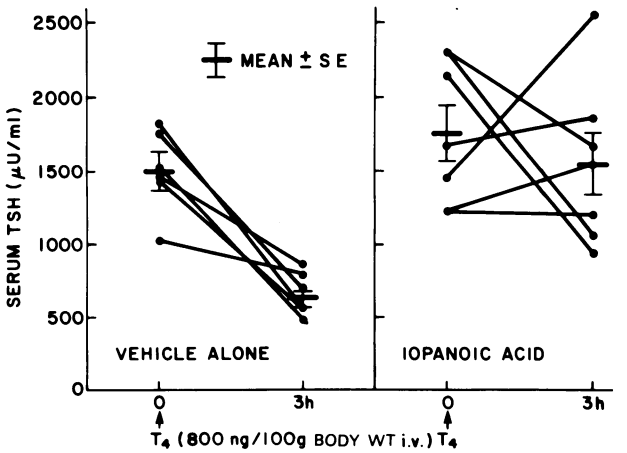

FIGURE 1 Acute response of chronically hypothyroid rats to 800 ng T_4/100 g body weight i.v. One group received vehicle alone (alkaline 50% solution of propylene glycol). The other group received three injections of iopanoic acid, 5 mg/100 g body weight i.p., 24, 16, and 1.5 h before injection of T_4.

value 3 h after T_4 injection ($P < 0.005$). The magnitude of this suppression is similar to our previous results with this T_4 dose (1, 2, 18). In animals treated previously with three injections of iopanoic acid, the mean serum TSH 3 h after identical quantities of T_4 was not significantly altered. Thus, iopanoic acid pretreatment prevented the response of the pituitary to the dose of injected T_4.

Because the apparent blockade of TSH response might have been due to nonspecific effects of iopanoic acid, the response of chronically hypothyroid rats to 70 ng/100 g T_3 was also evaluated. Results of these studies are shown in Fig. 2. In vehicle-injected rats,

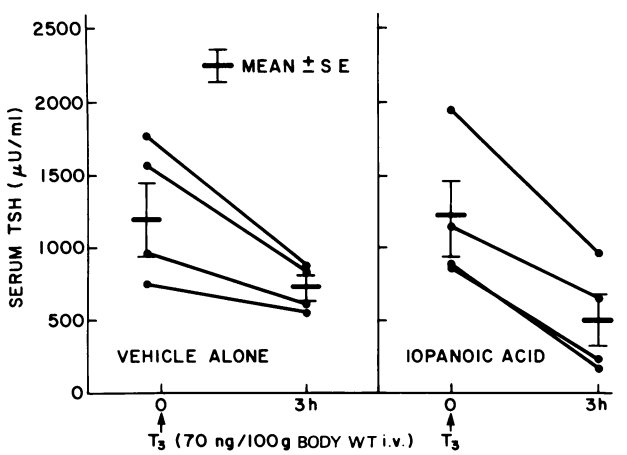

FIGURE 2 Acute response of chronically hypothyroid rats to injections of 70 ng T_3/100 g body weight i.v. One group of animals received vehicle alone (alkaline 50% propylene glycol solution); the second group received iopanoic acid, 5 mg/100 g body weight, i.p. 24, 16, and 1.5 h before injection of T_3.

TSH was suppressed by 40% ($P < 0.05$) in agreement with results of earlier studies (1, 18). The response to T_3 was not blocked by iopanoic acid pretreatment, because in the animals so treated T_3 caused suppression of TSH to 41% of the initial TSH level ($P < 0.01$) In other experiments not shown, the three iopanoic acid injections did not alter the basal TSH concentrations in these chronically hypothyroid rats.

DISCUSSION

The primary goal of these studies was to test the hypothesis that intrapituitary T_4 to T_3 conversion was required in order for T_4 to inhibit acutely the release of TSH in chronically hypothyroid rats (1). The blockade of the hepatic effect of T_4 (but not T_3) by PTU treatment has been shown by numerous investigators (19–22). Using tracer injections, Oppenheimer et al. demonstrated that this effect was due to impaired T_4 to T_3 conversion (3). The results of our own studies using radioimmunoassay of T_3 in T_4-treated animals confirmed these earlier studies (4, 18). The fact that we did not find inhibition of intrapituitary T_4 to T_3 in vivo conversion by pretreatment of animals with PTU was most puzzling (2). Although the same lack of effect has been recently demonstrated in vitro, the explanation is still unknown (9). Whatever the cause, the lack of effect of this agent obviated a test of the importance of intrapituitary T_4 to T_3 conversion in T_4-induced TSH suppression.

These results support our earlier speculation that intrapituitary T_4 to T_3 conversion is required for acute TSH suppression because pretreatment of rats with iopanoic acid prevented the response of the thyrotroph to injection of T_4 but not to T_3. Because the results of ^{131}I-T_3 injections demonstrated that there was no significant inhibition of ^{131}I-T_3 binding to the nuclei by iopanoic acid, the appropriate response of the hypothyroid rats to T_3 injection was not unexpected. The fact that the response to T_4 is blocked by pretreatment of animals with iopanoic acid in association with simultaneous inhibition of T_4 to T_3 conversion confirms the requirement for T_4-5′-monodeiodination in the production of this acute effect by T_4. Because the expected increase in nuclear T_3 after T_4 is blunted or inhibited in iopanoic acid-treated rats, the results are also consistent with the hypothesis that binding of T_3 to the nuclear receptor initiates the sequence of events leading to the inhibition of TSH secretion. However, other actions not mediated through the nucleus but also requiring T_3 could still be responsible for this effect.

Because the data from experiment C indicate that iopanoic acid inhibits T_4 to T_3 conversion in all tissues, documentation that it is the inhibition of intrapituitary T_4 to T_3 conversion which is responsible for

the marked decrease in pituitary nuclear T_3 is required. In our previous studies, we have provided data indicating that most of the nuclear T_3 3 h after T_4 administration to hypothyroid rats is the result of intrapituitary T_4-5′-monodeiodination. Although we analyzed the sources of serum and pituitary nuclear ^{125}I-T_3 as was done in experiment C (Table V), this aspect of the earlier studies was not emphasized (1, 2). For example, 3 h after injection of ^{125}I-T_4, we found ≅60% of the plasma ^{125}I-T_3 was derived from newly generated ^{125}I-T_3 in these experiments (2). This in turn accounted for about 20% of the noncontaminant pituitary nuclear ^{125}I-T_3 (Appendix of [2]). Therefore, 80% of the pituitary nuclear T_3 was locally generated 3 h after T_4 injection in this study. This is in close agreement with the estimate of 74% in Table V when data from animal one are excluded from the mean. At this time interval after T_4 injection, the major portion of pituitary nuclear ^{125}I-T_3 is derived from pituitary T_4-5′-conversion. The absence of nuclear ^{125}I-T_3 as in experiment C, or the marked decreases seen in experiments A and B, all indicate pituitary T_4-5′-conversion must be markedly reduced. This is in good agreement with the potent effect of iopanoic acid in vitro (8, 9). To summarize, the data suggest that at least 70–80% of pituitary nuclear T_3 at 3 h is derived from intrapituitary T_4 to T_3 conversion. Although inhibition of peripheral hepatic and renal T_4 to T_3 conversion also occurs as a result of iopanoic acid treatment, this source contributes in only a minor way to pituitary nuclear T_3 under these experimental circumstances.

Because T_4 to T_3 conversion is well recognized to occur in the liver and kidney, and iopanoic acid inhibits this process in vitro, the question may also be raised as to why no effects on nuclear ^{125}I-T_3 were apparent in these tissues. As we have previously demonstrated in both hypothyroid and euthyroid rats, intracellular T_4 to T_3 conversion in liver and kidney contributes only a small portion of the nuclear T_3 in these tissues (2, 12). For example, in euthyroid rats we calculated that roughly equal quantities of pituitary nuclear T_3 were derived from plasma T_3 and from intracellular T_4 to T_3 conversion (12). In liver and kidney, only 28 and 14% of the nuclear T_3 was estimated to derive from local intracellular T_4 to T_3 conversion, respectively. The reasons for these differences are not precisely known. Certainly one important influence is the fact that roughly 50% of T_3 in the pituitary is in the nucleus, while only 5–10% of intracellular T_3 is present on the nuclear receptor in liver and kidney cells (23). This means that a fivefold greater fraction of any T_3 generated will be bound to the nuclei of pituitary tissue as opposed to liver. Because intracellular T_4 to T_3 conversion is not a major source of nuclear T_3 in these tissues, inhibition of ^{125}I-T_4-5′-monodeiodination would not be expected to have an effect greater than that anticipated from the change in the plasma ^{125}I-T_3 concentration especially at relatively short time intervals after ^{125}I-T_4 injection. A further reason for the absence of a detectable effect in liver nuclei is that hepatic T_4 to T_3 conversion is reduced in hypothyroid rats, whereas pituitary T_4 to T_3 conversion is stimulated (8, 9, 24–26).

In these studies, we provide new data with respect to the sources of nuclear T_3 in cardiac tissue, which has not been studied previously. Apparently the nuclear receptors of the mycardial cells, like liver and kidney, are also predominantly dependent upon serum for nuclear T_3, because there appears to be no specific inhibitory effect of iopanoic acid on nuclear ^{125}I-T_3 in this tissue. All of the modestly lower results for nuclear ^{125}I-T_3 in iopanoic acid-treated rats in tissues other than anterior pituitary can be explained in terms of the somewhat lower concentrations of serum ^{125}I-T_3 in these animals. Although in none of the experiments was this difference in serum ^{125}I-T_3 statistically significant, the data in experiment C clarify a major reason for this phenomenon. At the same time that iopanoic acid inhibits T_4-5′-monodeiodination, it also appears to inhibit the metabolic clearance of T_3, perhaps through a similar inhibition of deiodination. This is reflected in the fact that in all three experiments, the serum ^{131}I-T_3 was lower in vehicle than in iopanoic acid-treated rats. Although this might be interpreted as being a result of an iopanoic acid-induced reduction in the volume of T_3 distribution, the fact that the difference between iopanoic acid-and vehicle-treated rats is greater at 3 h than at 1.5 h after injection suggests that it may be clearance that is affected. This process tends to reduce the differences in serum ^{125}I-T_3 in the two groups of animals. The iopanoic acid-treated rats have relatively complete impairment of T_4 to T_3 conversion, but serum ^{125}I-T_3 is present either due to impaired clearance of the contaminant or its distribution into a smaller volume than in controls. The vehicle-treated rats clear the contaminant more rapidly but generate new ^{125}I-T_3 in peripheral tissues. It should also be kept in mind that the percentage of the T_3 dose per milliliter is a function of weight, and therefore some of the variation in plasma ^{131}I-T_3 values in the groups in Table I can be explained in part by weight differences. For example, animal one in experiment A weighs almost 100 g more than animal two, and the serum ^{131}I-T_3 and ^{125}I-T_3 concentrations are the lowest of the three in that group. Similarly, animal three in experiment B is the smallest of the entire group, and this would certainly contribute to the fact that the serum ^{131}I-T_3 and ^{125}I-T_3 is substantially higher in that animal than in the other two.

The fact that nuclear T_4 may increase when intrapituitary T_4 to T_3 conversion is decreased (Table VI) supports the concept that the nuclear receptor sites

for T_3 and T_4 are the same and that there is competition between T_3 and T_4 for binding to the receptor (27). Apparently, the 10-fold higher binding affinity of T_3 for the nuclear receptor site together with the endogenous intracellular ratio of T_3 to T_4 results in little T_4 being bound to pituitary nuclei under normal circumstances. When the concentration of T_3 is reduced through inhibition of intrapituitary T_4 to T_3 conversion, then it is possible to demonstrate increased T_4 binding to the nucleus. Presumably the effect of iopanoic acid could be overcome by amounts of T_4 so large that the T_4 itself could induce a biological effect through occupancy of an appropriate number of nuclear receptors even if T_3 production was totally blocked.

In summary, these studies confirm our previous speculations regarding the requirement for conversion of T_4 to T_3 in the pituitary to obtain acute suppression of TSH release in chronically hypothyroid rats. The fact that the physiological effect of T_4 is prevented at the same time the increase in nuclear T_3 after T_4 is also inhibited supports the concept that this effect of T_4 is initiated by binding of locally produced T_3 to the pituitary nuclear receptor. The results raise the distinct possibility that the impressive changes in serum T_4 and TSH reported by Bürgi et al. (5) in euthyroid subjects given iopanoic acid are not only due to an inhibition of hepatic and renal T_4 to T_3 conversion, but also to inhibition of intrapituitary $T_4$5′-monodeiodination.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Richard Jove and Maurice Castonguay for their careful technical assistance and Ms. Anne Duli for preparation of the manuscript.

This work was supported by grant RO1 Am18616 from the National Institute of Arthritis, Metabolism, and Digestive Diseases and the William F. Milton Fund.

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