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SPECIFIC-NUCLEAR TRIIODOTHYRONINE BINDING SITES IN RAT LIVER AND KIDNEY[1]

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ABTRACT

Injection of increasing quantities of L-triiodothyronine (T_3) resulted in a progressive decrease in the proportion of T_3 bound to the nuclear fraction of rat liver and kidney cells. A similar dose-response relationship could not be demonstrated for other ultracentrifugal fractions. Only a modest reduction in nuclear binding of thyroxine was observed after injection of large amounts of this iodothyronine. The results indicate the presence of relatively specific binding sites for T_3 in the nuclei of rat liver and kidney.

Although previous studies have suggested that both L-thyroxine (T_4) L-triiodothyronine (T_3) are bound to a variety of subcellular constituents, the stereospecificity of these interactions has not been evaluated (1-4). In this communication, we shall present the first evidence that nuclei from rat liver and kidney possess a set of receptor sites which exhibit at least relative specificity for T_3. In contrast, other subcellular fractions separated by ultracentrifugation did not exhibit similar stereochemical specificity.

MATERIALS AND METHODS

Groups of male Sprague-Dawley rats (Carworth Farms) were injected through the tail vein with ^{125}I-labeled T_3 or T_4 (Abbott Laboratories) (40 μc/μg) together with increasing quantities of nonradioactive T_3 or T_4. Animals were killed 30 minutes and three hours after injection by exsanguination. Subcellular fractionation was carried out as previously described (3) except that a purified nuclear fraction was prepared by an additional centrifugation of the 700 X g fraction through 2.4M sucrose (5). The purity of the nuclear fraction was checked both by phase and electron microscopy. Plasma proteins were precipitated with 20% trichloroacetic acid in order to remove iodide. Descending chromatography of ethanolic extracts of nuclei was carried out in t-amyl alcohol:2N ammonia:hexane.

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RESULTS

Figure 1 illustrates one of three representative experiments in which animals were killed 3 hours after the injection of varying doses of nonradioactive T_3 and T_4 together with the corresponding iodothyronine labeled with ^{125}I. As the dose of triiodothyronine

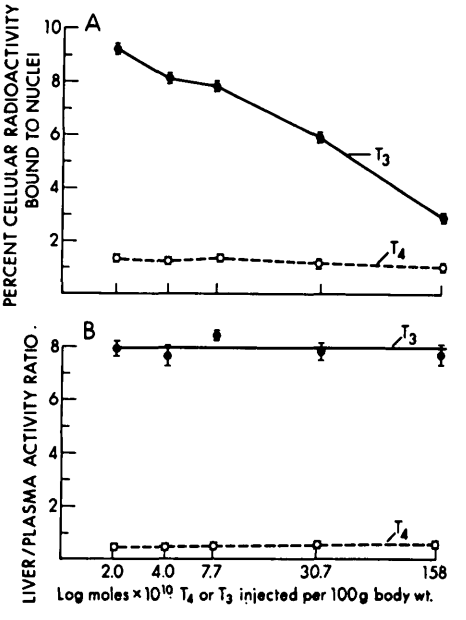

Fig.1. 5 rats for each point in 2 experiments, one with T_4, one with T_3. Mean ± SEM. Activity ratio=(cpm/g liver)/(cpm/ml TCA ppt serum).

was increased from 130 ng/100 g body weight, the percent of hepatic radioactivity associated with the purified nuclear fraction was reduced from 9.3 to 3.0 (Figure 1A). Over 98% of nuclear radioactivity could be extracted with ethanol and was demonstrated to migrate in the T_3 area of paper chromatograms. These findings suggest that loading doses of T_3 displace labeled T_3 from a set of stereospecific T_3 binding sites located in the hepatic nucleus. The apparent constancy of the liver:plasma T_3 ratio (Figure 1B) over the entire dose range of injected T_3 suggests that most of the hepatocellular T_3 binding sites do not exhibit similar stereospecificity. Previous studies have shown that in a rat secular equilibrium between plasma and liver iodothyronine is established within 15 minutes after intravenous injection of labeled hormone (6). Moreover, the overall binding of T_3 by plasma proteins, as determined by equilibrium dialysis, is not altered by the concentration of T_3 achieved in the present set of experiments. Conventional subcellular fractionation (Table 1) indicates that the reduction in the percentage of T_3 associated with the nuclear fraction is accompanied by a proportional increase in the percentage of T_3 associated with other subcellular fractions.

Table 1. Subcellular T_3

	% hepatic	radioactivity
ng/100 g bw	70	10,000
Nuclei	7.1	0.84
Mitochondria	11.6	14.3
Microsomes	17.7	19.4
Supernatant	25.9	28.4
700 X g-nuclei	22.6	23.0
% recovered	83	87

Mean of 5 animals indicated

Analogous studies in animals injected with T_4 (Figure 1A and B) indicate that a smaller percentage of hepatic iodothyronine is bound to the purified nuclear fraction and that a much smaller relative proportion of nuclear radioactivity is displaced by increasing doses of injected T_4. 1.4% was in the nuclear fraction when 150 ng T_4/100 g body weight was injected; 1.1% when the dose of T_4 was increased to 11,900 ng/100 g body weight. The actual molar quantities of injected iodothyronine reaching the hepatic cell was similar in the experiments with T_3 and T_4 (range after injection of T_3: 4.0-150 picomoles/gram; T_4 range: 4.4-430 picomoles/gram). Chromatography of extracts of nuclear radioactivity at the lowest dose of injected T_4 indicated that 85% of the radioactivity was in the form of T_4, and the 14% was in the form of T_3. T_3 radioactivity probably resulted from the selective accumulation by the nucleus of the 1-2% radioactive T_3 present in all commercial preparations of radioactive T_4 and to the small quantities of T_3 which were generated from T_4 during the previous 3 hours. The liver:plasma T_4 ratio did not change with increasing doses of T_4.

The results of these experiments suggest, therefore, that the nuclear binding sites exhibit at least a relative specificity for T_3. The modest reduction in T_4-binding by the nucleus could have resulted from either minimal crossreactivity of the nuclear sites for T_4 or displacement of ^{125}I-T_3 by nonradioactive T_3. Both radioactive and nonradioactive doses of T_4 are minimally (1-2%) contaminated with T_3. Entirely comparable results were demonstrated in six additional experiments in which the animals were killed within 30 minutes after the injection of graded doses of T_4 and T_3. During this period less than 6% of T_3 and less than 3% of T_4 are metabolized. Tracer T_3 added to liver homogenates 30 minutes after the injection of loading doses of T_3 did not distribute as the injected T_3 (Table 2). Moreover, a much larger proportion of injected T_3 was associated with the nuclear fraction.

Table 3 illustrates the results of experiments in which the radioactivity of kidney nuclei was examined

after the injection of labeled T_4 and T_3 together with increasing nonradioactive doses of the corresponding iodothyronines. The results were entirely comparable to those observed in the liver experiments.

Table 2. Nuclear binding of iodothyronine administered *in vivo* or added *in vitro*.

Dose injected (ng/100 g bw)		% hepatic radioactivity with nuclei	
		in vivo	*in vitro*
T_3	130	8.22	0.90
	10,000	1.32	0.68
T_4	150	1.44	1.10
	11,900	1.28	0.84

^{125}I-T_3 and ^{125}I-T_4 were injected with corresponding carrier iodothyronine. Rats (n=3) were killed 3 hrs. later and the corresponding ^{131}I-labelled tracer was added to the homogenate.

DISCUSSION

The results of these studies strongly suggest the existence of a set of easily saturatable T_3 binding sites in nuclei harvested from rat liver and kidney. Calculations based on the average concentration of T_3 in rat plasma (0.8 ng/ml) and the specific activity of injected T_3 indicate that only modest increments in the plasma T_3 (0.3 ng/ml) are accompanied by a significant displacement of the nuclear T_3. On the other hand, the extranuclear binding sites appear not to be easily saturable since the liver:plasma concentration ratio is essentially unaltered over a wide range of injected T_3. Only 8-9% of "tracer" quantities of liver T_3 are bound to the nuclei. Hence, even total saturation of these sites would results only in a 8 to 9% reduction in the total liver:plasma T_3 ratio, a reduction which would be difficult to detect experimentally. In contrast, the nuclear binding for T_4 is less avid, and only a small fraction of labeled T_4 is displaced from the nuclei as the injected dose of nonradioactive T_4 is increased for T_3.

In previous studies we have shown that distribution of labeled T_4 among subcellular components is the same, whether the iodothyronine is injected intravenously or is added directly to the homogenate (3). The results of the present experiments show that this is not the case for T_3 since major differences were demonstrable depending upon whether the tracer was administered *in vivo* or added *in vitro*. Moreover, *in vitro* addition of tracer, at least under the conditions used in these experiments, did not allow us to demonstrate the limited T_3 nuclear

Table 3. Nuclear Binding of T_3 and T_4 in kidney.

	n	Dose/100 g bw (picomoles)	kidney/plasma iodothyronine ratio	% kidney radioactivity in nucleus
T_3	12	177	12.1 ± 1.28	$2.56 \pm .56$
	8	400	12.7 ± 1.26	$1.17 \pm .31$
	12	1,442	10.8 ± 1.68	$0.42 \pm .08$
T_4	4	240	.50	.34
	4	460	.47	.30
	4	1,470	.50	.24

Animals were killed 30 minutes after injection of tracer and carrier doses of iodothyronine. Mean $\pm$ SEM indicated in T_3 experiments. Results of T_4 studies indicated are the mean of 2 sets of kidney homogenates, each consisting of two animals.

binding sites. Further studies are required to elucidate the underlying reasons for these differences.

Tata & associates (7) have suggested that a very early step in the action of the thyroid hormones is enhanced nuclear activity. Both the incorporation of orotic acid into nuclear RNA and the activity of DNA-dependent RNA polymerase are stimulated. The results of the current experiments are compatible with the concept that a direct interaction of T_3 or perhaps a T_3-cytosol complex with nuclear sites may initiate these early biological effects. Specific nuclear binding sites for number of steroid hormones have been identified (8,9). The apparent specificity of these iodothyronine binding sites for T_3 is also of interest in view of accumul- ng evidence that T_4 must be converted to T_3 before it becomes "metabolically active" (10,11).

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