Rapid Thyroxine to 3,5,3′-Triiodothyronine Conversion and Nuclear 3,5,3′-Triiodothyronine Binding in Rat Cerebral Cortex and Cerebellum

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ABSTRACT Thyroxine (T_4) to 3,5,3′-triiodothyronine (T_3) conversion was evaluated in vivo in cerebral cortex, cerebellum, and anterior pituitary of male euthyroid Sprague-Dawley rats. Tracer quantities of ^{125}I-T_4 and ^{131}I-T_3 were injected into controls and iopanoic acid-pretreated rats 3 h before isolation of nuclei from these tissues. Specifically-bound nuclear ^{131}I-T_3, denoted $T_3(T_3)$; ^{125}I-T_3, denoted $T_3(T_4)$; and ^{125}I-T_4 were extracted and identified by chromatography. Plasma iodothyronines were similarly quantitated. In control rats, nuclear $T_3(T_3)$ (percent dose per milligram DNA $\times 10^{-4}$) was 174±31 in cerebral cortex, 50±9 in cerebellum, and 932±158 in pituitary (all values, mean±SEM). Nuclear $T_3(T_4)$ (percent dose per milligram DNA $\times 10^{-4}$) was 23.3±3.3 in cortex, 3.5±0.6 in cerebellum, and 39.4±6.9 in pituitary. Two-thirds of nuclear $T_3(T_4)$ derived from local T_4 to T_3 conversion. Nuclear $T_3(T_4)$ in all tissues was reduced to less than 15% of its control value by iopanoic acid treatment and all of the residual nuclear $T_3(T_4)$ could be accounted for by plasma $T_3(T_4)$. Nuclear $T_3(T_3)$ binding was not inhibited by iopanoic acid. These results indicate there is rapid local T_4 to T_3 conversion in rat brain and nuclear binding of the T_3 produced. We have previously found that local $T_3(T_4)$ production is the source of ~50% of the T_3 in rat anterior pituitary. The present observations that the ratio of locally derived nuclear $T_3(T_4)$ to nuclear $T_3(T_3)$ is much higher in cerebral cortex (0.1) and cerebellum (0.04) than in anterior pituitary (0.015) suggest that this locally produced $T_3(T_4)$ is the predominant source of intracellular T_3 in these portions of rat brain.

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INTRODUCTION

Thyroid hormones have obvious functional and developmental effects on the mammalian brain. The mechanism by which these are produced has not been elucidated, though specific nuclear receptors for thyroid hormones have been identified in the brain of both adult and neonatal rats (1–4). The sources of thyroid hormones in brain tissue have not been well characterized. Our previous studies have indicated that there is a substantial contribution to rat anterior pituitary 3,5,3′-triiodothyronine (T_3)[1] arising from thyroxine (T_4) to T_3 conversion within the pituitary cells (5–7). This contrasts to the situation in liver, kidney, and heart where most intracellular T_3 appears to be derived directly from the plasma (5–7). Several investigators have found significant quantities of tracer T_3 in brain tissue within a relatively short time after injection of labeled T_4 (8, 9). Data of Obregon et al. (10) also have suggested that the ratio of the T_3 derived from injected tracer T_4 to the T_3 derived directly from plasma was significantly higher in brain than in liver, kidney, or heart. In the present experiments, we investigated T_4 to T_3 conversion in rat brain and evaluated the response to iopanoic acid, an agent which inhibits T_4 to T_3 conversion in rat anterior pituitary and liver (7, 11, 12).

METHODS

Euthyroid male Sprague-Dawley rats weighing 200–300 g were obtained from Zivic-Miller, Allison Park, Penn. 10 μCi/100 g body wt ^{131}I-T_3 (~3,300 μCi/μg, sp act) and about 100 μCi/100 g body wt ^{125}I-T_4 (2,800 μCi/μg, sp act) were given simultaneously by jugular injection with 200 μg NaI. Iopanoic acid, Telepaque, was supplied by Dr. F. C. Nachod, Winthrop Laboratories, Sterling Drug Co., New York. This was dissolved in alkalinized isotonic saline and 5 mg/100 g body

[1] *Abbreviations used in this paper*: IOP, iopanoic acid; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine.

wt i.p. was given 24, 16, and 1.5 h before iostope administration. Control animals received vehicle at the same times. Animals were killed after 3 h by exsanguination under light ether anesthesia and perfused with 30 ml of cold 0.15 M NaCl retrograde through the abdominal aorta to minimize the contribution of plasma iodothyronines to cellular radioactivity. Cell nuclei from anterior pituitary, cerebral cortex, and cerebellum were prepared as previously described (1, 11). At least two morphologically distinct types of nuclei were seen by phase contrast microscopy in brain preparations. Recovery of DNA in pituitary tissue was 80–90% and was 36 and 59% for cortex and cerebellum, respectively. Total DNA in the tissues was in agreement with previous estimates (1, 2, 6). In some experiments, 20 μg T_3/100 g body wt was injected simultaneously with tracer to determine nonspecific nuclear T_3 binding. Nonspecific T_3 binding was <8% of that bound at tracer doses for pituitary and cerebellum and 20% for cortex calculated as described (1). In all tissues, nonspecific nuclear binding of ^{125}I-T_4 in rats given excess T_3 was 21–25% of that bound when tracer was given alone.

Identification and quantitation of ^{131}I-T_3, ^{125}I-T_3, and ^{125}I-T_4 bound to nuclei and present in plasma was performed as previously described (7, 13). In brief, the nuclear pellets were counted to determine total ^{131}I-T_3 binding, extracted with ethanol-NH_4OH, and the iodothyronines chromatographed in tertiary amyl alcohol:hexane:2 N NH_4OH (5:1:6) with cold T_3, T_4 and I^-. The "T_3 and T_4 spots" were located by chemical staining and counted. Net ^{125}I-T_3 (above paper background and ^{131}I-T_3 crossover) was corrected for ^{131}I-T_3 losses (~30%) during extraction. ^{131}I-T_3 counts in the "T_3 spot" in control rats ranged from 7 to 18 $\times 10^3$ counts/40 min in cerebellum and pituitary and twice that number in cortex. Net nuclear ^{125}I-T_3 was 3–12 $\times$ 10^3, 4–12 $\times 10^3$, and 40–70 $\times$ 10^3 counts/40 min in cerebellum, pituitary and cortex, respectively. The counting error was <5% in all studies. Plasma ^{125}I-T_3 and ^{131}I-T_3 were isolated by affinity chromatography followed by paper chromatography (7, 13). T_3 recovery determined simultaneously with uninjected tracer was 15–25%. The contamination of ^{125}I-T_4 with ^{125}I-T_3 varied from 0.3 to 0.5% determined in a similar manner (7, 13).

The contribution of plasma ^{125}I-T_3 to nuclear ^{125}I-T_3 was estimated by multiplication of the plasma ^{125}I-T_3 concentration by the nuclear/plasma ratio for ^{131}I-T_3 corrected for nonspecific binding. Plasma ^{125}I-T_3 is derived from both ^{125}I-T_3 contaminant and ^{125}I-T_3 generated in tissues and returning to plasma. Since 3–3.5 h is required for complete equilibration of plasma T_3 with brain nuclear T_3 (1), this correction somewhat overestimates the contribution of newly generated plasma ^{125}I-T_3 to nuclear ^{125}I-T_3 and, therefore, underestimates the residual nuclear ^{125}I-T_3, which is that derived from local (intracellular) T_4 to T_3 conversion (5–7). However, this approach is sufficiently accurate for the present studies. To facilitate presentation of these results, we will denote ^{131}I-T_3 as $T_3(T_3)$ and ^{125}I-T_3 as $T_3(T_4)$ whether the latter was generated from T_4 *de novo* or present as a contaminant in the injected tracer T_4. Statistical significance was determined using unpaired Student's *t* test. All values are given as mean±SEM.

RESULTS

Specifically-bound nuclear iodothyronines in pituitary, cortex and cerebellum are shown in Table I. The fraction of the $T_3(T_3)$ dose specifically bound to nuclei was considerably higher in the pituitary than in the cortex and higher in cortex than in cerebellum ($P < 0.05$ for both comparisons). Nuclear $T_3(T_3)$ comprised 33±2.1, 5.4±0.3, and 8.5±0.5% of the total tissue $T_3(T_3)$ in pituitary, cortex, and cerebellum respectively. In contrast, nuclear $T_3(T_4)$ was not significantly different in pituitary and cortex but was substantially higher in both than in cerebellum. Specifically-bound nuclear ^{125}I-T_4 was found in all three tissues. Since over 90% of the nuclear $T_3(T_4)$–that not due to injected T_3·(T_4) contaminant–has been derived from T_4 labeled in the distal ring via 5′-monodeiodination, the specific activity of the cellular $T_3(T_4)$ is approximately half that of T_4. Thus, in all three tissues, $T_3(T_4)$ constitutes 70–80% of the ^{125}I-labeled nuclear iodothyronines.

TABLE I

Specifically Bound Nuclear Iodothyronines in Euthyroid Rat Brain and Anterior Pituitary 3 h after Simultaneous ^{131}I-T_3 and ^{125}I-T_4 Injection

Tissue	$T_3(T_3)$–^{131}I-T_3	$T_3(T_4)$–^{125}I-T_3	^{125}I-T_4
	% dose/mg DNA × 10^{-4}		
Pituitary	1,130±260	46±12	23±7.1
Cortex	290±89	28±4.6	24±7.8
Cerebellum	63±14	4.2±0.9	3.3±0.7

* All values are mean±SEM, $n = 5$.

To determine the quantity of $T_3(T_4)$ derived from local conversion within the tissue and to evaluate the effect of iopanoic acid (IOP) on this conversion in vivo, we performed the experiments shown in Table II. Nuclear $T_3(T_3)$ and the nuclear/plasma $T_3(T_3)$ ratios (not shown) were not affected by IOP pretreatment. This indicates that there is no inhibition of nuclear T_3 binding by IOP, consistent with our previous results in pituitary, liver, heart, and kidney of intact rats (7). Total nuclear $T_3(T_4)$ was reduced to <15% of control by IOP pretreatment. In Table II, the quantity of nuclear $T_3(T_4)$ contributed by local, i.e., intracellular, T_4 to T_3 conversion is calculated. About two-thirds of the nuclear $T_3(T_4)$ in cortex and cerebellum in control rats is derived from local T_4 5′-monodeiodination; the remainder derives from the plasma. In IOP-treated rats, all of the residual nuclear $T_3(T_4)$ could be accounted for by the $T_3(T_4)$ in plasma, indicating complete inhibition of local T_4 to T_3 conversion in the tissues examined. An average of 87±1% (SEM) of the plasma $T_3(T_4)$ in IOP-treated rats was due to injected $T_3(T_4)$ contaminant as opposed to 14±1% in controls.

DISCUSSION

The present results demonstrate that there is significant local T_4 to T_3 conversion in the brain of euthyroid rats. As previously demonstrated for anterior pituitary, the T_3 generated from T_4 is bound to limited-capacity nuclear binding sites (5–7). At present, it has not been shown that nuclear binding of T_3 or T_4 is required for

TABLE II

Specifically Bound Nuclear T_3 in Euthyroid Rat Brain and Anterior Pituitary 3 h after Simultaneous ^{131}I-T_3 and ^{125}I-T_4 Injection

		Cortex	Cerebellum	Pituitary
		% dose/mg DNA × 10^{-4}		
Nuclear ^{131}I-T_3	Control (9)	174±31	50±9	932±158
	IOP (7)	292±57	76±18	1160±170
Total nuclear ^{125}I-T_3	Control (9)	23.3±3.3	3.5±0.6	39.4±6.9
	IOP (7)	2.0±0.3*	0.3±0.1*	5.8±1.4*
Nuclear ^{125}I-T_3 from plasma	Control (9)	5.2±0.9	1.4±0.3	25.7±5.0
	IOP (7)	2.0±0.5‡	0.5±0.1‡	7.9±1.6‡
Nuclear ^{125}I-T_3 from local T_4 to T_3 conversion	Control (9)	18.1±2.6	2.1±0.3	13.7±3.5
	IOP (7)	-0-*	-0-*	-0-§

Number of animals is given in parentheses. In the text, ^{131}I-T_3 is denoted $T_3(T_3)$, and ^{125}I-T_3 as $T_3(T_4)$.
Significantly different from control:
* $P < 0.001$.
‡ $P < 0.05$.
§ $P < 0.01$.

the initiation of hormone action in brain. Therefore, nuclear T_3 in brain can only be said at this time to be a representative sample of the intracellular T_3. Furthermore, it is not known whether local T_4 to T_3 conversion and nuclear binding take place in neuronal cells, glial cells, or both cell populations. Schwartz and Oppenheimer have estimated that the binding capacity of solubilized receptor was 0.33 ng/mg DNA in cortex and 0.064 ng/mg DNA in cerebellum (2), and previous studies have shown that anterior pituitary nuclear T_3 binding capacity is 0.8 ng/mg DNA (1, 6). Therefore, the different quantities of nuclear $T_3(T_3)$ in the various tissues can probably be attributed to these differences in nuclear binding capacities.

It is apparent from Table II that total nuclear $T_3(T_4)$ substantially exceeds that which can be accounted for by plasma $T_3(T_4)$ alone, emphasizing the importance of local T_4 to T_3 conversion. Further substantiation of the important role of local conversion is indicated by the fact that nuclear $T_3(T_4)$ is virtually eliminated by pretreatment of rats with IOP. These results are similar to our observations of the effect of this agent on anterior pituitary T_4 to T_3 conversion both in vivo and in vitro (7, 11).

Local production of $T_3(T_4)$ in rat anterior pituitary does not provide a maximum contribution to nuclear T_3 until about 16 h after T_4 injection (6). The present studies do not establish when the quantities of $T_3(T_4)$ in cortex and cerebellum reach a maximum. Therefore, it is not possible to make a precise gravimetric comparison of the relative contributions of $T_3(T_3)$ and locally produced $T_3(T_4)$ to the total nuclear T_3 in brain. However, the data in Table II suggest that the contribution of $T_3(T_4)$ is substantial. The ratio of locally derived nuclear $T_3(T_4)$ to nuclear $T_3(T_3)$ in anterior pituitary in Table II is ~0.015, whereas the ratios in cortex and cerebellum are 0.10 and 0.04, respectively. This suggests that the contribution of local T_4 to T_3 conversion to nuclear T_3 (and total cellular T_3) would be even greater in cortex and cerebellum than the 50% that it contributes in anterior pituitary. Therefore, plasma T_4, through its local conversion to T_3 in the brain, may be the predominant source of intracellular T_3 in the cerebral cortex and cerebellum of the rat. Studies are currently underway to substantiate these estimates. If these speculations are confirmed, it would suggest that, analogous to the situation in anterior pituitary and unlike that in liver, kidney, or heart, establishment of normal intracellular T_3 concentrations in cortex and cerebellum of hypothyroid rats would require normalization of serum T_4 as well as serum T_3. If this proves to be the case in man as well, this concept would have special importance in the proper treatment of congenital hypothyroidism.

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