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 (T₄) to 3,5,3'-triiodothyronine (T₃) conversion was evaluated in vivo in cerebral cortex, cerebellum, and anterior pituitary of male euthyroid Sprague-Dawley rats. Tracer quantities of $^{125}$I-T₄ and $^{131}$I-T₃ were injected into controls and iopanoic acid-pretreated rats 3 h before isolation of nuclei from these tissues. Specifically-bound nuclear $^{131}$I-T₃, denoted T₃(T₃); $^{125}$I-T₃, denoted T₃(T₄); and $^{125}$I-T₄ were extracted and identified by chromatography. Plasma iodothyronines were similarly quantitated. In control rats, nuclear T₃(T₃) (percent dose per milligram DNA × 10⁻⁴) was 174±31 in cerebral cortex, 50±9 in cerebellum, and 932±158 in pituitary (all values, mean±SEM). Nuclear T₃(T₄) (percent dose per milligram DNA × 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 T₃(T₃) derived from local T₄ to T₃ conversion. Nuclear T₃(T₄) in all tissues was reduced to less than 15% of its control value by iopanoic acid treatment and all of the residual nuclear T₃(T₃) could be accounted for by plasma T₃(T₄). Nuclear T₃(T₃) binding was not inhibited by iopanoic acid. These results indicate there is rapid local T₄ to T₃ conversion in rat brain and nuclear binding of the T₃ produced. We have previously found that local T₃(T₄) production is the source of ~50% of the T₃ in rat anterior pituitary. The present observations that the ratio of locally derived nuclear T₃(T₄) to nuclear T₃(T₃) is much higher in cerebral cortex (0.1) and cerebellum (0.04) than in anterior pituitary (0.015) suggest that this locally produced T₃(T₄) is the predominant source of intracellular T₃ in these portions of rat brain.

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 (T₃) arising from thyroxine (T₄) to T₃ conversion within the pituitary cells (5–7). This contrasts to the situation in liver, kidney, and heart where most intracellular T₃ appears to be derived directly from the plasma (5–7). Several investigators have found significant quantities of tracer T₃ in brain tissue within a relatively short time after injection of labeled T₄ (8, 9). Data of Obregon et al. (10) also suggested that the ratio of the T₃ derived from injected tracer T₄ to the T₃ derived directly from plasma was significantly higher in brain than in liver, kidney, or heart. In the present experiments, we investigated T₄ to T₃ conversion in rat brain and evaluated the response to iopanoic acid, an agent which inhibits T₄ to T₃ 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 $^{125}$I-T₃ (~3,300 µCi/µg, sp act) and about 100 µCi/100 g body wt $^{125}$I-T₄ (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 alkalginized isotonic saline and 5 mg/100 g body

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Abbreviations used in this paper: IOP, iopanoic acid; T₃, 3,5,3'-triiodothyronine; T₄, thyroxine.
wt i.p. was given 24, 16, and 1.5 h before isotope administration. Control animals received vehicle at the same time. 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 T₃/100 g body wt was injected simultaneously with tracer to determine nonspecific nuclear T₃ binding. Nonspecific T₃ binding was <5% 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 ¹²⁵I-T₃ in rats given excess T₃ was 21–25% of that bound when tracer was given alone.

Identification and quantitation of ¹²³I-T₃, ¹³¹I-T₃, and ¹³¹I-T₄ bound to nuclei and present in plasma was performed as previously described (7, 13). In brief, the nuclear pellets were counted to determine total ¹²⁵I-T₃ binding, extracted with ethanol-NH₄OH, and the iodothyronines chromatographed in tertiary amyl alcohol:hexane:2 N NH₄OH (5:1:6) with cold T₃, T₄, and I-1. The “T₃ and T₄ spots” were located by chemical staining and counted. Net ¹²³I-T₃ (above paper background and ¹³¹I-T₃ crossover) was corrected for ¹²⁴I-T₃ losses (~30%) during extraction. ¹²³I-T₃ counts in the “T₃ spot” in control rats ranged from 7 to 18 × 10⁶ counts/min in cerebellum and pituitary and twice that number in cortex. Net nuclear ¹²³I-T₃ was 5–12 × 10⁸, 4–12 × 10⁷, and 40–70 × 10⁶ counts/min in cerebellum, pituitary, and cortex, respectively. The counting error was <5% in all studies. Plasma ¹²³I-T₃ and ¹³¹I-T₃ were isolated by affinity chromatography followed by paper chromatography (7, 13). T₃ recovery determined simultaneously with uninjected tracer was 15–25%. The contamination of ¹²⁴I-T₃ with ¹²³I-T₃ varied from 0.3 to 0.5% determined in a similar manner (7, 13).

The contribution of plasma ¹²³I-T₃ to nuclear ¹²³I-T₃ was estimated by multiplication of the plasma ¹²³I-T₃ concentration of the nuclear/plasma ratio for ¹²⁴I-T₃ corrected for nonspecific binding. Plasma ¹³¹I-T₃ is derived from both ¹²³I-T₃ contaminant and ¹³¹I-T₃ generated in tissues and returning to plasma. Since 3–3.5 h is required for complete equilibration of plasma T₃ with brain nuclear T₃ (4), this correction somewhat overestimates the contribution of newly generated plasma ¹²³I-T₃ to nuclear ¹²³I-T₃ and, therefore, underestimates the residual nuclear ¹²³I-T₃, which is that derived from local (intracellular) T₄ to T₃ conversion (5–7). However, this approach is sufficiently accurate for the present studies. To facilitate presentation of these results, we will denote ¹²¹I-T₃ as T₃(T₃) and ¹⁵¹I-T₄ as T₄(T₄) whether the latter was generated from T₄ or T₃ de novo or present as a contaminant in the injected tracer T₄. 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 1. The fraction of the T₃(T₃) 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 T₃(T₃) comprised 33±2.1, 5.4±0.3, and 8.5±0.5% of the total tissue T₃(T₃) in pituitary, cortex, and cerebellum respectively. In contrast, nuclear T₃(T₄) was not significantly different in pituitary and cortex but was substantially higher in both than in cerebellum. Specifically-bound nuclear ¹²³I-T₄ was found in all three tissues. Since over 90% of the nuclear T₃(T₄)–that not due to injected T₃(T₄) contaminant–has been derived from T₄ labeled in the distal ring via 5’-monodeiodination, the specific activity of the cellular T₃(T₄) is approximately half that of T₄. Thus, in all three tissues, T₃(T₄) constitutes 70–80% of the ¹²³I-labeled nuclear iodothyronines.

To determine the quantity of T₃(T₄) 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 T₃(T₃) and the nuclear/plasma T₃(T₃) ratios (not shown) were not affected by IOP pretreatment. This indicates that there is no inhibition of nuclear T₃ binding by IOP, consistent with our previous results in pituitary, liver, heart, and kidney of intact rats (7). Total nuclear T₃(T₃) was reduced to <15% of control by IOP pretreatment. In Table II, the quantity of nuclear T₃(T₄) contributed by local, i.e., intracellular, T₄ to T₃ conversion is calculated. About two-thirds of the nuclear T₃(T₄) in cortex and cerebellum in control rats is derived from local T₄ 5’-monodeiodination; the remainder derives from the plasma. In IOP-treated rats, all of the residual nuclear T₃(T₄) could be accounted for by the T₃(T₄) in plasma, indicating complete inhibition of local T₄ to T₃ conversion in the tissues examined. An average of 87±1% (SEM) of the plasma T₃(T₄) in IOP-treated rats was due to injected T₃(T₄) contaminant as opposed to 14±1% in controls.

**DISCUSSION**

The present results demonstrate that there is significant local T₄ to T₃ conversion in the brain of euthyroid rats. As previously demonstrated for anterior pituitary, the T₃ generated from T₄ is bound to limited-capacity nuclear binding sites (5–7). At present, it has not been shown that nuclear binding of T₃ or T₄ is required for

<table>
<thead>
<tr>
<th>Tissue</th>
<th>T₃(T₃)</th>
<th>T₃(T₄)</th>
<th>m/I-T₄</th>
<th>% dcp DNA × 10⁴</th>
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<tr>
<td>Pituitary</td>
<td>1.13±0.26</td>
<td>46±12</td>
<td>23±7.1</td>
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<tr>
<td>Cortex</td>
<td>290±80</td>
<td>28±4.6</td>
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<td>Cerebellum</td>
<td>63±14</td>
<td>4.2±0.9</td>
<td>3.3±0.7</td>
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</table>

* All values are mean±SEM, n = 5.
the initiation of hormone action in brain. Therefore, nuclear T₃ in brain can only be said at this time to be a representative sample of the intracellular T₃. Furthermore, it is not known whether local T₄ to T₃ 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 T₃ binding capacity is 0.8 ng/mg DNA (1, 6). Therefore, the different quantities of nuclear T₃(T₃) in the various tissues can probably be attributed to these differences in nuclear binding capacities.

It is apparent from Table II that total nuclear T₃(T₄) substantially exceeds that which can be accounted for by plasma T₃(T₄) alone, emphasizing the importance of local T₄ to T₃ conversion. Further substantiation of the important role of local conversion is indicated by the fact that nuclear T₃(T₄) 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 T₄ to T₃ conversion both in vivo and in vitro (7, 11).

Local production of T₃(T₄) in rat anterior pituitary does not provide a maximum contribution to nuclear T₃ until about 16 h after T₄ injection (6). The present studies do not establish when the quantities of T₃(T₄) in cortex and cerebellum reach a maximum. Therefore, it is not possible to make a precise gravimetric comparison of the relative contributions of T₃(T₃) and locally produced T₃(T₄) to the total nuclear T₃ in brain. However, the data in Table II suggest that the contribution of T₃(T₄) is substantial. The ratio of locally derived nuclear T₃(T₄) to nuclear T₃(T₃) 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 T₄ to T₃ conversion to nuclear T₃ (and total cellular T₃) would be even greater in cortex and cerebellum than the 50% that it contributes in anterior pituitary. Therefore, plasma T₄, through its local conversion to T₃ in the brain, may be the predominant source of intracellular T₃ 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 T₃ concentrations in cortex and cerebellum of hypothyroid rats would require normalization of serum T₄ as well as serum T₃. If this proves to be the case in man as well, this concept would have special importance in the proper treatment of congenital hypothyroidism.

ACKNOWLEDGMENTS

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REFERENCES

1. Oppenheimer, J. H., H. L. Schwartz, and M. I. Surks. 1974. Tissue differences in the concentration of triodo-

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TABLE II

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Pituitary</th>
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<tr>
<td>% dose/mg DNA × 10⁻⁶</td>
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<tr>
<td>Nuclear ¹³¹I-T₃</td>
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<tr>
<td>Control (9)</td>
<td>174±31</td>
<td>50±9</td>
<td>932±158</td>
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<td>IOP (7)</td>
<td>292±57</td>
<td>76±18</td>
<td>1160±170</td>
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<td>Total nuclear ¹³¹I-T₃</td>
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<td>Control (9)</td>
<td>23.3±3.3</td>
<td>3.5±0.6</td>
<td>39.4±6.9</td>
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<tr>
<td>IOP (7)</td>
<td>2.0±0.3*</td>
<td>0.3±0.1*</td>
<td>5.8±1.4*</td>
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<td>Nuclear ¹³¹I-T₃ from plasma</td>
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<td>Control (9)</td>
<td>5.2±0.9</td>
<td>1.4±0.3</td>
<td>25.7±5.0</td>
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<tr>
<td>IOP (7)</td>
<td>2.0±0.5</td>
<td>0.5±0.11</td>
<td>7.9±1.6†</td>
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<tr>
<td>Nuclear ¹³¹I-T₃ from local T₄ to T₃ conversion</td>
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<tr>
<td>Control (9)</td>
<td>18.1±2.6</td>
<td>2.1±0.3</td>
<td>13.7±3.5</td>
</tr>
<tr>
<td>IOP (7)</td>
<td>-0-*</td>
<td>-0-*</td>
<td>-0-§</td>
</tr>
</tbody>
</table>

Number of animals is given in parentheses. In the text, ¹³¹I-T₃ is denoted T₃(T₃), and ¹³¹I-T₃ as T₃(T₄).

Significantly different from control:
* P < 0.001.
† P < 0.05.
§ P < 0.01.


