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# POSSIBLE ROLE OF T4 BINDING TO CYTOPLASMIC FRACTION OF PITUITARY CELLS ON THE CONTROL OF TSH RESPONSE TO TRH IN THE RAT

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ABSTRACT

It has previously been shown that thyroxine (T4) can inhibit the thyrotropin (TSH) response to TSH-releasing hormone (TRH) before a significant intrapituitary conversion of T4 to triiodothyronine (T3) had occurred in euthyroid rats. The present study has investigated the in vivo binding of T4 and T3 to cytoplasm and nucleus of pituitary cells of hypothyroid rats. The results indicate that $^{125}$I-T4 was specifically bound to cytoplasms and non-specifically-bound to nuclei. On the other hand, nuclear binding of $^{125}$I-T3 was specific whereas cytoplasmic binding was non-specific. The data suggest that the inhibition of TRH stimulation of TSH by T4 previously reported may involve the binding of T4 to extranuclear cell components, perhaps those located in the cytoplasmic fractions.

KEYWORDS

Pituitary binding of thyroid hormones; control of TSH response to TRH.

## INTRODUCTION

Previous studies (Larsen and co-workers, 1979) have demonstrated that the binding of T3 to pituitary cells nuclei was a necessary step to decrease the basal plasma TSH in hypothyroid rats. However, work from our laboratories have shown that T4 could inhibit the TSH response to TRH within 30 minutes of its i.v. administration and before a significant intrapituitary conversion to T3 had occurred (Boado, Ulloa and Zaninovich, 1982). We have no investigated the in vivo binding of $^{125}$I-T4 and $^{125}$I-T3 to pituitary cells of hypothyroid rats.

## MATERIAL AND METHODS

### Intrapituitary conversion of T4 to T3

Male Wistar rats weighing approximately 200 g made hypothyroid by the intraperitoneal injection of $^{131}$I. Animals were used when plasma levels of T4 and T3 were undetectable. Groups of rats were treated with iopanoic acid (IOP) to inhibit the conversion of T4 to T3, in a dose of 5 mg/100g BW i.p., 24, 12 and 1.5 hours preceding the study. Control rats were injected with the vehicle

alone. Intrapituitary conversion of T4 to T3 was measured as described by Segall-Blank, Connolly and Inhbar (1982). Rats were exsanguinated and the hypophyses removed immediately and individually homogenized in 300 µl phosphate buffer 0.075 M pH 7.4 (Boado, Ulloa and Zaninovich, 1983). Two hundred microliters of each homogenate were incubated with a tracer dose of $^{125}$I-T4 (s.a. 1500 µCi/µg) containing 20 mM of dithiothreitol during 3 hours at 37 C. Incubation was stopped by addition of 300 µl of methanol:$NH_4OH$ 2N (99:1). Homogenates were chromatographed in $NH_4OH$ 1N: tertiary amyl acohol:hexane system (6:5:1).

Pituitary Binding of $^{125}$I-T4 and $^{125}$I-T3

Control and IOP-treated rats were injected with 150 nCi of 3', 5'-$^{125}$I-T4 (25 ng, 6000 µCi/µg). Another group received, in addition, a loading dose of 25 µg of non-radioactive T4 i.v.. Parallel studies were carried out in rats injected with 50 nCi of $^{125}$I-T3 (controls) or the isotope plus a loading amount of 17 µg of non-radioactive T3. Thirty minutes later animals were exsanguinated and killed by cervical dislocation. Hypophyses were removed and homogenized individually in 200 µl of a solution containing 0.3 M sucrose and 20 mM $MgCl_2$. Nuclei were purified as described elsewhere (Cheron, Kaplan and Larsen, 1979). Cytoplasms, nuclei, aliquots of plasma and standards were extracted in 200 µl ethanol:$NH_4Cl$ (99:1) containing 100 µg of each T4, T3 and KI/ml, and chromatographed as described above. Double-labelled $^{125}$I-T4 was prepared as described previously (Boado, Ulloa and Zaninovich, 1982). The purity of $^{125}$I-T4 was at least 98.3% and that of $^{125}$I-T3 was 99.7%. Statistical analyses were made by the analysis of variance and the Student's t-test.

RESULTS

Table 1 shows the percentage of $^{125}$I-T3 produced in pituitary homogenates incubated with $^{125}$I-T4 in vitro. In control rats, the fraction

TABLE 1 Pituitary Conversion of T4 to T3 in IOP-Treated Hypothyroid Rats

| | $^{125}$I | $^{125}$I-T4 | $^{125}$I-T3 |
|---|---|---|---|
| | (% of total radioactivity) | | |
| Control (5)[1] | 10.5 ± 7.3[2] | 77.3 ± 12.5 | 11.2 ± 5.8 |
| IOP (6) | 2.7 ± 2.0 | 94.6 ± 2.0 | 1.2 ± 0.6 |
| P Value | $< 0.05$ | $< 0.01$ | $< 0.005$ |

[1]Number of experiments; [2]Mean ± S.D.

of $^{125}$I-T3 generated by $^{125}$I-T4 conversion during 3 hours was 11.2 ± 5.8% of the total radioactivity. The administration of IOP markedly reduced the production of $^{125}$I-T3 ($P < 0.005$) and $^{125}$I ($P < 0.05$) while increasing the intact $^{125}$I-T4 in the homogenate ($P < 0.01$). In experiments where $^{125}$I-T3 was added to pituitary homogenates, no significant deiodination of this hormone was observed.

The in vivo binding of labelled T4 is shown in Table 2. In control animals the total radioactivity in cytoplasmic fractions was 0.013 ± 0.001% of the injected $^{125}$I-T4, and it was mostly due to T4 (76 ± 6%) whereas 17 ± 3% was due to labelled T3 which derived from T4 conversion. The administration of IOP induced a significant increase in total radioactivity ($P < 0.05$) and in $^{125}$I-T4 ($P < 0.01$)

accompanied by a pronounced reduction in labelled T3 ($P < 0.01$). When A T4 load was administered, total radioactivity ($P < 0.05$) and $^{125}$I-T3 ($P < 0.05$) were both reduced as compared to control animals.

TABLE 2 Pituitary Radioactivity 30 Minutes After I.V. Injection of 150 uCi of 3'5'-$^{125}$I-T4 to Hypothyroid Rats

| | Total Radioactivity (% injected dose) | $^{125}$I-T4 (% of total) | $^{125}$I-T3 (% of total) |
|---|---|---|---|
| | Cytoplasm | | |
| Control (6) | 0.013 ± 0.001 | 76 ± 6 | 17 ± 3 |
| IOP (6) | 0.019 ± 0.002 | 94 ± 3[2] | 1 ± 0.7[2] |
| T4 load (6) | 0.008 ± 0.0006[1] | 76 ± 4 | 5 ± 1 |
| | Nucleus | | |
| Control | 0.0042 ± 0.0036 | 51 ± 4 | 28 ± 6 |
| IOP | 0.0023 ± 0.0008 | 65 ± 8 | 15 ± 6 |
| T4 load | 0.0040 ± 0.0004 | 60 ± 6 | 16 ± 3 |

Significance vs. control group: [1] 0.05; [2] 0.01

Total nuclear radioactivity and the proportion of both labelled T4 and T3 found in control rats were not significantly altered by IOP or by the non-radioactive T4 load, thus suggesting a non-specific binding of T4 to nuclei.

TABLE 3 Pituitary Radioactivity 30 Minutes After I.V. Injection of 50 µCi of $^{125}$I-T3 to Hypothyroid Rats

| | Total Radioactivity (% injected dose) | $^{125}$I-T3 (% of total) |
|---|---|---|
| | Cytoplasm | |
| Control (5) | 0.053 ± 0.011 | 96 ± 8 |
| 17 ug T3 (5) | 0.051 ± 0.011 | 98 ± 6 |
| | Nucleus | |
| Control | 0.0075 ± 0.0035 | 96 ± 8 |
| 17 ug T3 | 0.0010 ± 0.0001[1] | 98 ± 2 |

[1] $P < 0.01$ vs. control group

Table 3 describes the pituitary radioactivity in rats injected with 50 µCi of $^{125}$I T3 i.v. In the cytoplasmic fraction of control rats, total radioactivity was 0.053% of the injected dose, 96% of it being labelled T3 and the rest was $^{125}$I. A non-radioactive T3 load failed to displace radioactive T3 from cytoplasm, while significantly reducing $^{125}$I-T3 in nuclei ($P < 0.01$). These findings may be taken to indicate that binding of T3 to nuclear sites was specific, whereas cytoplasmic binding was non-specific, effects which appear to be opposite to those observed in the case of $^{125}$I-T4. No other labelled compounds other than radioactive T3

(98 ± 2%) and radioactive iodine were detected in nuclei of animals treated with 17 µg of non-radioactive T3. The same applies to other groups depicted in Table 3.

## DISCUSSION

The data of Larsen and co-workers (1979) indicated that T4 had to be deiodinated to T3 in the pituitary nuclei in order to regulate the secretion of TSH in hypothyroid rats. The contention found support in their observation that IOP, which inhibited the intrapituitary conversion of T4 to T3, suppressed the T4 effect on TSH secretion in the same animals, albeit no data on the response to TRH was reported. Nevertheless, our cited studies (Boado, Ulloa and Zaninovich, 1982) have clearly shown that T4 is capable of blocking the stimulatory effect of TRH On TSH secretion without prior conversion to T3. In the present study, labelled T4 was displaced from cytoplasm by a T4 load, which might reflect specific binding of this hormone to cytoplasmic receptors, as previously reported by Galton (1977). Moreover, the specific binding of T3 to nuclear sites and its apparent lack of binding to cytoplasm may be a reflection that nuclear T3 would control the basal secretion of TSH whereas the stimulatory effect of TRH would be mediated by pituitary cytoplasmic binding of T4.

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