Effects of L-thyroxine treatment on pituitary GH content of adult rats with neonatal thyrotoxicosis

A. M. Pascual-Leone, E. Besa, F. Hervás, F. Escrivá and C. Alvarez

Departamento de Bioquímica del C. S. I. C., Facultad de Farmacia de la Universidad Complutense, Madrid-3 and Departamento de Endocrinología de la Seguridad Social (Ciudad Universitaria), Madrid-3

Abstract. Rats receiving large doses of thyroxine (30 µg/5 doses) during their first days of life develop an apparently permanent alteration of the hypothalamic-pituitary-thyroid complex. This neonatal thyrotoxicosis has been called neo-T₄ syndrome. A state of permanent but not very severe hypothyroidism seems to be induced, accompanied by a decrease in pituitary GH content at least until day 22. In this work, growth hormone content has been measured by a specific radioimmunoassay in the anterior pituitary of 45 and 78 day old neo-T₄ and control (saline-injected) rats. GH content of the adult neo-T₄ treated animals was significantly lower than that of the adult controls. Administration of different doses of T₄ (1.7 µg/100 g body weight/3 doses or 2.5 µg/100 g body weight/8 doses, to 70 day old rats, and 5 µg/100 g body weight/3 doses to 42 day old rats) to adult neo-T₄ rats did not alter these decreased pituitary GH levels. This differs from hypothyroid rats, in which T₄ administration has been shown to increase pituitary GH content. A third approach was to thyroidectomize neo-T₄ and control rats and administer 5 µg T₄/100 g body weight, which produced the same increase in pituitary GH in both groups of animals. These results seem to indicate that changes in pituitary GH content of neo-T₄ rats are not due to hypothyroidism. Thus, it would appear that treatment with large T₄ doses during the early perinatal period not only deranges the hypothalamic-pituitary-thyroid axis but other pituitary functions as well.

It has repeatedly been observed that large doses of thyroxine produce an alteration of the hypothalamo-pituitary-thyroid axis (Eayrs & Holmes 1964; Bakke & Lawrence 1966; Bakke et al. 1974). There is a decrease in body and brain weight and slight hypothyroidism with reduction in pituitary TSH content. This neonatal thyrotoxicosis has been called neo-T₄ syndrome, and was defined as a kind of pseudohypothyroidism (Bakke & Lawrence 1966). A marked decrease in pituitary GH content has been shown by radioimmunoassay in young neo-T₄ animals (Pascual-Leone et al. 1976). Eayrs & Holmes (1964) had previously reported that rats treated with large doses of T₃ showed a decrease in the number of pituitary acidophils. A decrease in TSH reserve and an alteration in TRH response have been described (Azizi et al. 1974), but whether the disturbance takes place in the pituitary or whether it affects the development of the hypothalamo-pituitary system is yet to be established.

Hervás et al. (1975) studied the effects of thyroid deprivation and restitution on growth hormone economy in adult rats. They concluded that the increase in pituitary GH content of thyroidectomized animals produced by exogenous thyroxine treatment was such a quick and sensitive effect that it could be a useful parameter to measure the biological effect of thyroid hormone in vivo.

We thought it would be interesting to check whether adult neo-T₄ rats still have low pituitary GH levels, as these have only been measured in young animals, and, if so, to try to find out if the persistent, irreversible alteration in pituitary GH content was due to a possible slight hypothyroidism or to perturbances in the development of the hypo-
thalamo-pituitary GH complex. With this aim, high exogenous T₄ doses were administered to neo-T₄ animals to see if they increased pituitary GH levels, as has been described for hypothyroid animals. Experiments were also carried out to see if neo-T₄ animals deprived of circulating T₄ by thyroidectomy (and thus definitely hypothyroid), react in the same way as control thyroidectomized animals, by increasing pituitary GH content in response to exogenous T₄ doses.

Material and Methods

**Animals**

Wistar rats were used throughout the study. They were fed the normal stock diet containing 1–2 µg iodine/g.
The day of birth of the pups was designated day 0 and day 1 was 24 h later. On the day of birth the number of pups was adjusted to 8 per mother.

**Treatments**

L-thyroxine sodium pentahydrate purchased from Sigma Chemicals Co. (St. Louis, MO) was dissolved at a concentration of 30 µg/0.1 ml of 0.9% NaCl (saline) containing diluted NaOH. Either 0.1 ml of this solution or an equal volume of the slightly alkaline saline were injected under the skin of the neck using a short 26 gauge needle. These injections were started on days 1 or 3, and continued in each case for 5 consecutive days, to induce the neo-T₄ syndrome. Rats receiving saline during the same period were used as controls.

**Experiment A**

A population of neo-T₄ and control rats, injected from the third day of life, were allowed to grow until day 70 and were then divided into two groups receiving the following treatments: a) 1.7 µg T₄/100 g body weight in 3 days, by ip injection; b) an equivalent injection of saline. Rats were sacrificed at 73 days of age by ether inhalation. Pituitary GH content was determined.

**Experiment B**

Neo-T₄ and control rats, injected from the first day of life, were divided into three groups on day 70, receiving the following treatments: (a) none; (b) 2.5 µg T₄/100 g body weight during 8 days, by ip injection; (c) an equivalent injection of saline. Rats were sacrificed by decapitation at 78 days of age. Pituitary GH content was determined.

**Experiment C-I**

Neo-T₄ and control rats, injected from the first day of life, were divided into three groups on day 42, receiving the following treatments: (a) none, (b) 5 µg T₄/100 g body weight by ip injection during 3 days; (c) the equivalent injection of saline. Rats were sacrificed by decapitation at 45 days of age. Pituitary GH content was determined. Both in this experiment and in C-II, 42 day old rats were used instead of 70 day old, since pituitary GH levels were just as low at both ages and the length of the experiments could thus be shortened.

**Experiment C-II**

This was similar to experiment C-I, groups (a) and (b), except that neo-T₄ and control rats had been thyroidectomized on day 25.

| Table 1. | Body, brain and pituitary weights and pituitary GH content of rats at 45 or 78 days of age, treated with saline (0.1 ml) or T₄ (30 µg T₄/0.1 ml) starting injection at day 1 of age. Group (a) Experiments B, C-I. |
| --- | --- | --- | --- | --- | --- |
| Beginning of treatment | Days of life | Treatment | Number of rats | Body weight (g) | Brain weight (g) | Pituitary weight (g) | µg (GH)* pituitary |
| 1 | 45 | Saline | 10 | 136 ± 25 | 1.09 ± 0.08 | 0.0041 ± 0.0014 | 600 ± 50 |
| | | Neo-T₄ | 10 | 98 ± 11 | 0.998 ± 0.11 | 0.0048 ± 0.0016 | 220 ± 30 |
| | | | ××× | × | ×× | ××× |
| 1 | 78 | Saline | 8 | 213 ± 15 | 1.23 ± 0.06 | 0.0065 ± 0.001 | 630 ± 80 |
| | | Neo-T₄ | 8 | 174 ± 34 | 1.11 ± 0.07 | 0.0076 ± 0.008 | 321 ± 141 |
| | | | × | ×× | × | ×× |

*The GH contents given are referred to one pituitary gland and expressed in µg of the NIAMDD Rat GH-RP-1 preparations, mean values ± sd. × = P < 0.05, ×× = P < 0.01, ××× = P < 0.001.
Pituitary GH content* in 73 or 78 day old neo-T4 rats treated with 1.75 µg T4/100 g body weight (3 doses starting on day 70) or 2.5 µg T4/100 g body weight (8 doses starting on day 70). Experiments A and B.

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<th>Sacrificed</th>
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<td>3rd day</td>
<td>saline</td>
<td>(ether)</td>
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<td>Neo-T4</td>
<td>954 ± 46 (8)</td>
<td>n.s.</td>
<td>754 ± 36 (5)**</td>
<td>73 days of life</td>
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<td>Saline</td>
<td>1061 ± 76 (8)</td>
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<td>878 ± 194 (5)</td>
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<td>1st day</td>
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<td>Neo-T4</td>
<td>295 ± 95 (8)</td>
<td>n.s.</td>
<td>378 ± 103 (8)</td>
<td>78 days of life</td>
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<td>Saline</td>
<td>586 ± 106 (10)</td>
<td>n.s.</td>
<td>630 ± 80 (10)</td>
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Sacrificed = number of rats, * = GH expressed as in Table 1, ** = rats sacrificed by ether inhalation.

Analytical procedures

Plasma PBI and thyroid I content were determined by modifications of the semiautomated procedure described by Benotti & Benotti (1963).

Pituitary and plasma GH content was determined by a specific radioimmunoassay (RIA) for rat GH, using reagents generously supplied by Dr. A. Parlow from the Rat Pituitary Agency of the National Institutes for Arthritis, Metabolic and Digestive Diseases (NIAMDD) at the National Institutes of Health (NIH), Bethesda, Md. The more sensitive and rapid procedure previously described (Hervás & Morreale de Escobar 1974) was used. Pituitary samples were assayed at 2 or more dilutions, samples from one experiment being assayed simultaneously.

Statistics

Mean values and standard deviations of the means are given. These and the significance of differences between mean values were calculated as outlined by Snedecor & Cochran (1956).

PBI in adult neo-T4 and control rats was 0.049 ± 0.02 µg I/ml plasma and 0.056 ± 0.005 µg I/ml plasma, respectively.

Table 2 shows the results of experiments A and B, groups (b) and (c). From experiment A it can be concluded that 3 consecutive daily injections of 1.75 µg T4/100 g body weight starting on day 70 do not change the pituitary GH content of control or neo-T4 rats treated from the third day of life. The known effect of ether in increasing pituitary GH levels can be observed in this experiment, so that all other rats were sacrificed by decapitation. The results of experiment B (b) and (c), show that 8 consecutive daily injections of 2.5 µg T4/100 g body weight, starting on day 70, did not change the pituitary GH content of control or neo-T4 rats treated from the first day of life.

In experiments C-I and C-II, rats were sacrificed at 45 days because, as can be seen in Table 1, adult pituitary GH levels had already been reached at this age in both neo-T4 and control rats and experiments could thus be shortened. In experiments B, C-I and C-II, rats were treated with high thyroxine doses (30 µg/5 doses) starting on the first day of life, since this makes the neo-T4 syndrome more irreversible. However, this also caused a high mortality so that larger numbers of rats had to be used in order to have enough survivors. In these experiments rats were sacrificed by decapitation. Fig. 1 depicts the results of experiments C-I, groups (b) and (c) and C-II. T4 injection to 42 day
old neo-T₄ and control rats lowered pituitary GH content, while the same treatment in rats thyroidectomized at 25 days of age produced an increase in pituitary GH content in both neo-T₄ and control rats.

PBI in these experiments was 0.024 ± 0.006 µg I/ml plasma for thyroidectomized neo-T₄ rats and 0.038 ± 0.005 µg I/ml plasma for non-thyroidectomized neo-T₄ rats, P < 0.01. Saline-injected control rats had a PBI of 0.047 ± 0.004 µg I/ml plasma, while in thyroidectomized saline-injected rats, it was 0.016 ± 0.005, P < 0.001. PBI was also smaller in neo-T₄ non-thyroidectomized rats than in saline intact controls, P < 0.01.

Discussion

Rats used in these experiments clearly showed the neo-T₄ syndrome in adult life, as demonstrated by a decrease in body and organ weights and of pituitary GH levels (Table 1). The pituitary GH content in adult neo-T₄ rats was always lower than in controls, corroborating the importance and irreversibility of this intervention. Moreover, it is interesting to observe that the decrease in pituitary GH in neo-T₄ animals is a more permanent change than the decrease in pituitary TSH (Pascual-Leone et al., unpublished). Thus, it seems of interest to find the cause of the low pituitary GH content in neo-T₄ rats in order to understand the disturbances produced by high doses of T₄ during immature stages of the central nervous system (CNS).

The low pituitary GH content found in the neo-T₄ syndrome can be explained in part by the studies of Eayrs & Holmes (1964) who observed a decrease in the number of pituitary acidophils in this syndrome. On the other hand, a similar decrease is always found in hypothyroid conditions in the rat, both in the adult and in the perinatal periods (Kikuyama et al. 1974; Coiro et al. 1979). However, hypothyroid rats manifest an increase in pituitary GH when they receive T₄ doses. The results in Table 2 indicate that exogenous physiological doses of T₄ (1.7 µg/100 g body weight) or larger doses (2.5 µg/100 g body weight) and longer treatments did not alter pituitary GH content. Experiment C-I (groups (b) and (c)) was made to confirm the previous results by giving neo-T₄ adult rats and controls a high dose of T₄ (5 µg/100 g body weight) for a period of 3 days which seemed adequate to produce maximal increase in pituitary GH content in normal thyroidectomized adult rats (Hervás et al. 1975). Fig. 1 shows that in the control rats an injection of T₄ decreased GH content and when thyroidectomized control rats received T₄, the expected large increase in GH was seen. Neo-T₄ rats responded with a similar decrease of GH content when thyroxine was administered. Thyroidectomized neo-T₄ rats increased their GH pituitary content with an injection of T₄ in a similar way to thyroidectomized controls. Thus, the fact that neo-T₄ rats, whose PBI is slightly lower than in controls, do not react to T₄ treatment in the same way as thyroidectomized animals, which are truly hypothyroid, indicates that the low pituitary GH content of neo-T₄ rats can not be explained by the slightly low circulating T₄ levels. These results also confirm that the hypothyroid state of neo-T₄ rats is not very severe.

These studies suggest that the alterations in pituitary GH content of neo-T₄ rats are not a consequence of the relative hypothyroidism of the animals but rather of a possible impairment of development of pituitary GH function. This impair-
ment could be related to the major disturbance of carbohydrate metabolism of neo-T₄ rats in the neonatal period, with low liver glycogen, blood glucose and insulin levels, which implies a lack of energy substrates for the neonatal brain (Escrivá & Pascual-Leone 1981; Dickerman et al. 1969). On the other hand these alterations in pituitary GH content could be a consequence of treatment with thyroid hormones, since chronic neonatal hyperthyroidism, though produced by much smaller doses of T₄, has been shown to alter the developmental pattern of serum GH (Poland et al. 1979).

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References


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