The influence of growth hormone and thyroxine on iodothyronine deiodinase activity in the liver, kidney and brown adipose tissue in hypophysectomized rats

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Abstract. The effects of GH and T₄ substitution on peripheral iodothyronine deiodinase activity in the liver, kidney and brown adipose tissue of hypophysectomized rats were investigated. Animals were treated with GH (140 μg hGH/day), T₄ (3 μg/day), GH plus T₄ (same doses), or saline. Rats were killed 0, 4, 7 or 11 days after treatment was started. Non-hypophysectomized, age-matched rats were killed after 0 and 11 days and served as controls. GH plus T₄ restored body weight gain to normal, whereas GH alone and T₄ alone did not. Tissue deiodinase activity and T₃ concentrations were severely depressed in the hypophysectomized rats compared with non-hypophysectomized controls (to less than 10%). GH substitution in hypophysectomized rats led to a slight but significant elevation in tissue iodothyronine deiodinase activity in the liver and kidney, without concomitant increases in T₃. Deiodinase activity in brown adipose tissue did not differ from that in saline-treated controls. T₄ administration normalized deiodinase activity and tissue T₃ content in all the evaluated tissues. GH plus T₄ resulted in a lesser increase in deiodinase activity than T₄ alone in the liver and kidney (p<0.01 at day 11), whereas no significant difference was observed in brown adipose tissue. In conclusion, GH stimulates iodothyronine deiodinase activity of the liver and kidney in hypophysectomized rats. Moreover, when GH is administered together with T₄, the T₄-stimulated enzyme activity in the liver and kidney is downregulated, suggesting that GH attenuates (or modulates) the T₄ effect on this specific enzyme activity.

Previous investigations on the direct effect of GH on the peripheral tissue thyronine deiodinase have been confounded by the influence of pituitary hormones. Treatment of man with GH has been shown to depress the TSH response to TRH stimulation, sometimes leading to a state of hypothyroidism (1-4). On the other hand, according to several authors, the observed changes in serum thyroid hormones indicate a GH- or IGF-I mediated elevation of peripheral thyronine deiodinase (1,5-9).

In the present experiment we used hypophysectomized rats in order to abolish the GH-mediated effects on TSH and other pituitary hormones. This design made it possible to study directly the peripheral tissue effect of GH and T₄ substitution on thyronine deiodinase.

Material and Methods

Animals
Male 3-week-old Wistar rats (from Charles River Wiga GmbH, Sulzfeld, FRG) were acclimatized for 1 week in a 12-h light, 12-h dark cycle, controlled temperature (22±2°C) and humidity (55±10%). The animals had free access to food (Altromin, Lage, FRG) and tap water. Hypophysectomy was performed under anesthesia with ip injections of amylene hydrate (400-500 mg/kg) and metohexical (40-50 mg/kg). After hypophysectomy, the rats were treated for three days with two daily injections of buprenorphine. Weighing was performed before and 14 days after hypophysectomy. Only animals with a weight loss <4 g and a weight gain <10 g were included.
in the experiment. Normal control animals (N=12) were age-matched with those having undergone hypophysectomy. At the beginning of the treatment period, the hypophysectomized animals weighed between 90 and 95 g, whereas normal control animals weighed between 170 and 185 g. Fourteen days after hypophysectomy (Hx) the rats were randomly divided into four groups: Hx+GH (N=18), Hx+T4 (N=18), Hx+GH+T4 (N=18), and Hx+NaCl (N=24), respectively. The first group received twice daily a sc injection of 0.5 ml human growth hormone (GH) (Norditropin®, 140 mg/l), the second group received twice daily a sc injection of 0.5 ml L-thyroxine (T4) (3 mg/l, dissolved in 0.9% NaCl), the third group received twice daily the same doses of GH plus T4, as those in the two first groups. Doses were given to reach physiological levels (10,11). The fourth (placebo) group received once daily an injection of 0.5 ml 0.9% NaCl. Treatment of hypophysectomized rats was continued for up to 11 days. Non-hypophysectomized controls were killed at day 0 and day 11. Weighing was performed daily, and 6 rats from each group were killed at day 0 (Hx+NaCl only), 4, 7 and 11 by exsanguination using the same anesthetic procedure as during hypophysectomy. The liver, kidney and brown adipose tissue were weighed, immediately frozen in liquid nitrogen and stored at −60°C until assays. Blood was collected for serum and stored at −20°C until further analysis.

Determinations of tissue thyronine deiodinase
5-thyronine deiodinase was determined according to the method of Boye et al. (12), modified as previously described (13). In brief, 0.5 g tissue was washed free of blood and homogenized twice in 5 volumes of ice-cold buffer (25 mmol/ TRIS-HCl, 3 mmol/L dithiothreitol, 1 mmol/L EDTA, pH 7.2). After homogenization and centrifugation, 50 µl of the supernatant (0.25-0.5 mg protein) were incubated (1 h at 37°C) with 100 µl substrate 125I-rT3 (specific activity 5500 cpm/3 pg, 50% counting efficiency), prepared according to Laurberg (14). Each incubate was applied to 1-ml Sephadex columns (G-25 Fine, Pharma¬cia). All analyses were carried out in triplicate. 125I derived by deiodinating activity was eluted by 0.5 and 1.5 ml 50 mmol/l phosphate buffer (pH 7.5). The last 1.5 ml eluate was collected and counted for 5 min (LKB-Wallac 1277 Gammamaster). The liberated 125I was used as a relative index of thyronine deiodinase enzyme activity.

Serum T3, serum T4 and tissue T3
These variables were determined in triplicate on the undiluted serum and homogenate by RIA (15), with the modification that PEG separation was used instead of wic-chromatography. We compared the recovery of tissue T3 by our method with the one described by Silva & Larsen (16), using butanol/CH3COOH (9:1) extraction. Results showed that there was no significant differences in T3 recovery (data not shown). Protein content was measured by the Bio-Rad kit, with bovine serum albu¬min as standard.

Calculations and statistics
Results are given as mean values ±SEM unless otherwise noted. Each value represents experiments performed on 6 different animals. Differences between group means were assessed by the two-tailed t-test for non-paired obser¬vations and by the Kruskal-Wallis test. In cases of vari¬ance inhomogeneity the Mann-Whitney test was used. Level of significance: *p<0.05.

Results
Body, liver and kidney weights during the different treatment regimens are depicted in Table 1. Hx+GH rats had a body weight gain about half of

Table 1
Changes in body liver and kidney weight in hypophysectomized rats after treatment with GH, T4, or GH+T4. Body, liver and kidney weights in hypophysectomized (Hx) rats after 4, 7 and 11 days on different treatment regimens. Controls (non-hypophysectomized) were killed on day 0 and 11 (N=6 on each day). Placebo group: Hx+NaCl; GH only: Hx+GH; T4 only: Hx+T4, GH plus T4: Hx+GH+T4. Δ%: Weight gain from day 0 to day 11. Statistical comparisons are made between Hx+NaCl at day 0 and the other treated groups. Values are given as mean ± SD. Level of significance: *p<0.05.
that seen in normal controls (28 vs 46%), liver weight gain was 24 vs 34%, whereas kidney weight gain did not differ in the Hx+GH and control group (27 vs 28%) after 11 days. Hx+T_4 rats differed only slightly from Hx+NaCl rats during the 11 days as to body and liver weight gain (−4 and −8% vs −12 and −7% respectively), whereas kidney weight gain was considerable in the Hx+T_4 group compared with the Hx+NaCl rats (19 vs −17%). In the Hx+GH+T_4 rats, weight gain increased in a more than additive way (54, 70 and 73%, respectively), which was also above control values (46, 35 and 28%).

Serum T_4 and T_3 are shown in Fig. 1a and 1b. Hypophysectomy resulted in a depressed thyroid state, T_4 being <25% of that seen in the normal rats (p<0.001), and T_3 <5%. Hx+GH rats did not differ in serum T_4 or T_3 from Hx+NaCl, whereas serum T_4 rose above normal in Hx+T_4 rats at day 4 (p<0.05), then normalized. Hx+GH+T_4 rats showed a greater increase in serum T_4 than those receiving T_4 alone (p<0.05 at day 4, 7 and 11, when comparing Hx+GH+T_4). In contrast, no difference between these two groups were observed in the serum T_3 levels.

5’thyronine deiodinase and T_3 concentrations in the liver are shown in Fig. 2a and 2b. At day 7 and 11, Hx+GH rats showed an elevation of 5’thyronine deiodinase by 109% (p<0.001) and 55% (p<0.005) above Hx+NaCl rats, although still far from being normalized (less than 40% of control levels, p<0.05). Hx+T_4 rats showed normalization of 5’thyronine deiodinase, whereas in the Hx+GH+T_4 group, a smaller elevation of thyronine deiodinase was observed (p<0.02 at day 7 and p<0.01 at day 11, when comparing Hx+T_4 and Hx+GH+T_4). Changes in liver T_3 (Fig. 2b) reflected the changes in thyronine deiodinase. After an initial steep rise, T_3 reached control levels at day 7 and onwards in the Hx+T_4 rats and the Hx+GH+T_4 group.

Much the same pattern was observed in the kidney (Fig. 3a and 3b). Hx+GH resulted in an elevation of thyronine deiodinase after 7 (52%, p<0.01) and 11 days (62%, p<0.01) when compared with Hx+NaCl rats, whereas Hx+GH+T_4

![Fig. 1.](image)

Serum T_4 (Fig. 1a) and T_3 (Fig. 1b) in hypophysectomized (Hx) rats. Day 0 represents the values obtained in rats 14 days after hypophysectomy. Subgroups of rats were killed 0 (N=6), 4 (N=24), 7 (N=24) and 11 (N=24) days after treatment with Hx+GH ( ), Hx+T_4 ( ), Hx+GH+T_4 ( ) and Hx+NaCl ( , placebo). Normal, non-hypophysectomized rats ( ) were killed after 0 (N=6) and 11 days (N=6) as controls. Each point represents separate experiments performed on 6 rats. Values are given as mean ± SEM. Statistical comparisons are made only between Hx+T_4 and Hx+GH+T_4 rats. Level of significance: P<0.05.
rats showed a more discrete increase in enzyme activity (367 and 461%) than those receiving T$_4$ alone (471 and 634%, after 7 and 11 days, respectively, p<0.02 and 0.002). The pattern of changes in kidney T$_3$ was the same as those observed in the liver, showing no difference between Hx+NaCl and Hx+GH.

In brown adipose tissue (Fig. 4a and 4b), as in the liver and kidney, thyronine deiodinase and T$_3$ concentrations increased in the Hx+T$_4$ and Hx+GH+T$_4$ rats, returning to control levels by day 11. However, in contrast to in the liver and kidney, brown adipose tissue thyronine deiodinase in Hx+GH rats seemed to be lower than in the Hx+NaCl group, although the difference is not statistically significant. There were no significant differences between Hx+T$_4$ and Hx+GH+T$_4$ concerning thyronine deiodinase, in contrast to in the other tissues.

Discussion

The new finding of our study is that GH seems to exert, directly or indirectly, a modulating effect on the T$_4$-stimulated increase in peripheral thyronine deiodinase of hypophysectomized rats. When administered to hypophysectomized rats in replacement doses, we found that GH alone was able to increase liver and kidney thyronine deiodinase about 1.6 times above the initial low levels. This finding is quite in accordance with other investigators (4,6,7). The fact that GH alone was not able to increase thyronine deiodinase and tissue T$_3$ up to normal levels may partly be due to the low levels of tissue substrate T$_4$ present in these hypophysectomized animals. When T$_4$ was administered alone, thyronine deiodinase increased about 6.5 times, whereas when GH and T$_4$ were administered together, thyronine deiodinase increased significantly less (about 5.1 times). It would seem reasonable to expect an additive or permissive effect of GH on the T$_4$-mediated thyronine deiodinase action, but according to our findings, GH rather exerts a depressing or modulating effect on thyronine deiodinase when substrate T$_4$ is present in sufficient amounts. The experimental conditions in this study may play a role, and it should be noted that Hx+GH+T$_4$ rats did not differ significantly from non-hypophysectomized control animals concerning thyroid hormone metabolism, whereas the

![Fig. 2.](image-url)

Liver iodothyronine deiodinase (5'D) (Fig. 2a) and T$_3$ (Fig. 2b) in hypophysectomized (Hx) rats. For further details, see legend to Fig. 1. Statistical comparisons are made between Hx+T$_4$ and Hx+GH+T$_4$ or between Hx+GH and Hx+NaCl (*). Mean ± SEM, level of significance: p<0.05).
Fig. 3.
Kidney iodothyronine deiodinase (5'D) (Fig. 3a) and T₃ (Fig. 3b) in hypophysectomized (Hx) rats. For further details, see legend to Fig. 1. Statistical comparisons are made between Hx+GH+T₄ and Hx+T₄ or between Hx+GH and Hx+NaCl (*). Mean ± sem, level of significance: p<0.05.

Fig. 4.
Brown adipose tissue iodothyronine deiodinase (5'D) (Fig. 4a) and T₃ (Fig. 4b) in hypophysectomized (Hx) rats. For further details, see legend to Fig. 1. Statistical testing was only performed between Hx+GH+T₄ and Hx+T₄ (*). Mean ± sem, level of significance: p<0.05.
$T_4$-treated ones tended to rise towards hyperthyroid levels within the observation period. Further, the manipulations with $T_4$ and/or GH may have influenced the non-deiodinative pathways in the liver, and in this way masking some effects on the thyronine deiodinase activity.

The fact that GH influences thyroid hormone metabolism at different levels makes it difficult to establish what is a direct and what is an indirect effect of GH on the peripheral tissue deiodination. By using hypophysectomized rats, we avoided the influence of some regulatory hormones on peripheral thyronine deiodinase. Only a few studies have been reported, where thyronine deiodinase was measured directly in tissues and related to the changes in serum thyroid hormones, but to our knowledge, never in hypophysectomized animals. De Luze & Leloup (6) determined liver thyronine deiodinase after 10 $\mu$g of tilapia GH in the hormonally intact eel. They found that enzyme activity was increased 2-3 times above control level after 24 and 48 hours. In addition, serum $T_3$ was elevated and $T_4$ depressed, reflecting the increased deiodination. Kuhn et al. (7) investigated liver thyronine deiodinase in normal fed and fasted chicken. One hour after injecting 100 $\mu$g ovine GH iv, they found that thyronine deiodinase was elevated, serum $T_3$ elevated and $T_4$ depressed, but only in the fasted animals. Experimental conditions differ highly from ours, as they used normal, fasted chicken with intact pituitary function, a clearly supraphysiological dose, and an observation time of only 2 hours. Sato et al. (1) treated 8 idiopathic hypotuitary children and adolescents with hGH for 12 months, of whom three had signs of central hypothyroidism. They suggested that peripheral thyronine deiodinase may be elevated during treatment with GH, as they found serum levels of $T_3$ elevated and $T_4$ depressed. Jorgensen et al. (5) followed 22 GH-deficient adults in a double blind, placebo-controlled cross-over study. Half of the patients received $T_4$ replacement therapy as well as GH throughout the study period (of each four months). Serum $T_4$ was found to be depressed, r$T_3$ and $T_3$ elevated, indicating that thyronine deiodinase was elevated during GH therapy in both groups. They suggested that the mechanism may be mediated via an increased IGF-I level, as serum IGF-I increased during GH treatment from a clearly subnormal level to reference levels. Everts et al. (17), on the other hand, measured IGF-I levels in hypophysectomized rats receiving $T_4$, GH or GH plus $T_4$ substitution. They showed that IGF-I did not differ in serum of $T_4$-treated rats and those receiving placebo, whereas rats receiving GH or GH plus $T_4$ had normalized IGF-I levels after 11 days. From their findings, it seems unlikely that the normalized thyronine deiodinase in the $T_4$ treated rats of our study should be mediated via changes in IGF-I levels.

In the present study, $T_4$ treatment as well as combined GH and $T_4$ administration normalized brown adipose tissue thyronine deiodinase after 7 days. This is in opposition to what was observed by Silva & Larsen (18). They found that the specific thyronine deiodinase (Type II thyronine deiodinase) (insensitive to propylthiouracil, PTU) in this tissue is strongly depressed by, among other factors, $T_4$ and GH. The discrepancy may be due to different experimental designs, as their rats were supplied with glucose in their drinking water after hypophysectomy, and further, the animals were treated with bGH where ours were treated with hGH. It seems rather puzzling that in the present study, $T_4$ alone and GH+$T_4$ treatment normalized brown adipose tissue thyronine deiodinase, whereas GH alone seemed rather to depress enzyme activity (although not statistically significant), as would be expected. We did not measure the thyronine deiodinase type II by the addition of PTU, so it may be difficult to rule out the contribution of thyronine deiodinase type II to the results.

After 11 days of $T_4$ substitution, no body weight increase was observed in hypophysectomized rats. This is in agreement with findings of Bowen et al. (19) and Glasscock & Nicoll (20). On the other hand, Bowen found that GH treatment alone did not increase weight in thyroidectomized chickens, whereas when $T_3$ was added, the weight gain normalized. In our study we found a marked weight gain after GH treatment alone, but in contrast to the experiment of Bowen, our rats still had a remnant thyroid production which may explain the conflicting results. When GH and $T_4$ were administered together, weight gains more than normalized, probably owing to a "catch-up" growth spurt, indicating that even though $T_4$ did not by itself induce weight gain (except in the kidney, Table I), the hormone elicits a permissive action on the GH effect, as suggested by others (19,21).

In conclusion, we find that GH by itself exerts some effect on peripheral thyronine deiodinase in hypophysectomized rats, probably limited by the

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small amounts of substrate T₄. When administered together with T₄, GH exerts a moderately depressing effect on T₄-stimulated thyronine deiodinase, indicating some kind of negative interaction between the two hormones. In man and various hormonally intact animal species, evidence has been brought that GH may stimulate peripheral thyronine deiodinase, both acutely and after chronic treatment. The conflicting results may be due to different experimental designs, including the way of GH administration (22). More speculatively, the stimulated thyronine deiodinase described in other reports may have been due to the influence of pituitary hormones, most likely TSH.

Acknowledgments

The study was supported by The Danish Heart Foundation. We are indebted to Karen Mathiassen for excellent laboratory assistance.

References


Received November 22nd, 1990.
Accepted April 8th, 1991.

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