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Studies on the thyroid in transgenic mice expressing the genes for human and bovine growth hormone

A. Mayerhofer[a], S. Easterly[b], A. G. Amador[b], J. Gher[b], A. Bartke[b], J. Yun[c] and T. E. Wagner[c]

[a] Abteilung Anatomie und Zellbiologie, Universität Ulm, D-7900 Ulm (Federal Republic of Germany), [b] Southern Illinois University, Department of Physiology, School of Medicine, Carbondale (Illinois 62901-6512, USA), and [c] Ohio University, Edison Center, Athens (Ohio 45701, USA)
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Summary. The thyroid glands of transgenic mice (TM) expressing the genes for human (*h*) and bovine (*b*) growth hormone (GH) were studied. The percentages of larger follicles in *h*GH TM of either sex were significantly greater than in the corresponding normal littermates, and follicles ranging up to 350 µm in diameter were present in male *h*GH TM. In contrast, thyroid follicles were only slightly enlarged in male *b*GH TM, and were unchanged in female *b*GH TM. The serum concentrations of T4 were significantly decreased in male *b*GH TM and not altered in the other groups. Serum concentrations of T3 were slightly, but significantly increased in female *h*GH TM and female *b*GH TM, but were unaffected in male TM of either type. Since the principal difference between these foreign GHs in rodents is the additional lactogenic activity of human GH, these results may indicate that the effects of prolactin can influence the development of the thyroid.
Keywords. Thyroid; transgenic mouse; human growth hormone; thyroid hormones.

Transgenic mice (*Mus musculus*) expressing the genes for the human (*h*) or bovine (*b*) growth hormones (GH) fused with the mouse methallothionein (mMT) promoter show marked stimulation of body growth. These transgenic mice (TM) synthesize the foreign GHs at several ectopic sites, especially in liver and kidney, and have measurable amounts of heterologous GH in the peripheral circulation[1-3]. The incorporation of the GH genes in TM is permanent and is passed to their progeny as a dominant trait.

Underdeveloped thyroid glands in mutant dwarf mice with inherited lack of prolactin and GH suggest a role for GH and possibly for prolactin in the development of the thyroid[4]. Since in rodents *b*GH shows purely somatotrophic effects, whereas *h*GH has, in addition, a prolactin-like action[5,6], TM expressing the genes for these hormones offer the opportunity to examine further the role of GH and prolactin in the development of the thyroid.

Materials and methods

Transgenic mice. Transgenic mice of both types were originally produced by microinjection of gene constructs (mMT/GH) into pronuclei of fertilized eggs, as described previously[3,7]. All TM used for the present study were derived from one fertile *h*GM TM, male and one fertile *b*GH TM male produced in the original experiments. These founder males and their male transgenic progeny were mated with B6C3F1 hybrid females (Jackson Laboratory, Bar Harbor, ME). Their offspring, normal mice

and their transgenic littermates, were housed together under controlled light (12 h light: 12 h dark) and temperature (23 ± 2 °C) conditions, with free access to food and water. No additional supplement of heavy metals to food or water was given. At the age of 3–4 months, mice were weighed and killed by exsanguination under ether anesthesia.

Histology. Segments of larynx with the thyroid glands were removed in toto and immersed in Bouin's fixative for 12 h. The tissue was then embedded in paraffin and 4 µm sections were cut and stained with hematoxylin-eosin (H.-E.) and periodic acid Schiff reagent (PAS). Sections through the central region of the thyroids, with at least 50 follicles/section, were selected for determination of follicular diameters. With an ocular micrometer, calibrated with a stage micrometer, the mean of the largest and the smallest diameter (from basal membrane to basal membrane) was determined for each follicle per section.

Measurements of hormones and statistics. Thyroid hormones (thyroxine = T4 and 3,5,3′-triiodothyronine = T3) in serum were measured by RIA, using commercial kits (Pantex, Santa Monica, CA). Dilution curves from a mouse pool were found to be parallel to the standard curves of the kits (for T4: standard curve: slope = −2.91, y-intercept = 3.04, r = −99.11%; mouse pool: slope = −2.01, y-intercept = 3.03, r = −100%; for T3 standard curve: slope = 2.46, y-intercept = 6.34, r = −98.83; mouse pool: slope = −2.50, y-intercept = 6.43, r = −100%). The sensitivity of the assays was 0.894 nmol/l for T4 and 0.038 nmol/l for T3. Samples from *h*GH and *b*GH (each with their normal littermates) were measured in different assays.

All values are reported as means ± SEM. Data were compared using Student's t-test.

Results

Both types of TM showed the expected increase in body weight (table).

At the microscopic level, follicular lumina of the thyroid of TM and normal mice of either sex contained colloid that stained positive for PAS. Follicular cells in all groups ranged from approximately 5–12.5 µm in diameter, with high epithelium being associated with smaller follicles and flat epithelium being present in large follicles. Follicular cells contained PAS-positive material (fig. 1 a, b, c). In general, the gross architecture of the thyroids of TM of either type was normal, except for conspicuous fat cells in the stroma of *h*GH TM and the variability in the size of the follicles in these TM. After arbitrarily dividing the follicles into groups according to their mean diameter (< 50 µm; 51–100 µm; 101–150 µm; 151–200 µm; 201–250 µm; 251–300 µm; 301–350 µm) the percent-

Body weights (BW; g) and hormone values (nmol/l serum) of transgenic mice (TM)[1]

	BW	T4	T3
Male mM1-bovine GH-TM	48.3 ± 2.2*	3.55 ± 0.32*	0.32 ± 0.01
Male control littermate	33.8 ± 1.2	4.78 ± 0.15	0.31 ± 0.03
Female mM1-bovine GH-TM	37.0 ± 1.2*	3.85 ± 0.58	0.37 ± 0.05
Female control littermates	24.3 ± 0.7	3.77 ± 0.73	0.23 ± 0.03*
Male mM1-human GH-TM	58.7 ± 2.6*	5.95 ± 0.29	0.23 ± 0.01
Male control littermates	37.9 ± 0.8	5.32 ± 0.59	0.29 ± 0.03
Female mM1-human GH-TM	50.3 ± 3.1*	4.75 ± 0.31	0.20 ± 0.01
Female control littermates	30.6 ± 1.4	5.24 ± 0.48	0.16 ± 0.01*

[1] (Values are means ± SEM; values with asterisks are significantly different from corresponding controls; $p < 0.05$).

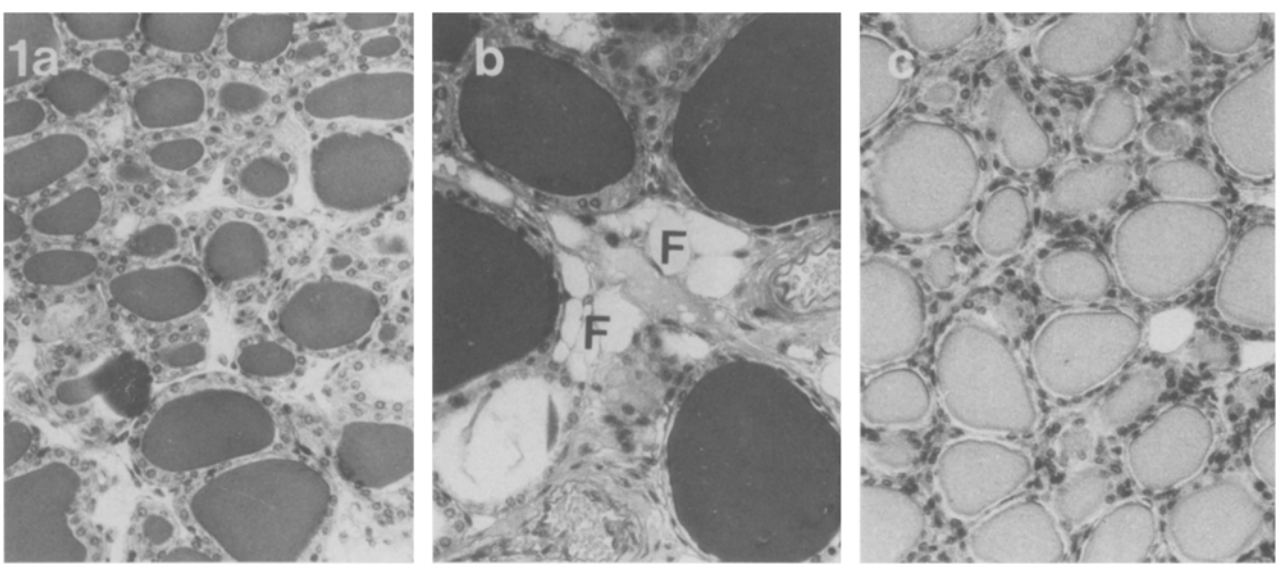

Figure 1. Paraffin-embedded sections of the thyroids of *a* a control mouse, *b* a human GH TM, and *c* a bovine GH TM; F: fat cells; PAS (*a, b*) and H.-E. (*c*).

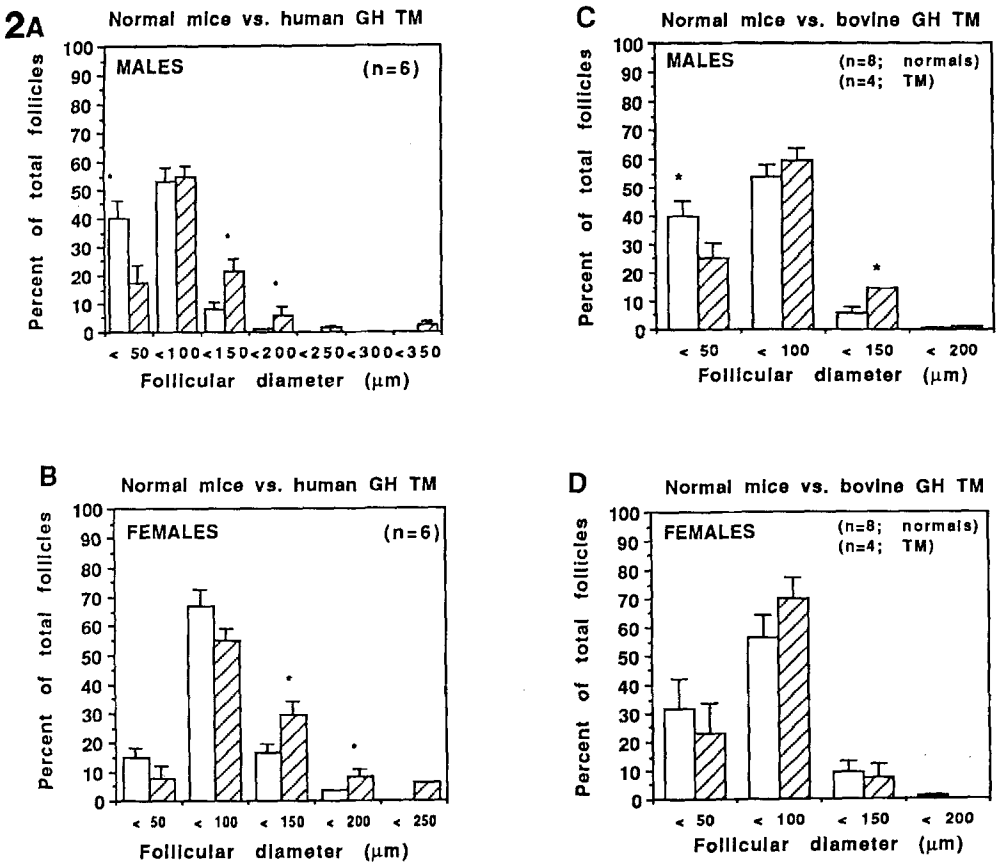

Figure 2. The numerical distribution of thyroid follicles of different diameters in control (white columns) and transgenic mice (TM; hatched columns), expressed as a percentage of total follicles [controls vs *A* human GH TM males; *B* human GH TM females; *C* bovine GH TM males; *D* bovine GH TM females]. Values are means ± SEM (number of animals per group are indicated in each graph). Values with different superscripts are significantly different ($p < 0.05$) from their corresponding controls.

ages of large follicles (101–150 μm and 151–200 μm) in female and male *h*GH TM were shown to be significantly higher than in normal littermates ($p < 0.05$). Follicles with larger diameters were present in *h*GH TM (fig. 2a, b), but absent in *b*GH TM, in which only males had increased percentages of follicles ranging from 100–150 μm, while females were not different from their normal controls (fig. 2c,d). Serum concentrations of T4 were decreased in male *b*GH TM but not altered in the other groups. Serum concentrations of T3 were slightly, but significantly increased in female *h*GH TM and female *b*GH TM, but unaffected in male TM of either type (table).

Discussion

The present results show that the expression of the *h*GH gene in a line of TM was associated with conspicuous increases in the average size of thyroid follicles, without an increase in follicular cell sizes. Comparable enlargements were not evident in the thyroids of *b*GH TM, although some changes in the follicular composition of male *b*GH TM were found. Both lines of TM that were used for the present study have elevated levels of the foreign growth hormones[9] (and Bartke and Buonomo, unpublished data). Since in cultured thyroid cells GH and its mediator, insulin-like growth factor-1 (IGF-1), can stimulate protein- and DNA-synthesis, a role for these substances in the growth of the thyroid has been suggested[8].

One may conclude that the observed changes in male *b*GH TM with increased plasma levels of GH (*b*GH levels in this line of TM averaged in a previous study 11.3 ± 0.9 [n = 9] vs 2.2 ± 0.4 ng/ml in control animals [n = 6]; Bartke and Buonomo, unpublished data) may therefore be caused solely by GH action. However, the main difference between the effects of *h* and *b*GH is the additional lactogenic action of *h*GH in rodents. It is therefore likely that the enlargement of the thyroid follicles in *h*GH TM is a result of combined prolactin-like action and increased levels of GH/IGF-1[2,9,10]. Alternatively, the results could be explained by possible insertional mutagenesis of the foreign GH genes, local (ectopic) GH gene expression or abnormal patterns of GH release. It is of interest that the effects of *h*GH gene expression on the average size of thyroid follicles are opposite to changes in mutant dwarf mice with inherited lack of GH and prolactin, which exhibit marked underdevelopment of the thyroid[4]. In the light of the data obtained from these dwarf mice, the present results in *h*GH TM therefore indicate that prolactin can influence

the development of the thyroid. These effects presumably take place during fetal or early postnatal life and may include regulatory action on the organization of the structure of the thyroid.

In spite of the strikingly enlarged thyroid follicles in *h*GH TM, the presence of many normal sized follicles led us to expect the finding that the serum levels of T4, which is synthesized in the thyroid, were not elevated over levels found in normal mice. Together with the fact that TSH cells of the anterior pituitary in these mice are comparable in number and size to those of normal mice[11], one can therefore also assume normal TSH levels (in contrast, T4 was significantly decreased in male *b*GH TM). Thus, as suggested by their large diameters and their narrow follicular epithelium, most of the enlarged follicles of *h*GH TM are probably not actively secreting T4, but rather may serve as stores.

The level of circulating T3, the most potent metabolite of T4, was slightly but significantly increased although only in female TM of either kind. These changes are consistent in that we have also observed a significant increase of T3 in female *h*GH TM in a pilot study using plasma rather than serum (0.163 ± 0.032 vs 0.089 ± 0.028 nmol/l; $p < 0.025$). These findings suggest increased peripheral deiodination of T4 in female TM, an effect which could be due to GH action, because in the chick embryo, hepatic 5'monodeiodination activity is stimulated by GH[12]. However, the reason for the sexual dimorphism in T3 values observed in the present study remains unclear. The main contributors of T3 are kidney, brown fat and liver[13,14]. In TM, the liver and the kidney are also the main sites of expression of the foreign GH genes. Thus, one cannot rule out the possibility that locally produced foreign GHs could have direct or indirect influence on the deiodination process of T4 in these organs. The results of a pilot study, which showed increased T3 concentrations in the livers and kidneys of *h*GH TM compared with the values obtained in normal mice, support this assumption (T3: 8.2 ± 1.8 pg/mg of liver homogenate of female *h*GH TM vs 7.5 ± 1.8 pg/mg in control females; 3.30 ± 0.57 pg/mg of kidney homogenate of female *h*GH TM vs 2.24 ± 0.49 pg/mg in female controls; n = 6/group; means $\pm$ SEM; Mayerhofer, Amador, Bartke, unpublished data).

In summary, the TM expressing foreign GHs from fetal life appear to be unique models for the study of the effects of GH alone (*b*GH TM) and the effects of GH combined with prolactin (*h*GH TM) on the thyroid. Thus, the effects of *h*GH on both the follicular composition of the thyroid in the mouse and on the basal thyroid function, as judged by serum T4, differ from the effects of *b*GH. In the light of findings in mutant dwarf mice[4], the present results in GM TM may indicate a stimulatory role for prolactin in the structural development of the thyroid.

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