Thyrocyte-specific \( G_q/G_{11} \) deficiency impairs thyroid function and prevents goiter development

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Thyrocyte-specific Gq/G11 deficiency impairs thyroid function and prevents goiter development

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The function of the adult thyroid is regulated by thyroid-stimulating hormone (TSH), which acts through a G protein–coupled receptor. Overactivation of the TSH receptor results in hyperthyroidism and goiter. The Gq-mediated stimulation of adenylyl cyclase–dependent cAMP formation has been regarded as the principal intracellular signaling mechanism mediating the action of TSH. Here we show that the Gq/G11-mediated signaling pathway plays an unexpected and essential role in the regulation of thyroid function. Mice lacking the α subunits of Gq and G11 specifically in thyroid epithelial cells showed severely reduced iodine organization and thyroid hormone secretion in response to TSH, and many developed hypothyroidism within months after birth. In addition, thyrocyte-specific Gαq/Gαι-deficient mice lacked the normal proliferative thyroid response to TSH or goitrogenic diet, indicating an essential role of this pathway in the adaptive growth of the thyroid gland. Our data suggest that Gq/G11 and their downstream effectors are promising targets to interfere with increased thyroid function and growth.

Introduction
Thyroid hormone plays a central role in maintaining the basal level of metabolism in the body. It regulates O2 consumption as well as lipid and carbohydrate metabolism and is required for normal growth and maturation (1, 2). The primary regulator of thyroid gland growth and function in the adult organism is the thyroid-stimulating hormone (TSH). Lack of TSH or TSH action results in a reduced weight of the adult thyroid gland and almost hyperthyroidism and goiter (6).

TSH regulates thyroid function through a G protein–coupled receptor on thyrocytes (7–9). TSH receptor–dependent activation of the Gs/adenyl cyclase–mediated pathway has been suggested to account for most of the biological effects of TSH on thyroid cells, such as the stimulation of iodine uptake, hormone secretion, and proliferation (7). Consistent with this, thyroid glands of mice lacking the TSH receptor have defects in producing iodinated thyroglobulin, but the ability to take up iodine and to organize it can be restored by the adenylyl cyclase activator forskolin (4).

Nongoitrous hypothyroidism has also been observed in patients with one defective allele of the gene encoding Gαq (GNAS) and pseudohyoparathyroidism type 1a (10) as well as in at least one mouse model with Gαq deficiency (11). In addition, constitutive activation of the thyrocyte cAMP cascade in humans carrying activating somatic mutations of GNAS or in transgenic mice overexpressing the Gq-coupled adenosine A2 receptor, a constitutively active mutant of Gαq, or cholera toxin in thyroides causes hyperfunctioning thyroid adenomas (12–17).

In various species, including humans, TSH can also induce the Gq/G11-mediated stimulation of phospholipase C–β (PLC–β), leading to mobilization of intracellular Ca2+ ([Ca2+]i) by inositol 1,4,5-trisphosphate and formation of diacyl glycerol (18–20). However, the role of the Gq/G11-mediated signaling pathway in thyroid function is unclear. There is evidence that the constitutive activation of the Gq/G11/PLC–β pathway in thyrocytes of mice overexpressing an active mutant of the α1a adrenergic receptor further promotes malignant transformation of the thyroid gland (21), but it is unclear whether Gq/G11 mediate a growth-promoting effect under more physiological conditions.

In order to understand the role of Gq/G11-mediated signaling in thyroid follicular cells, we have generated mice lacking the α subunits of Gq/G11 selectively in thyrocytes. Because Gαq/Gαι-deficient mice die in utero (22), we used a floxed allele of the gene encoding Gαq (gnaq), which can be used to inactivate Gαq function in a Gαι-deficient background (23), using the Cre/loxP system. Our data indicate that the Gq/G11-mediated signaling pathway is dispensable for thyroid development but is required for TSH-induced thyroid hormone synthesis and release in the adult and that the lack of Gq/G11 leads to hypothyroidism. In addition, Gq/G11 deficiency prevented the development of goiter induced by blockade of thyroid function or TSH treatment.

Results
Generation of thyroid-specific Gαq/Gαι deficiency. In order to inactivate the Gq/G11-mediated signaling pathway in thyrocytes, we generated a transgenic mouse line expressing Cre recombinase under the control of the thyrocyte-specific thyroglobulin promoter using a P1-derived bacterial artificial chromosome (PAC) harboring the thyroglobulin gene (see Methods). After crossing with the Gq(ROSA)26Sor Cre reporter mouse line (24), 3 of the 4 tested transgenic founder
indication of altered thyroid histology or serum TSH and thyroid hormone levels in mice expressing Cre compared with wild-type littermates (Figure 1A and data not shown). However, Cre-mediated recombination was observed by P2. No significant difference in basal or TSH-induced cAMP levels was seen between thyrocytes prepared from Tc-Gαq/Gα11−/−KO mice and wild-type littermates at 1–2 months of age, indicating no significant alteration in the Gq-mediated signaling pathway in the absence of Gαq/Gα11 (Figure 1C). In contrast, thyrocytes from Tc-Gαq/Gα11−/−KO mice lacked a functional Gq/Gα11-mediated signaling pathway, as demonstrated by determination of [Ca2+] concentrations using the fluorescent calcium indicator Fluo-2. While in wild-type thyrocytes, TSH, ATP, and other stimuli acting via Gαq/G11-coupled receptors induced an increase in [Ca2+], (Figure 1D and data not shown), in thyrocytes from Tc-Gαq/Gα11−/−KO mice, only the Ca2+ ionophore ionomycin induced a response (Figure 1D).

Hypothyroidism in thyrocyte-specific Gαq/Gα11-deficient mice. The development of the thyroid gland in the absence of the Gq/G11-mediated signaling pathway was normal, as indicated by normal thyroid histology and normal thyroid hormone and TSH plasma levels during the first 2 months of age (Figures 2 and 3). Similarly, no change in the size or form of thyroid follicles was observed in mice up to 2 months of age (Figure 3 and data not shown). There was also no difference in body weight or reproductive performance in Tc-Gαq/Gα11−/−KO mice compared with littermate controls (data not shown). However, after 2 months of age, the TSH plasma levels in the thyrocyte-specific Gαq/Gα11−/−KO mice gradually increased, differing significantly at 6 months of age. Eventually, about half of the Tc-Gαq/Gα11−/−KO animals developed overt hypothyroidism, with low T4 levels and strongly increased TSH levels, at 6 months of age or older (Figure 2 and data not shown). The proportion of males to females was very similar in groups with normal and altered TSH and T4 plasma levels (data not shown), which indicates that there were no sex-specific differences. There was also no difference in thyroid weights of Tc-Gαq/Gα11−/−KO mice with normal (<150 ng/ml) and elevated TSH levels (>500 ng/ml) (0.12 ± 0.01 and 0.11 ± 0.01 mg/g body weight, respectively).
To test whether the slowly progressing hypothyroidism in some animals was due to incomplete recombination of the floxed \( \alpha_{11} \) allele or a defect in the \( \alpha_{11} \)-mediated regulation of cAMP levels, we compared thyrocytes from 5- to 6-month-old Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice with normal and elevated TSH levels. As shown in Figure 2C, \( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO as well as \( \alpha_{11} \) protein levels were indistinguishable between the groups. Also, the ability of TSH to induce an increase in cAMP levels in wild-type mice was similar to that in Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice with normal and elevated TSH levels (Figure 2D).

The histology of the Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO thyroid glands at 1 month of age showed no obvious difference in follicle size or form or staining of the colloid compared with control mice. However, at 6 months of age, concomitant with elevated TSH and reduced thyroid hormone levels, the thyroid histology of the thyroid-specific \( \alpha_{11} \)-deficient mice was altered (Figure 3). In these animals the normal thyroid follicular structure was disturbed, with few normal follicles left. Follicle cells were often enlarged and columnar and had large nuclei. Despite the long-term elevation of TSH levels and the follicular cell changes, the thyroids of the Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice were not significantly larger than those of controls. Nor was the thyroid weight increased: thyroid weights were 1.65 ± 0.09 mg in control mice (\( n = 14 \)) and 1.58 ± 0.15 mg in Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice (\( n = 8 \)).

Defect of TSH-induced regulation of thyroid function in the absence of \( \alpha_{q/\alpha_{11}} \). To analyze potential defects in thyrocytes resulting from \( \alpha_{q/\alpha_{11}} \)-deficiency, we determined several cellular functions required for thyroid hormone formation, storage, and release. The functional studies were performed at the age of 1-2 months, when thyroid morphology was still normal and animals were euthyroid with normal TSH levels. TSH has previously been shown to increase iodine uptake in thyrocytes (6). As shown in Figure 4A, there was no significant difference between basal and TSH-stimulated iodine uptake between 6-week-old control and thyrocyte-specific \( \alpha_{q/\alpha_{11}} \)-deficient mice, indicating that the

\[ \text{Ga}_{\alpha_{q}}/\text{Ga}_{\alpha_{11}} \]-mediated signaling pathway is not required for TSH-stimulated iodine uptake.

To test whether the stimulation of iodine coupling to thyroglobulin by TSH is dependent on \( \alpha_{q}/\alpha_{11} \), we measured the amount of iodine incorporated into thyroid proteins. While basal levels of iodine incorporation were the same in wild-type and Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO animals, stimulation of incorporation by TSH was abrogated in the absence of \( \alpha_{q}/\alpha_{11} \) (Figure 4B).

In order to evaluate the role of \( \alpha_{q}/\alpha_{11} \)-mediated signaling for thyroid hormone release, TSH was administered to 4-week-old mice, which are still euthyroid with no apparent alteration in thyroid histology. At this stage, there was also no difference in the T\(_{4}\) content of thyroids from wild-type and Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice (7.5 ± 0.6 and 8.05 ± 2.1 \mu g/dl, respectively). As expected, TSH led to an increase in total T\(_{4}\) plasma levels in wild-type mice, with a maximal effect after 6 hours (Figure 4C). However, in Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice, the TSH-induced thyroid hormone release was almost completely abrogated (Figure 4C). There was no difference between wild-type and Tg-Cre mice (data not shown).

To test whether the impaired thyroid hormone release in Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice was due to an impaired pinocytotic uptake of colloid, we challenged control and euthyroid Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice with TSH and measured the formation of intracellular pinocytotic vesicles by counting the colloid droplets in the thyrocytes. In wild-type animals, TSH stimulation resulted in the formation of multiple pinocytotic vesicles in thyroid follicular epithelium cells within 5 hours (Figure 4, D and E). However, the number of pinocytotic vesicles after TSH treatment was severely reduced in thyrocytes from Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice and amounted to less than 20% that of wild-type animals.

Lack of goiter development in the \( \alpha_{q}/\alpha_{11} \)-deficient thyroid. To study the role of the \( \alpha_{q}/\alpha_{11} \)-mediated signaling pathway in thyroid gland growth, weights of thyroid glands were determined. At the age of 1 month, when there is no significant difference in TSH levels between Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO and control mice, there was no significant difference in thyroid weight either. Interestingly, at the age of 1 year, despite the highly elevated serum TSH levels in the thyrocyte-specific \( \alpha_{q/\alpha_{11}} \)-deficient mice, there was no significant increase in thyroid weight compared with control animals (data not shown). This suggested that thyroids from Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO mice did not respond to elevated levels of TSH by growing.

To test the acute effects of TSH on thyroid growth, we treated 6- to 8-week-old wild-type and Tc-\( \alpha_{q/\alpha_{11}} \)/\( \alpha_{11} \)-KO animals for 1 week
with TSH. While the weight of wild-type thyroid increased by about 100%, no increase was observed in Gαq/Gα11-deficient thyroids in response to TSH (Figure 5, A and B). Instead, thyroids of Tc-Gαq/Gα11-KO mice showed decreased weight, caused by a slight reduction in follicle lumen size (see below). In wild-type animals, TSH treatment resulted in an increase in cell number as well as in cell size, while in Tc-Gαq/Gα11-KO mice, no increase in cell proliferation was observed (Figure 5C). While the average number of thyrocytes per follicle increased from 13.3 to 17.2 after treatment of wild-type animals with TSH, the number of thyrocytes in Tc-Gαq/Gα11-KO mice did not increase after treatment with TSH (11.8 and 10.9, respectively). However, Gαq/Gα11-deficient thyrocytes still showed a hypertrophic response to TSH (Figure 5D). In addition, unlike wild-type thyroids, the colloid area in Tc-Gαq/Gα11-KO mice was reduced after TSH treatment (Figure 5E).

To test the response of Gαq/Gα11-deficient thyrocytes to inhibition of thyroid function, 1- to 2-month-old animals were fed a goitrogenic diet containing sodium perchlorate and methimazole. After 3 weeks of goitrogenic diet, the thyroid weight of the wild-type animals increased more than 2-fold compared with animals that received control diet (Figure 6A). Interestingly, in the Tc-Gαq/Gα11-KO mice, there was no significant difference in thyroid weight between the treated and untreated groups, indicating that Gαq/Gα11 proteins are required for goiter development. TSH values increased 20-fold in wild-type and about 30-fold in Tc-Gαq/Gα11-KO mice 2–3 weeks after starting the diet (data not shown).

The failure of Tc-Gαq/Gα11-KO mice to respond to goitrogenic diet with an enlargement of the thyroid was accompanied by a lack of corresponding changes in the morphology of the thyroid (Figure 6B). While wild-type mice showed hyperplastic goiters characterized by colloid-depleted follicles, large vessels, and varying epithelial cell size, thyroids of Tc-Gαq/Gα11-KO animals did not show cell proliferation and showed much less vascularization than did thyroids from wild-type animals fed the goitrogenic diet (Figure 6B). The lack of cell proliferation in Tc-Gαq/Gα11-KO mice fed the goitrogenic diet was verified by the determination of BrdU incorporation (Figure 6C). While wild-type animals showed 10 ± 2.1 BrdU-positive cells per visual field in thyroid sections, less than 1 BrdU-positive cell per field was observed in Tc-Gαq/Gα11-KO mice and in mice fed normal diet. Because goiter-associated angiogenesis requires the synthesis of various vascular growth factors by thyrocytes (25), we determined the amount of VEGF in thyroids from wild-type and Tc-Gαq/Gα11-KO mice before and 3 weeks after starting the goitrogenic diet. As seen in Figure 6D, wild-type thyroids showed an increase in VEGF levels after treatment with goitrogenic diet. In contrast, in the absence of Gαq/Gα11 there was already a lower basal level of VEGF in thyroid lysates, and treatment with goitrogenic diet did not induce any change in VEGF levels.

Discussion

In this study we have addressed the role of Gαq/Gα11-mediated signaling in the regulation of thyroid function by generating a conditional mouse model lacking both Gαq and Gα11 proteins in thyrocytes. While the deletion of genes encoding Gαq and Gα11 proteins occurred perinatally, the lack of Gαq/Gα11 did not lead to any obvious defect in postnatal development of the thyroid gland, as indicated by normal thyroid histology and normal serum TSH and thyroid hormone levels for up to 2 months. However, by that age, the thyroid-specific Gαq/Gα11-deficient mice showed impaired thyroid hormone formation and secretion when acutely challenged with TSH. Starting at 2 months of age, most of the Tc-Gαq/Gα11-KO animals slowly developed hypothyroidism, with elevated serum TSH levels and alteration in thyroid histology appearing later in life. Despite the highly elevated TSH levels at 6 months of age, the thyroid weight of the Gαq/Gα11-deficient mice was not increased. In addition, the lack of Gαq/Gα11 proteins in thyrocytes prevented thyroid growth when challenged with TSH or goitrogenic diet.

While basal and TSH-stimulated iodine uptake was normal, the incorporation of iodine into thyroid proteins in response to TSH was impaired in Tc-Gαq/Gα11-KO mice. This indicates that the rapid TSH-dependent stimulation of iodine uptake via the Na+/I- symporter is not mediated by the Gαq/Gα11-dependent pathway, but rather involves Gα11-mediated cAMP formation (26). In contrast to the TSH-dependent regulation of iodine uptake, iodination in response to TSH requires signaling through the Gαq/Gα11-mediated pathway. This is consistent with earlier reports suggesting a regulation of peroxidase primarily through Ca²⁺ and PKC (27, 28).

Mice lacking the Gαq/Gα11-mediated signaling pathway show impaired thyroid hormone secretion in response to TSH. TSH-
induced thyroid hormone secretion is initiated by internalization of thyroglobulin via macropinocytosis (29–31). There is evidence that TSH-induced pinocytotic uptake of thyroglobulin and thyroid hormone release are mediated by the cAMP-dependent pathway (7, 32). However, other mediators have also been suggested to play a role in processes leading to thyroid hormone secretion. In sheep thyroid cells, for example, [Ca^{2+}] has been shown to regulate hormone secretion in vitro (33). Our results indicate that the G_{q}/G_{11}-mediated signaling pathway in mice is required for the macropinocytotic uptake of thyroglobulin in response to TSH.

In addition to macropinocytosis, endocytotic processes can contribute to the uptake of thyroglobulin by thyrocytes (34). The recently described endocytosis of thyroglobulin via megalin is followed by the apical to basolateral transcytosis of thyroglobulin with low hormone content (35, 36). The transcytotic removal of hormone-poor thyroglobulin is believed to increase lysosomal degradation of hormone-rich thyroglobulin and hence hormone secretion. There is, however, no evidence that megalin or other endocytic receptors are regulated via G protein–mediated signaling pathways (37). During and after pinocytotic uptake of thyroglobulin, thyroid hormone is released via enzymatic digestion by cathepsins (38, 39). The effect of the G_{q}/G_{11}-mediated signaling pathway on proteolysis was not studied here, but our data indicate that the lack of G_{q}/G_{11} proteins in thyrocytes impairs the process of secretion already at the level of colloid uptake via pinocytosis.

The fact that serum TSH levels in 2-month-old animals were normal while their response to TSH treatment was impaired suggests that under normal in vivo conditions the thyroid can fully compensate the partial defect in TSH responsiveness for a variable time period. However, when high, supraphysiological TSH concentrations revealed the defect even when thyroid function in vivo was completely normal and the defect was compensated.

Abnormal thyrocyte cell proliferation underlies a variety of diseases, including various forms of goiter and thyroid neoplasia. In many cases thyroid proliferation has been shown to be under the control of TSH, and the cAMP-mediated signaling pathway is believed to play a predominant role in the mitogenic effects exerted by TSH (6, 40, 41). The induction of thyroid growth by a goitrogenic diet consisting of thyrostatic drugs is thought to be initiated by increased pituitary secretion of TSH, which results in thyroid cell hyperplasia accompanied by hypervascularization caused by angiogenesis within 1–2 weeks (42, 43). The lack of thyrocyte proliferation in Tc-G_{q}/G_{11}-KO mice in response to TSH or goitrogenic diet indicates that downstream mediators of the G_{q}/G_{11}-mediated signaling pathway are required for TSH-induced thyroid cell proliferation as well as for thyroid growth in response to goitrogenic diet. This is consistent with previous data indicating that G_{q}/G_{11} can mediate mitogenic effects in different cell types (44, 45).

Other than the follicular cell hyperplasia, thyroid enlargement caused by nontoxic goiter is also characterized by an early vacu-
lar response resulting in hypervascularization and abnormally enlarged blood vessels. There is good evidence that the goiter-associated angiogenesis is initiated by the production of endothelial growth factors including VEGF by thyrocytes exposed to increased TSH levels (25, 46, 47). The fact that no increase in VEGF levels in response to goitrogenic diet was observed in Tc-\(\alpha_q/\alpha_{11}\)-KO mice indicates that induction of VEGF expression requires an intact \(\alpha_q/\alpha_{11}\)-mediated signaling pathway.

The analysis of the intracellular signaling mechanisms regulating thyroid follicular cell function and growth has led to the concept that the \(\alpha_q\)-dependent cAMP-mediated signaling pathway is the principal mechanism through which TSH and other factors acting via G pro-
tein–coupled receptors increase thyroid function and growth. Our data, based on a thyrocyte-specific knockout of the Gαq/Gα11-mediated signaling pathway, reveal an essential role of these G proteins in mediating the regulation of thyroid function. We show that Gαq/Gα11-mediated signaling processes are required for thyroid hormone formation and release as well as for the adaptative growth of the thyroid. Thus, inhibition of Gαq/Gα11-mediated signaling processes or their downstream effectors may be a promising strategy to interfere with diseases characterized by increased thyroid function and/or growth.

**Methods**

**Experimental animals and treatments.** Procedures of animal care and use in this study were approved by the Regierungspräsidium Karlsruhe (Karlsruhe, Germany). For the analysis of serum hormone levels, 100 μl of blood was drawn from the retroorbital plexus under anesthesia with xylazine hydrochloride (3 mg/kg body weight) and ketamine hydrochloride (100 mg/kg body weight). For collecting tissue samples, mice were sacrificed with CO2. To induce goiter, a group of mice was treated with 5 g/l sodium perchlorate–KO mice after 2 weeks of goitrogenic treatment. Arrows indicate blood vessels. (C) After 20 days of goitrogenic treatment, wild-type and Tc-Gαq/Gα11–KO mice were injected with BrdU, and thyroid sections were prepared and stained with anti-BrdU antibodies to determine cell proliferation. Scale bars: 125 μm. (D) Thyroid homogenates from wild-type and Tc-Gαq/Gα11–KO mice treated with normal or goitrogenic diet for 8 weeks were separated on SDS-PAGE and immunoblotted. Shown is an immunoblot with an anti-VEGF antibody. Con, control (Panc-1 cell lysates).

**Figure 6**

Role of Gαq/Gα11 in goiter development. (A) Wild-type and Tc-Gαq/Gα11–KO mice were treated with goitrogenic diet 0–8 weeks, and thyroid weights were determined thereafter. Inset: thyroids of wild-type and Tc-Gαq/Gα11–KO mice after 8 weeks of treatment. Values are mean ± SEM. ***P < 0.001 versus wild-type. (B) Sections of thyroids from wild-type and Tc-Gαq/Gα11–KO mice after 2 weeks of goitrogenic treatment. (C) After 20 days of goitrogenic treatment, wild-type and Tc-Gαq/Gα11–KO mice were injected with BrdU, and thyroid sections were prepared and stained with anti-BrdU antibodies to determine cell proliferation. Scale bars: 125 μm. (D) Thyroid homogenates from wild-type and Tc-Gαq/Gα11–KO mice treated with normal or goitrogenic diet for 8 weeks were separated on SDS-PAGE and immunoblotted. Shown is an immunoblot with an anti-VEGF antibody. Con, control (Panc-1 cell lysates).
Pluronic F-127. After a 30-minute incubation at 37°C, the cells were washed twice and incubated for another 30 minutes. To monitor the changes in [Ca2+], the coverslips were mounted in a holder and the fluorescence at 510 nm was measured. The excitation wavelength alternated between 340 and 380 nm in intervals of 500 ms. Changes in [Ca2+] are given as the ratio of 340-nm to 380-nm intensities.

Western blotting. For Western blot analyses, thyroid gland lysates were analyzed by Western blotting using anti-Goα/Goβ1 and anti-VEGF antibodies (Santa Cruz Biotechnology Inc.) and antibodies against ERK1/2 (Cell Signaling Technology).

Iodine uptake and iodination. For iodine uptake experiments, Na125I was administered i.p. (0.1 μCi/10 g body weight) with or without TSH (100 mIU/10 g body weight) twice, 24 and 12 hours before administration of 125I, together with a third dose of TSH; 5 hours later, animals were sacrificed. The whole thyroid gland was dissected and weighed, and counted in a γ counter. An uptake index was calculated as counts of thyroid per weight of liver. Organization was determined after trichloroacetic acid (10%/w/v) precipitation of thyroid homogenates as previously described (55).

Statistics. The Statview program (Windows version 4.57, Abacus Concepts Inc.) was used for ANOVA and for Fisher’s protected least-significant-difference post-hoc tests. A P value less than 0.05 was considered significant. Results are presented as mean ± SEM.

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