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A Retinoid Antagonist Increases the Hypothalamic-Pituitary-Thyroid Set Point in Mice and Thyrotrope Cells

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Abstract

Retinoid X Receptor (RXR) signaling influences thyrotrope function. Synthetic RXR agonists, rexinoids, can cause central hypothyroidism. To test the hypothesis that endogenous rexinoids contribute to the TSH ‘set point’, TαT1 mouse thyrotrope cells were treated with a rexinoid antagonist, LG101208. Increasing concentrations of LG101208 significantly increased TSHβ mRNA levels, indicating that the rexinoid antagonist may interfere with RXR-signaling by an endogenous rexinoid in thyrotropes. When the same experiments were repeated in the presence of charcoal-stripped serum the effect of the rexinoid antagonist was lost. Pretreatment with the transcription inhibitor DRB blocked the increase of TSHβ mRNA levels by rexinoid antagonist, indicating the primary effect is at the level of gene transcription. Mice treated with LG101208 had higher levels of serum T4, T4/TSH ratios as well as pituitary α-subunit and TSHβ mRNA compared with vehicle treated mice. Hypothalamic TRH levels were unchanged. In summary, the rexinoid antagonist, LG101208, increases TSH subunit mRNA levels in thyrotrope cells and mouse pituitaries, primarily at the level of gene transcription. These data suggest that an “endogenous rexinoid” contributes to the TSH ‘setpoint’ in thyrotropes.

Keywords

Thyrotrope; TSH; retinoid; rexinoid; RXR

1. Introduction

Vitamin A and retinoids have a well recognized, but poorly understood effect on the hypothalamic-pituitary-thyroid (HPT) axis (Haugen 2004; Morley 1980). Retinoids affect cell growth, differentiation, metabolism and function through two sets of nuclear hormone receptors, retinoic acid receptors (RAR) and retinoid X receptors (RXR). Recent studies

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indicate that HPT function is affected primarily by RXR-selective retinoids (called rexinoids) (Sharma et al 2006; Sherman et al 1999). Our group and others have demonstrated that rexinoids can suppress TSH using *in vitro* and animal models (Liu 2002; Sharma 2006) as well as human studies (Dabon-Almirante 1999; Golden 2007; Sherman 1999; Smit 2007).

If an exogenous rexinoid can suppress TSH and T4 levels in animal models and human studies as well as TSH β mRNA levels in thyrotropes, one might predict that an endogenous rexinoid could play a role in the HPT axis 'setpoint'. While 9-*cis* retinoic acid (9-*cis* RA) has been used as the classic rexinoid agonist in various studies, many believe this is not a significant endogenous rexinoid (Wolf 2006). Investigators have more recently shown that unsaturated fatty acids such as docosahexaenoic, linoleic, linolenic and arachadonic acid may act as endogenous rexinoids when produced locally in cells and tissues (de Urquiza 2000; Goldstein 2003; Lengqvist 2004). It is still unclear which molecules may serve as endogenous rexinoids and if they are different in certain cells, tissues or under specific conditions.

In order to explore the hypothesis that an endogenous rexinoid affects TSH levels in the thyrotrope and the HPT axis 'set point', we took a reverse pharmacologic approach by treating mice and thyrotrope-derived cells with a rexinoid antagonist, LG101208. In the search for RXR-specific modulators, a number of RXR antagonists have been synthesized (Michelly 2003), but very few have been studied in cell-based experiments. One specific RXR antagonist, LG101208 has been shown to directly inhibit the effects of the fatty acid eicosapentaenoic acid in macrophages (Selvaraj 2006). In this report, we show for the first time that the rexinoid antagonist LG101208 increases TSH β mRNA levels in thyrotrope cells and affects the HPT axis in mice, suggesting an endogenous rexinoid may contribute to the physiologic 'TSH set point' *in vivo*.

2. Materials and Methods

2.1 Cell Culture

Murine T α T1 thyrotrope cells were generated from transgenic mouse pituitary tumors and were generously provided by Dr Pamela Mellon (Yusta 1998). T α T1 cells were grown in DMEM (Invitrogen, Life Technologies, Carlsbad, CA) containing 10% fetal bovine serum (FCS, Hyclone, Logan, UT), 10 mM HEPES buffer solution, 20 U penicillin-streptomycin (Invitrogen). Hypothyroid media from thyroidectomized bovine serum (Rockland Immunochemicals, Gilbertsville, PA) and charcoal-stripped serum (Gemini Bio-Products, West Sacramento, Ca) were used in selected experiments. T α T1 cells were seeded on Matrigel-coated plates (BD Biosciences, Bedford, MA), which facilitated adhesion. Matrigel was first diluted 30-fold with PBS before coating the plates, which were allowed to dry before plating cells. The cells were maintained at 37°C in an environment of 5% CO₂. Replacement with the same medium containing a specified amount of rexinoid was done either 24 or 48 h before harvesting the cells. TSH levels in the media of cultured cells were measured after 24 or 48 hours of treatment with rexinoid or vehicle (DMSO). For inhibition of new mRNA synthesis, cells were pretreated for 30 minutes with media containing 100 μ M DRB (5,6-Dichlorobenzimidazole riboside, RNA polymerase II inhibitor) prior to adding media with vehicle, 100 nM LG100268 or 100 nM LG101208 for 24 hours (kindly provided by Ligand Pharmaceuticals, San Diego, CA). DRB blocks the elongation of RNA polymerase II transcripts (Yusta 1998).

RNA measurement by quantitative RT-PCR—Total RNA was isolated from cells using the RNeasy Mini Kit (Qiagen) as recommended by the manufacturer. Total RNA was isolated from murine pituitaries and hypothalami by homogenizing in 1 mL of Tri Reagent

(Applied Biosystems), followed by addition of 0.2 mL of chloroform and centrifugation at 13,000 g for 15 minutes. The aqueous layer was then mixed with an equal volume of RNeasy Lysis buffer (RLT) with added β ME (Qiagen). mRNA levels of mouse (m) TSH β and prepro-TRH were measured by real-time quantitative RT-PCR using the ABI Prism 7700 sequence detection system (PerkinElmer/Applied Biosystems, Foster City, CA). The sequences of forward and reverse primers as designed by Primer Express (PE/Applied Biosystems) were 5'-CCTGACCATCAACACCACCA-3' and 5'-TGGAAGAAACAGTTTGCCAT-3' (mTSH β), 5'-CTCCAGCGTGTCGAGG-3' and 5'-TCCCTTTTGCCCGGATG-3' (mTRH), and 5'-GCCTACAAACAGGTTAACTG-3' and 5'-CCTGATTCAGGATTGGAG-3' (mDeiodinase type 2), respectively. The TaqMan fluorogenic probe used was 5'-6FAM-GATATCCCGTCATACAATACCCAGCACAG-TAMRA-3' for mTSH β and 5'-6FAM-CTTGGTGCTGCCTTAGATTCTGGA-TAMRA-3' for mTRH. Deiodinase type 2 amplification was performed with SYBR green according to the manufacturer's protocol. Amplification reactions were performed in MicroAmp optical tubes (PE/Applied Biosystems) in a 25- μ L mix containing 8% glycerol, 1 $\times$ TaqMan buffer A [500 mM KCl, 100 mM Tris-HCl, 0.1 M EDTA, 600 nM passive reference dye ROX (pH 8.3) at room temperature], 300 μ M each of dATP, dGTP, dCTP, and 600 μ M deoxyuridine 5-triphosphate, 5.5 mM MgCl₂, 900 nM forward primer, 900 nM reverse primer, 200 nM probe, 0.625 U AmpliTaq Gold DNA polymerase (PerkinElmer, Foster City CA), 6.25 U Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD), 10 U RNAs in ribonuclease inhibitor (Promega Corp., Madison, WI), and the template RNA. Thermal cycling conditions were as follows: reverse transcription was performed at 48°C for 30 min followed by activation of TaqGold at 95°C for 10 min. Subsequently 40 cycles of amplification were performed at 95°C for 15 sec and 60°C for 1 min. A standard curve was generated using the fluorescent data obtained from 10-fold serial dilutions of a positive strand of mTSH β RNA that was synthesized as described (Sharma 2006). TRH and α -subunit standard curves were generated using control plasmid. Deiodinase type 2 standard curve was generated from dilution of the T α T1 cell mRNA. Quantities of TSH β and TRH in samples were normalized to the corresponding 18S rRNA (PerkinElmer/Applied Biosystems, P/N 4308310). The primers used for alpha subunit amplification were:

Fwd: 5'-TGGCCAAAGCATTACTAAGGC-3';

Rev: 5'-AAGTGCTACAGTGGCACTCCG-3'

The final PCR mixture contained 200 nM each of forward and reverse primers, 1 $\times$ SYBR PCR mix (Applied Biosystems), and 5 μ L of cDNA template. Real-time PCR was performed with an ABI Prism 7700 sequence detector (Applied Biosystems). The thermal cycling conditions were: 2 min at 50°C, 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Dissociation curves were recorded after each run, and the amplified products were visualized by 2% agarose gel electrophoresis.

2.2 Animals

Wild type, male 129SvJ mice (Jackson Laboratories) were housed in a pathogen-free facility at the Animal Care Facility of the University of Colorado Denver. All animal protocols were approved by the Animal Care and Use Committee. Mice were studied between 8 and 12 wk of age on a standard *ad libitum* chow diet. LG101208 (kindly provided by Ligand Pharmaceuticals, San Diego, CA) was prepared in wet granulation vehicle. The vehicle consisted of 0.085% povidone, 1.5% lactose, 0.026% Tween 80, and 0.2% Antifoam. This was used as a vehicle to completely dissolve the rexinoids that is not toxic to mice. Either LG101208 or vehicle alone was administered by daily gavage (200 μ L, 5 mg/kg) at 8 am daily. On the morning of the fourth day, mice were again treated by oral gavage, fasted for four hours, anesthetized with Avertin (Winthrop Laboratories), blood was collected for

plasma by cardiac puncture and retrieved tissues (brain, liver, pituitary and a hypothalamic fragment containing paraventricular nucleus) were snap frozen. The hypothalamic fragment was dissected using the optic chiasm as the rostral border, the caudal side of the pituitary stalk as it exited the median eminence as the caudal border, the lateral edge of the fornix for the lateral border, and the top of the fornix for the rostral border.

2.3 Thyroid function tests

Murine plasma and cell media TSH values were measured by RIA (performed by Dr. Samuel Refetoff, University of Chicago, Chicago, IL). Mouse TSH was measured in 50 μ L of plasma using a sensitive, heterologous, disequilibrium, double-antibody precipitation radioimmunoassay (Pohlenz 1999). Standards were diluted in plasma from mice treated with thyroid hormone for the plasma measurement, and standards were diluted in media [10% fetal bovine serum (FBS)-DMEM] for measurement of TSH secreted into the media. The lower limit of detection for the TSH assay was 10 mU/L.

Plasma total T₄ levels were measured by standard RIA (Diagnostic Products Corp., Los Angeles, CA).

2.4 Statistics

Statistical analyses were performed with paired Student's *t* test, analysis of variance (Dunnett's method, Dunn's method), or Mann-Whitney Rank Sum Test as appropriate. Analyses were performed using the program SigmaStat 2.03 (Point Richmond, CA). Unless otherwise noted, the results are presented as means $\pm$ SD. All statistical tests were two-sided. *P* < 0.05 was considered to be statistically significant.

3. Results

3.1 Rexinoid antagonist increases TSH β mRNA in T α T1 thyrotrope cells

LG101208 is a pure RXR antagonist (Selvaraj and Klasing 2006). LG101208 significantly increased TSH β mRNA levels in T α T1 cells at both 24 and 48 hours (Fig. 1A, *p* < 0.01). Maximal increases of 71% and 81% with 1000 nM LG101208 were seen at 24 and 48 hours respectively. Maximal increases of 53% and 47% of α -subunit mRNA levels were also seen with 1000 nM LG101208 at 24 and 48 hours respectively (Fig 1B, *p* < 0.01). Type 2 deiodinase mRNA was also significantly increased by 50% at 48 hours with 1000 nM LG101208 (Fig. 1C, *p* = 0.01).

We have previously shown that the RXR-specific agonist, LG100268, significantly suppresses TSH β mRNA levels in T α T1 cells (Sharma 2006). Increasing concentrations of the RXR-specific antagonist, LG101208, was able to overcome and reverse the TSH β mRNA suppression by the rexinoid agonist (Fig. 2). TSH β mRNA levels were significantly higher compared with agonist alone in the presence of 1000 nM LG101208 (*p* < 0.05).

In order to determine if LG101208 is reversing TSH β mRNA suppression by an endogenous rexinoid, we repeated experiments in T α T1 cells in the presence and absence of charcoal-stripped serum which may contain endogenous rexinoids. Fig. 3 shows that the suppressive effect of rexinoid agonist (LG100268) is preserved in the presence of charcoal-stripped serum, but the ability of the rexinoid antagonist (LG101208) to increase TSH β mRNA is lost in the presence of charcoal-stripped serum. These data indicate that the rexinoid antagonist may be interfering with an endogenous rexinoid that is affecting the set point of TSH β mRNA levels in thyrotropes. The same experiments were repeated in the presence of hypothyroid calf serum which lacks thyroid hormone but should theoretically still contain endogenous rexinoid. Baseline TSH β mRNA levels were lower in the T α T1 cells grown in

hypothyroid calf serum compared with control serum (Fig 3), which may reflect the overall poor growth of these cells in hypothyroid serum. Treatment with the rexinoid antagonist did significantly increase TSH β mRNA levels, indicating that there is an endogenous rexinoid effect even in the absence of thyroid hormone. An alternative explanation is that hypothyroid serum contains higher amounts of endogenous rexinoid than normal euthyroid serum.

3.2 The effect of rexinoid agonist and antagonist on TSH β mRNA levels occurs primarily at the level of transcription in T α T1 cells

T α T1 cells were treated with DRB (5,6,-dichloror-1- β -ribofuranosyl benzimidazole, an analog of adenosine that selectively blocks synthesis of RNA polymerase II transcripts without affecting cell viability, prior to treatment with the rexinoid agonist or antagonist. The effect of agonist (LG100268) and antagonist (LG101208) on TSH β mRNA levels were both significantly attenuated by pretreatment with the transcription inhibitor DRB (Fig. 4), indicating that the effect of these rexinoids on TSH β mRNA occurs primarily at the level of transcription.

TSH levels in the media of cultured T α T1 cells was measured after 24 and 48 hours of treatment with LG101208. We observed no significant differences in TSH protein in the media of cells treated with the rexinoid antagonist compared with vehicle (data not shown).

3.3 Mice treated with the rexinoid antagonist LG101208 demonstrate altered serum thyroid function tests

We have previously shown that the rexinoid agonist, LG100268, lowers serum TSH and total T4 in mice after three days of treatment (Sharma 2006). Based on these data and our studies above in thyrotrope cells, we predicted that mice treated with rexinoid antagonist (LG101208) would have higher serum TSH and total T4 levels if an endogenous rexinoid was affecting the 'TSH setpoint' *in vivo*. Mice were treated by daily oral gavage with LG101208 (5 mg/kg/day) or vehicle for three days. Fig. 5 shows that serum total T4 levels and T4 to TSH ratio (T4/TSH $\times$ 10) were significantly higher in the mice treated with LG101208 as predicted ($p < 0.05$). Serum TSH levels were slightly, but not significantly higher in the treated mice ($p=0.12$). These data may indicate that rexinoid antagonist increased TSH production in mice which increased T4 levels which, in turn, lowered TSH levels back toward baseline (ie – a new 'set point'). Pituitary TSH β and α -subunit mRNA levels were higher in the treated mice, but pituitary deiodinase type 2 (D2) and hypothalamic preproTRH levels were not different between treated and untreated animals (Fig. 6). TRH may be unaffected by rexinoid antagonist in this *in vivo* model, although one would predict that preproTRH levels would be lower (due to the higher T4 levels) if LG101208 had no effect on the hypothalamus.

4. Discussion

In this report, we show for the first time that a rexinoid antagonist increases TSH β mRNA in thyrotropes using an *in vitro* and *in vivo* model. The rexinoid antagonist affects TSH β mRNA levels, at least in part, directly at the level of gene transcription. These data strongly suggest that the 'TSH setpoint' in thyrotropes is likely influenced by an endogenous rexinoid.

Vitamin A affects thyroid function and the main effect on thyrotrope function appears to be through an RXR-mediated or rexinoid pathway (Golden 2007; Haugen 2004; Morley 1980; Sharma 2006; Sherman 1999). We have previously shown that mice lacking the RXR γ isotype have higher serum TSH and T4 levels than wild-type littermates, indicating that this

isotype is important in rexinoid signaling in the thyrotrope (Brown et al 2000). We also demonstrated high levels of the RXR γ 1 isotype in mouse pituitaries and no compensatory increase in the RXR α and RXR β isotypes in our RXR γ knock-out animals. These data suggest that rexinoid signaling and RXR γ play a role in the hypothalamic-pituitary-axis and that an endogenous rexinoid may be an important regulator of TSH in thyrotropes.

Macchia and colleagues demonstrated that mice lacking thyroid hormone receptor TR β , which is critical for thyroid hormone signaling in the thyrotrope, still exhibited suppression of TSH with the rexinoid AGN194204 (Macchia 2002). Our group and others further showed that the effects of thyroid hormone and rexinoids on TSH β promoter activity were mediated by different regions of the TSH β promoter (Breen 1997; Sharma 2006). Together, these data indicate that thyroid hormone and rexinoids have distinct and independent effects on TSH regulation in the thyrotrope.

It is now clear that exogenous rexinoids suppress TSH in human, animal and *in vitro* studies. 9-*cis* retinoic acid (9-*cis* RA) was the first identified endogenous rexinoid, but it is believed that 9-*cis* RA likely does not play a significant role in physiologic rexinoid signaling (Wolf 2006). Others have shown that unsaturated fatty acids including oleic acid, linoleic acid, arachadonic acid and docosaheptaenoic acid are putative endogenous rexinoids in multiple tissues (de Urquiza 2000; Goldstein 2003).

We chose a reverse pharmacology approach to explore rexinoid signaling in thyrotropes using both cell culture (T α T1 cells) and intact organism mouse models. We hypothesized that if an endogenous rexinoid contributed to TSH regulation in the thyrotrope, treatment with a rexinoid antagonist would raise TSH β mRNA and TSH levels in these models. The rexinoid antagonist LG101208 significantly increased TSH β mRNA levels in T α T1 cells and antagonized the effect of the exogenous rexinoid, LG100268. One potential mechanism could be an indirect effect through a decrease in deiodinase type 2 (mD2), which would lead to decreased local T3 production and increased TSH β mRNA. The rexinoid antagonist did not decrease mD2 and actually increased mRNA levels at 48 hours, suggesting that the effect of LG101208 was not through effects on mD2 levels. We further showed that the effect of rexinoid antagonist on raising TSH β mRNA levels in our thyrotrope model was lost in the presence of charcoal-stripped serum, indicating that a lipophilic endogenous rexinoid is present in serum. Charcoal treatment of serum efficiently removes many free fatty acids (Chen 1967), which may serve as endogenous rexinoids. This is further supported by experiments showing that hypothyroid serum, which does not remove lipophilic compounds like free fatty acids, does not abrogate this rexinoid effect. These experiments do not rule out the possibility that the endogenous rexinoid is an intracellular fatty acid whose level is controlled by signaling from another serum factor.

It appears that rexinoid agonist suppression of TSH β mRNA and rexinoid antagonist elevation of TSH β mRNA in T α T1 thyrotropes occurs primarily at the level of gene transcription since both of these effects were abrogated in the presence of DRB, an RNA polymerase II inhibitor. Our group and others have previously shown in humans and animal models that rexinoids suppress TSH and T4 levels and that this effect appears to occur at the level of the pituitary and not the hypothalamus (Golden 2007; Liu 2002; Sharma 2006; Sherman 1999). We therefore predicted that inhibition of rexinoid signaling by the antagonist LG101208 would increase serum TSH and T4 levels and increase pituitary TSH β mRNA levels in mice with an intact hypothalamic-pituitary-thyroid (HPT) axis. After three days of rexinoid antagonist treatment, mice had significantly higher serum T4 levels and slightly higher TSH levels (although not statistically significant) suggesting an altered 'TSH setpoint' in these animals with an intact HPT axis. We further showed that pituitary levels of TSH β mRNA were higher in these animals while PVN levels of preproTRH mRNA were

unchanged. These data suggest that the effects of rexinoid signaling are primarily at the level of the pituitary although a small contribution from hypothalamic TRH cannot be ruled out since these animals had higher T4 levels and no compensatory decrease preproTRH mRNA in the PVN. These data for endogenous rexinoid signaling in the mouse are further supported by our studies of the RXR γ deficient mouse which has increases in serum TSH and T4 that are comparable to this rexinoid antagonist pharmacologic model (Brown 2000). We have also shown that RXR γ levels are similar to or slightly higher than RXR α and RXR β levels in mouse pituitaries, and that gene deletion of RXR γ does not affect levels of the other RXR isotypes (Brown 2000).

Patients with nonthyroidal illness commonly have low serum TSH and T4 levels and appear to have suppression of the HPT axis and the level of the pituitary and/or hypothalamus. Potential mechanisms include alterations in deiodinase function or cytokine signaling (Adler 2007; Koenig 2008). We believe that our data showing the important role of rexinoid signaling in the thyrotrope may be another feasible mechanism for the observed alterations in thyroid function in patients with nonthyroidal illness, which is a testable hypothesis.

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Fig. 1. Effect of the rexinoid antagonist LG101208 on TSH β mRNA levels in T α T1 thyrotrope cells

5×10^5 T α T1 cells were grown to 70% confluence in DMEM/10% FBS. Fresh media was added with vehicle (DMSO) or increasing amounts of LG101208 (x-axis). After 24 (open bars) or 48 hours (closed bars), cells were collected for total RNA extraction. Quantitative RT-PCR for TSH β and α -subunit mRNA was performed as previously described (Brown, Smart, Sharma, Brinkmeier, Greenlee, Camper, Jensen, Eckel, Krezel, Chambon, and Haugen 2000). **A)** mRNA levels are expressed as attograms (ag) of TSH β per ng of ribosomal RNA (rRNA). Results are an average (+SEM) of four separate experiments. **B)** mRNA levels are expressed as picograms (pg) of α -subunit per ng of ribosomal RNA (rRNA). Results are an average (+SEM) of two separate experiments performed in triplicate. **C)** mRNA levels are expressed as pictograms (pg) of deiodinase type 2 (D2) per ng of ribosomal RNA (rRNA). Results are an average (+SEM) of two separate experiments performed in triplicate. Asterisk (*) indicates significant stimulation by rexinoid antagonist compared with vehicle ($p < 0.01$).

Fig. 2. The rexinoid antagonist LG101208 competitively reverses TSHβ mRNA suppression by the rexinoid agonist LG100268 TaT1 thyrotrope cells

5×10^5 TaT1 cells were grown to 70% confluence in DMEM/10% FBS. Fresh media was added with vehicle (DMSO), 100 nM LG100268 alone or LG100268 with increasing amounts of LG101208 (x-axis). After 24 (open bars) or 48 hours (closed bars), cells were collected for total RNA extraction. Quantitative RT-PCR for TSHβ mRNA was performed as previously described (Brown 2000). mRNA levels are expressed as attograms (ag) of TSHβ per ng of ribosomal RNA (rRNA). Results are an average (+SEM) of four separate experiments. Asterisk (*) indicates significant increased TSHβ mRNA by rexinoid antagonist in the presence of rexinoid agonist compared with agonist alone at both 24 and 48 hours ($p < 0.05$).

Fig. 3. The rexinoid antagonist LG101208 increases TSH β mRNA levels in T α T1 thyrotrope cells in the presence of control media and hypothyroid media, but not charcoal-stripped media

5×10^5 T α T1 cells were grown to 70% confluence in DMEM/10% FBS. Fresh media with 10% fetal bovine serum (control media), 10% charcoal-stripped fetal bovine serum (charcoal-stripped media) or 10% bovine serum from thyroidectomized calves (hypothyroid media) was added with vehicle (DMSO, black bars), 100 nM LG100268 (rexinoid agonist, gray bars) or 100 nM LG101208 (rexinoid antagonist, white bars). After 48 hours, cells were collected for total RNA extraction. Quantitative RT-PCR for TSH β mRNA was performed as previously described (Brown 2000). mRNA levels are expressed as attograms (ag) of TSH β per ng of ribosomal RNA (rRNA). Results are an average (+SEM) of three separate experiments. Asterisk (*) indicates significant increased TSH β mRNA by rexinoid antagonist compared with untreated (vehicle, DMSO) cells ($p < 0.05$).

Fig. 4. The effects of rexinoid agonist and antagonist to increase TSH β mRNA levels in T α T1 thyrotrope cells occurs primarily at the level of transcription

5×10^5 T α T1 cells were grown to 70% confluence in DMEM/10% FBS. Cells were pretreated for 30 min with either vehicle (DMSO) or the transcription inhibitor DRB (100 μ M) for 30 min before adding vehicle (DMSO, gray bars), 100 nM LG100268 (rexinoid agonist, black bars) or 100 nM LG101208 (rexinoid antagonist, white bars). After 24 hours, cells were collected for total RNA extraction. Quantitative RT-PCR for TSH β mRNA was performed as previously described (Brown 2000). mRNA levels are expressed as attograms (ag) of TSH β per ng of ribosomal RNA (rRNA). Results are an average ($\pm$ SEM) of four separate experiments. Asterisk (*) indicates significant decreased TSH β mRNA by rexinoid agonist compared with untreated (vehicle, DMSO) cells ($p < 0.01$), and (**) indicates significantly increased TSH β mRNA by rexinoid antagonist ($p < 0.05$).

Fig. 5. Effect of the rexinoid antagonist LG101208 on serum hormone measurements in mice Mice (10 in each group) were given wet granulation vehicle (open bars) or LG101208 (5 mg/kg, closed bars) daily for 3 days by oral gavage. Serum was collected 4 hours after the last dose. Data are expressed as TSH mU/L, total T4 (μ g/dl) and T4 to TSH ratio ($\times 10$) + SEM. Asterisk (*) represents a significant increase ($p < 0.05$) in hormone levels compared with vehicle. TSH values < 10 mU/L were assigned an average value of 7 mU/L for statistical analysis.

Fig. 6. Effect of the rexinoid antagonist on pituitary TSH β , α -subunit and hypothalamic prepro-TRH mRNA in mice

Mice (9 in each group) were given vehicle or LG101208 (5 mg/kg/d) for 3 days by oral gavage. The mice were euthanized 4 hours after the last dose and pituitaries and hypothalami were collected for total RNA extraction. 100 ng total RNA was used for quantitative RT-PCR (ABI PRISM 7700) using specific cDNA for standard curves. Data are expressed as femtograms target mRNA level (TSH β $\times$ 100, α -subunit, D2 $\times$ 100, or TRH) corrected for an internal standard (ng rRNA). Asterisk (*) indicates a significant difference ($p < 0.05$) between vehicle and LG101208.