Evidence for a negative feedback in the control of eel growth hormone by thyroid hormones

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Abstract

The regulation of growth hormone (GH) by thyroid hormones (THs) has been shown to present species variation. We investigated the regulation of GH in the eel, a representative of an ancient group of teleosts. In vivo administration of triiodothyronine (T3) or thyroxine (T4) significantly reduced pituitary and serum GH levels, as measured by homologous RIA. In order to investigate the ability of THs to regulate GH production directly at the pituitary level, we used a long-term, serum-free primary culture of eel pituitary cells. Both T3 and T4 inhibited GH release in a concentration-dependent manner, producing up to 50% inhibition at 10 nM, with an ED50 of <0.2 nM, within the range of their physiological circulating levels. Other hormones also acting via the nuclear receptor superfamily, such as sex steroids (testosterone, estradiol and progesterone) and corticosteroid (cortisol), had no effect on GH release in vitro, underlining the specificity of the regulatory effect of THs on GH. Measurement of both GH release and cellular content for calculation of GH production in vitro indicated that THs not only inhibited GH release but also GH synthesis. Dot-blot assay of GH messenger RNA (mRNA) using an homologous eel cDNA probe showed a decrease in GH mRNA levels in cells cultured in the presence of T3, as compared with control cells. This demonstrated that the inhibition of T3 on GH synthesis was mediated by a decrease in GH mRNA steady state levels. In conclusion, we demonstrate inhibitory regulation of eel GH synthesis and release by THs, exerted directly at the pituitary level. These data contrast with the rat, where THs are known to have a stimulatory effect and suggest that the pattern observed here in an early vertebrate and also found in birds, reptiles and some mammals including humans, may represent an ancestral and more generalized vertebrate pattern of TH regulation of pituitary GH.

Introduction

Thyroid hormones (THs) and growth hormone (GH) are thought to play synergistic roles in the control of growth and development in vertebrates (McNabb 1992). Several physiological crosslinks have been demonstrated between thyrotropic and somatotropic axes at peripheral and central levels. In humans (both normal and GH deficient), GH has been shown to act on thyroid function by inducing extrathyroidal conversion of thyroxine (T4) to the biologically active hormone, triiodothyronine (T3) (Grunfeld et al. 1988). GH has also been reported to increase circulating T3 levels by stimulating peripheral 5'-monodeiodination in birds (Kühn et al. 1987) and teleosts (European eel: de Luze & Leloup 1984, rainbow trout: MacLatchy et al. 1992). At the pituitary level, thyrotropin-releasing hormone (TRH) was shown to be able to stimulate not only thyrotropin (TSH) but also GH release in various vertebrates including mammals, birds, reptiles, amphibians (Harvey 1990a) and teleosts (Peng & Peter 1997). Moreover, corticotropin–releasing hormone (CRH) has been shown not only to stimulate corticotropin release but also to act as a GH–releasing factor and TSH–releasing factor in reptiles (Denver & Licht 1989) and in teleosts (Larsen et al. 1998, Rousseau et al. 1999).

It is well established that THs are required for normal pituitary GH secretion in man and several other mammalian species (for review: Valcavi et al. 1992, Müller et al. 1999). In the rat and human, hypothyroidism results in impaired basal and stimulated GH secretion and reduced pituitary GH content, effects which are overcome by restoration of euthyroidism (Williams et al. 1985). However, various reports including data in humans indicated that THs may also exert inhibitory effects on GH.

Children with thyrotoxicosis have normal GH responses to provocative stimuli (Katz et al. 1969) but diminished 24-h GH secretion (Finkelstein et al. 1974). In hyperthyroid patients, sleep-related GH release is decreased...
The action of T₃ varies across species with reported stimulation in rats (Evans et al. 1982), and inhibition in humans (Cattini et al. 1986) and in bovines (Silverman et al. 1988). This suggests that the well-documented positive regulation by T₃ of rat GH may not be a common feature among mammalian species.

In birds, T₃ exerts a strong inhibitory effect on GH. In vivo, hypothyroidism is a potent stimulator of GH secretion, and exogenous THs markedly suppress GH release (Harvey 1990b, Harvey et al. 1991). A negative effect of T₃ on GH synthesis and release has also been documented in vitro in birds (Denver & Harvey 1991) and similarly, T₃ is reported to exert inhibitory effects on GH release in vitro in chelonid reptiles (turtles: Denver & Licht 1988). These data from cattle, humans, birds and reptiles suggest that the more generalized vertebrate pattern of thyroidal regulation of pituitary GH may be inhibitory (Denver & Harvey 1991). In teleosts, the few data available on the action of THs on GH production are contradictory, reporting no effect (Nishioka et al. 1985, Luo & McKeown 1991, Moav & McKeown 1992), inhibitory (Baker & Ingleton 1975) or stimulatory (Moav & McKeown 1992) effects on GH.

Here, we investigated the action of THs on GH release and synthesis in the European eel. The phylogenetic position of the eel, as a member of the Elopomorphs group, an ancient group among teleosts, may provide information on ancestral endocrine regulatory processes in vertebrates (De Pinna 1996). We used a long-term, serum-free system to investigate the direct pituitary effect of THs on eel GH in vitro. The effects of THs on GH were followed at the protein GH level by homologous RIA of GH cell content and release and at the messenger RNA (mRNA) level by dot-blot using an homologous complementary DNA (cDNA) probe for eel GH. The ability of THs to inhibit GH was validated by an in vivo study, analyzing their effects on GH serum level and pituitary content.

**Materials and Methods**

**Fish**

Female European eels, Anguilla anguilla L., were netted in ponds in the north and west of France. Animals were transferred to the laboratory and kept in running aerated freshwater for a short time (1 to a few weeks) until experimentation. Animal manipulations were performed according to the recommendations of the French ethical committee and under the supervision of authorized investigators.

**Hormones**

T₃, T₄, cortisol, estradiol, progesterone, testosterone and somatostatin (SRIH-14) were obtained from Sigma Chemical Co. (Saint-Quentin Fallavier, France).

In vivo experiments

Hormonal treatments were administered to female silver eels, netted at the time of the downstream migration (November) in ponds in the north of France (Peronne). Eels were transferred to the laboratory and kept in 100 l aquaria (8 eels/aquarium) in running aerated freshwater (12 ± 2 °C). Since at the silver stage eels have initiated a period of physiological fasting, fish were not fed during the experiment. Fish (8 eels/group) received one perivisceral injection per week of T₃ or T₄ (2 µg/g body weight) suspended in saline (0–15 M NaCl) or of saline alone (controls) for 3 months (December–February). No mortality occurred during the experiment. Body weights (282 ± 11 g, 288 ± 10 g, 285 ± 10 g for controls, T₃- and T₄-treated groups respectively at the beginning of the experiment) were reduced by about 10% at the end of the experiment, with no significant variations between groups (254 ± 10 g, 263 ± 9 g, 258 ± 10 g respectively). The day after the last injection, eels were killed by decapitation. Sera were obtained from blood samples allowed to clot overnight. Pituitaries were quickly removed and kept frozen in 0–15 M NaCl until extraction.

**Primary cultures of eel pituitary cells and in vitro treatments**

In vitro experiments were performed on pituitary cells from batches of yellow (sedentary growth stage) or silver (migratory stage) female eels (body weight 200–300 g) netted the year round in ponds in the west or north of France. A batch of about 100 eels was used for each cell preparation. Each experiment was repeated in three independent studies (performed on different cell preparations from various eel batches). Similar results were obtained with pituitary cell cultures from yellow and silver eels. Results of representative experiments are shown.

Dispersion of pituitary cells was performed using an enzymatic and mechanical procedure as previously described (Rousseau et al. 1998). The number of viable cells, as ascertained by Trypan Blue (Sigma Chemical Co.) exclusion test, represented more than 90% of the total dispersed cells. Cells were cultured on poly-l-lysine (Sigma Chemical Co.) precoated plates (Costar, Cambridge, MA, USA) in a serum-free medium (CM; medium 199 with Earle’s salt, sodium bicarbonate, 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 ng/ml fungizone; Life Technologies, Cergy-Pontoise, France) at 18 °C under 3% CO₂ and saturated humidity. Cells were plated on 96-well plates (Costar) at a density of 62 500 cells/well for measuring GH by RIA (Montero et al. 1998, Rousseau et al. 1998), or on 12-well plates at a density of 500 000 cells/well for measuring mRNAs by dot-blot (Huang et al. 1999a). After 1 day of culture to allow attachment, medium was changed, and treatments were started (day 0).

Stock solutions (10⁻³ M) were diluted in 0·1 M acetic acid (T₃ and T₄), 100% ethanol (cortisol, estradiol,
progesterone, testosterone) or in pure sterile water (SRH-14). They were further diluted with CM just before culture medium renewal. The final concentration of acetic acid or ethanol in culture wells never exceeded 0.2%; control wells were treated with the same concentration of acetic acid or ethanol in CM. Media were collected and treatments were renewed at each sampling time for up to 12 days. Collected media were kept frozen (−20 °C) until RIA of GH. Cultures were stopped before the addition of hormones (day 0) and at the end of the culture for measuring GH cell content or mRNA levels for GH.

Radioimmunoassays

Pituitaries were extracted by sonication (Bioblock Scientific, Illkirch, France) in 0.15 M NaCl, and for measurement of GH cellular contents from in vitro experiments, cells were submitted to osmotic shock (distilled water) and two repeated cycles of freezing and thawing (Rousseau et al. 1998, Huang et al. 1999b).

GH levels from pituitary extracts, sera, cellular contents, and culture media were assayed using an homologous RIA for eel GH (Marchelidon et al. 1996). Results from in vivo studies were expressed as μg/pituitary and ng/ml. For in vitro studies, total GH released into the culture medium was determined as cumulative GH release between each sampling point. GH synthesis in culture was calculated as described (Rousseau et al. 1998, Huang et al. 1999b) and reported as the sum of the cumulative release for 12 days and the variation of cell contents between day 0 and day 12. Results were expressed as nanograms GH per 62 500 cells.

GH cDNA probe

A European eel pituitary cDNA library constructed into λgt10 vector (Quérat et al. 1990) was used to amplify a GH cDNA by PCR. Two primers were designed based on the nucleotide sequences of the Japanese eel GH cDNA (Saito et al. 1990). The conditions for PCR were as follows: 1 µl amplified library lysate was mixed with 0.4 µM of each primer, 200 µM deoxynucleotidetriphosphates, 10 mM Tris–HCl, 1.5 mM MgCl2, 50 mM KCl, pH 8.3 and 1 unit Taq DNA polymerase (Boehringer-Mannheim, Meylan, France) in a final volume of 50 µl. Cycling parameters were 3 min denaturation at 94 °C followed by 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 53 °C and 1 min elongation at 72 °C, the final elongation step was extended to 10 min. PCR products were separated by 1.5% agarose gel electrophoresis, bands excised, purified with JET quick gel extraction kit (JENOMED, Bad Oeynhausen, Germany) and subcloned into PGEM T Easy (Promega-France, Charbonnières, France). PCR products of three independent PCR amplifications were cloned, sequenced on both strands using a dye terminator cycle sequencing kit (Amersham Life Science-France, Les Ulis, France) and sequence identity performed using the BLAST program (Genbank, NCBI, USA). One major fragment of 481 bp was amplified from the pituitary cDNA ligated into λgt10 vector using primer 1 and primer 2, subcloned and sequenced. Comparison of the nucleotide sequences showed a 98% nucleotide identity with the Japanese eel GH cDNA (Saito et al. 1988).

Dot-blot assay of GH mRNAs

Cells were collected and total RNAs were extracted according to the method described by Huang et al. (1999a). Cells were scraped in ice-cold PBS (Life Technologies) using a cell scraper (Costar), centrifuged (12 000 g) for 5 s at 4 °C, the supernatant was removed, and 45 µl TE (10 mM Tris and 1 mM EDTA, pH 7.2) buffer were added. Cells were then kept at −80 °C until RNA extraction. Total RNA was extracted by adding 5 µl 5% Nonidet P-40 (Sigma Chemical Co.) to disrupt cell walls, centrifuged (12 000 g) for 15 min at 4 °C, and the supernatant containing total RNA was collected and denatured at 60 °C for 15 min after adding 30 µl 20 × SSC (standard saline citrate) and 20 µl 37% formaldehyde. RNA was blotted onto Hybond-N+, Nylon membrane (Amersham Pharmacia Biotech, Les Ulis, France) through a Hybri-blot manifold (Life Technologies). The membrane was air-dried and RNAs were immobilized by baking at 80 °C for 2 h.

The PCR-derived cDNA fragment of eel GH was [32P]dCTP-labeled by random priming with the NEBlot-Kit (New England Biolabs, Hitchin, Herts, UK), to a specific activity of 1 × 108 c.p.m./μg and used as a probe. The membrane was prehybridized for 5 h at 42 °C in hybridization solution (ULTRAhybTM, Ambion, Clinsciences, Montrouge, France) and then hybridized with the 32P-labeled eel GH cDNA overnight at 42 °C. After high stringency washing (0.1 × SSC and 0.1% SDS for 30 min at 60 °C), the membranes were autoradiographed. Scanning densitometry and data processing were performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA). The membranes were stripped by boiling in 0.1% SDS and subsequently hybridized under the same conditions with 32P-labeled eel β-actin probe.

Statistical analysis

For in vitro experiments, replicates of six wells (62 500 cells/well) for RIA studies or of four wells (500 000 cells/well) for mRNA studies, were used for each treatment, and the means ± s.e.m. are given. For in vivo experiments, groups of eight eels were used for each treatment, and the means ± s.e.m. are given.
of variance was assessed by Bartlett’s test, and data compared by one-way ANOVA followed by Student-Newman-Keul’s multiple comparison test, using InStat (GraphPad Software, Inc., San Diego, CA, USA).

**Results**

In vivo effects of T₃ and T₄ on pituitary GH content and serum GH level (Fig. 1)

GH pituitary content was high in control eels (102 ± 16 µg/pituitary), in agreement with previous studies (Marchelidon et al. 1996). Chronic treatment with T₃ or T₄ significantly (P<0·05) reduced eel pituitary GH content by about 50% of control values (Fig. 1a). Serum GH level (3·4 ± 0·6 ng/ml) was also significantly (P<0·05) reduced by 50% with T₃ or T₄ treatment (Fig. 1b).

**Kinetics of in vitro effects of T₃ and T₄ on GH release - comparison with SRIH effect (Fig. 2)**

We investigated whether the inhibitory effects of T₃ and T₄ on GH levels could result from a direct pituitary action. Pituitary cells were cultured in serum-free medium to avoid interaction with hormones and growth factors contained in serum (Rousseau et al. 1998). In control wells, sustained GH release was observed throughout the duration of culture (Fig. 2). The total amount of GH released into the medium over 12 days (18 ± 1 µg/62 500 cells) represented 170% of the initial cell content (day 0: 11 ± 1 µg/62 500 cells), reflecting active synthesis of GH during the culture, as reported previously (Rousseau et al. 1998, 1999).

Addition of T₃ or T₄ (100 nM) significantly decreased GH release; the inhibitory effects of T₃ and T₄ were significant after 48 h of incubation (P<0·05) and were maintained up to the end of the culture on day 12 (P<0·001; Fig. 2). Total GH release over 12 days of culture was decreased by 50% by T₃ and T₄ (P<0·001).

The addition of SRIH (100 nM) significantly decreased GH release at each time period of incubation over 12 days (P<0·001), in agreement with our previous studies (Rousseau et al. 1998). After 12 days of culture, GH release was reduced by 95% as compared with controls, an inhibitory effect much stronger than that produced by T₃ or T₄ (P<0·001).

Measurement of GH cellular content at the beginning and at the end of the culture, and of total GH release, allowed us also to estimate the effect of T₃ on GH synthesis in vitro. Control cells showed a sustained synthesis of GH over 12 days of culture. Culture in the presence of 100 nM T₃ induced a significant reduction in GH synthesis, with a value after 12 days (4 ± 0·5 µg/62 500 cells) which was significantly lower (P<0·001) than in control cells (10 ± 1 µg/62 500 cells).

Concentration-dependence of T₃ and T₄ effects on GH release in vitro - comparison with SRIH (Fig. 3)

Cultured pituitary cells were incubated with various concentrations of T₃ or T₄ (0·001 to 1000 nM) for 12 days. The release of GH in the medium was decreased in a concentration-dependent manner by both T₃ and T₄ (Fig. 3). Both T₃ and T₄ produced similar maximal inhibitory effects (about 45% of control values) and maximal inhibition was produced at T₃ or T₄ concentrations of
The median effective dose (ED$_{50}$) was $<0.2$ nM for both hormones, with no significant differences between T$_3$ and T$_4$.

For comparison, the effects of various concentrations of SRIH (0.001 to 1000 nM) were tested in the same conditions. SRIH induced a concentration-dependent inhibition of GH release up to a maximal effect (5% of control values) significantly greater than the suppression by T$_3$ or T$_4$ ($P<0.001$) (Fig. 3).

Specificity of T$_3$ and T$_4$ effects on GH release - comparison with effects of other hormones in vitro (Fig. 4)

To test the specificity of TH action on GH release, we compared the effects of different concentrations (0.001 to 1000 nM) of various steroid hormones which also act via nuclear receptors. In contrast to the concentration-dependent inhibitory effects of T$_3$ and T$_4$, corticosteroid (cortisol) and sex steroids (testosterone, estradiol and progesterone) had no significant effect on GH release at any concentration tested, up to 12 days of culture (Fig. 4).

Effect of T$_3$ on GH mRNA levels in vitro (Fig. 5)

In order to further investigate the inhibitory action of T$_3$ on GH synthesis, we analyzed whether it implicated an action at the level of transcription.

Cell cultures were stopped for mRNA measurement before (day 0) or after various days in the presence or not of 100 nM T$_3$ (Fig. 5a,b). GH mRNA levels increased $\geq 10$ nM. The median efficient dose (ED$_{50}$) was $<0.2$ nM for both hormones, with no significant differences between T$_3$ and T$_4$.

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significantly with culture time in control cells (Fig. 5a; P<0.05 between day 2 and day 0 controls). The presence of T₃ blocked this increase in GH transcription, and this inhibitory effect was significant after 2 days of culture (Fig. 5a, × 0.75 control values, P<0.05). The inhibitory effect of T₃ was specifically exerted on GH mRNA levels (Fig. 5b; × 0.5 control values after 8 days of treatment, P<0.001), no change in β-actin mRNA levels being observed (Fig. 5b).

**Discussion**

Chronic administration of thyroid hormones (T₃ or T₄) significantly decreased both GH pituitary content and serum concentration, indicating that THs inhibit GH synthesis and release in vivo in the eel. The in vivo experiment was performed at the silver stage, when eels are physiologically fasting, in order to avoid possible indirect effects of THs on food intake and metabolism. Accordingly, no significant differences in body weight were observed between control and T₃- or T₄-treated fish at the end of the experiment. However, in vivo inhibitory effects of THs on GH could still reflect indirect actions such as changes in GH response to fasting (Marchelidon et al. 1996) or other indirect pathways.

In order to investigate whether THs were acting directly at the pituitary level to inhibit GH production, we used a serum-free primary culture of pituitary cells. T₃ and T₄ decreased GH release in vitro in a concentration-dependent manner with a maximal suppression of about 50%, similar to that observed in vivo. These data contrast with our earlier studies in which we demonstrated that THs had no effect on luteinizing hormone (LH) production by gonadotropes under the same culture conditions (Huang et al. 1998), assessing the specificity of TH inhibitory effect on somatotrophs. We further investigated the specificity of TH action by comparing the activities of various hormones also acting via the same molecular family of nuclear receptors, such as sex steroids and corticosteroids (Glass 1994). None of the steroids tested (androgen: testosterone; estrogen: estradiol; progesstagen: progesterone and glucocorticoid: cortisol) had any significant effect on GH release, in contrast to our earlier studies in which we demonstrated stimulatory effects of testosterone and cortisol on LH (Huang et al. 1999a).

In the eel, T₃ and T₄ had similar maximal inhibitory effects on GH release in vitro at concentrations within the physiological range (4 to 8 nM) (de Luze & Leloup 1984). These inhibitory effects of TH could be observed throughout the 12-day period of culture, indicating no desensitization. This suggests that THs may exert a physiological and chronic role in the negative regulation of GH levels in the eel. Support for this hypothesis comes from studies reporting significant inhibition of GH release by pituitaries cultured for 6 days in the presence of a high dose (1 µg/ml) of T₄ (Baker & Ingleton 1975). Our data on dispersed cultured cells indicate that the effect of TH acts directly on pituitary cells and is not mediated by the hypothalamic axonal endings which directly innervate the adenohypophysis in teleosts. These data are thus the first demonstration of an inhibitory effect of TH on GH release in vivo and in vitro in a teleost species. Indeed, previous studies in teleosts failed to show any significant alterations of GH in response to T₃ in vivo (rainbow trout: Moav & McKeown 1992) and in vitro (tilapia: Nishioka et al. 1985, carp: Luo & McKeown 1991).

These inhibitory effects of TH on GH in the eel are similar to those previously demonstrated in tetrapod vertebrates. In the chicken, exogenous TH markedly suppressed circulating GH levels, while thyroidectomy or goitrogen treatment resulted in a rise in the basal GH level.
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In the eel, comparison of T$_3$ and T$_4$ dose-dependent effects in vitro indicated that both hormones had a similar maximal effect and ED$_{50}$, suggesting that they were acting through similar mechanisms. In teleost pituitaries, nuclear TH binding sites have been described which bind T$_3$ with approximately 20–50 times greater affinity than T$_4$, properties closely resembling those of mammalian TH nuclear receptors (Bres & Eales 1990). These data suggest that T$_3$, because of its weaker affinity for the receptor, may act after deiodination into T$_3$ (McNabb 1992). Local deiodination of T$_4$ into T$_3$ is regulated by type II and type I 5′-deiodinases in rat anterior pituitary cells (Köhrle et al. 1995). Although deiodinase activities have been characterized in various tissues in teleosts, including liver, kidney, gill and brain (Eales et al. 1993), the existence of such enzymes in the pituitary has not yet been determined. The high capacity of T$_4$ to inhibit GH release in the eel in vitro likely reflects the presence of an active pituitary deiodinase. A similar conclusion was previously raised in view of the similar efficiency of both T$_3$ and T$_4$ to inhibit TSH mRNA levels in eel pituitary cells, in the same culture conditions (Pradet-Balade et al. 1997). In the turtle, T$_3$ and T$_4$ also had similar inhibitory effects on GH secretion in vitro (Denver & Licht 1988). In contrast, in the domestic fowl, T$_3$ not only inhibited GH release, but also blocked the accumulation of newly synthesized GH through a direct action on the pituitary (Denver & Harvey 1991). In the present study, we investigated whether the inhibitory effect of T$_3$ on GH synthesis could be mediated by an inhibition of GH mRNA levels. After cloning an homologous eel GH cDNA probe, we demonstrated that T$_3$ significantly decreased GH mRNA levels in eel cultured pituitary cells. These data indicate that the T$_3$ inhibitory effect may, at least partly, be mediated by a decrease in GH mRNA steady state levels. In contrast to the present study in the eel, data in other teleost species indicated a stimulatory effect of T$_3$ on GH mRNA levels. In the carp, T$_3$ increased GH mRNA levels in pituitary fragments in vitro (Farchi-Pisanty et al. 1995). In the rainbow trout, one-week T$_3$ treatment in vivo increased steady state levels of GH mRNA but produced no alterations in pituitary or plasma GH levels (Moxv & McKeown 1992). Species-related variations in the effects of T$_3$ on GH mRNA levels were also observed among mammals. In the rat, T$_3$ was shown to stimulate GH mRNA levels in cultured pituitary tumor cell lines (Samuels & Shapiro 1976). In contrast, T$_3$ reduced GH mRNA levels in bovine pituitary cells in vitro (Silverman et al. 1988), a situation similar to that found for human GH (Valcavi et al. 1992). In the rat, the positive effect of T$_3$ on GH mRNA levels has been attributed to a direct stimulatory effect on GH gene transcription.
mediated by the presence of positive thyroid responsive elements (TRE) (Theill & Karin 1993). In contrast, T₃ blocks human GH gene transcription even after transfection into rat pituitary cells (Cattini et al. 1986, Morin et al. 1990), indicating a regulation opposite to that of the rat GH gene. In an analysis of rat GH promoter constructs, Brent et al. (1991) were able to show that varying the position of TRE induced positive or negative regulation by T₃.

In conclusion, our study of a primitive teleost demonstrates negative regulation of GH expression and release exerted by THs acting directly on pituitary cells in vitro. This inhibition, confirmed by in vitro studies, may represent an ancestral negative feedback of THs on GH production, reflecting the crosslink between the thyro- tropic and somatotrope axes (Fig. 6). It is clear that variation in the effects of THs on GH regulation are observed amongst vertebrate species. The negative regulation demonstrated here in an early vertebrate is also observed in birds, reptiles and some mammals including humans. This may represent an ancestral and more generalized vertebrate pattern of TH regulation of pituitary GH.

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