Thyroidal inhibition of chicken pituitary growth hormone: alterations in secretion and accumulation of newly synthesized hormone

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ABSTRACT

Hypothyroidism reduces GH synthesis and release in several mammalian species, in which thyroid hormone directly stimulates GH gene transcription. In contrast, hypothyroidism stimulates GH secretion in birds, in which thyroid hormone directly inhibits pituitary GH release. We have, therefore, investigated the effects of thyroid status on the accumulation of newly synthesized GH in the pituitaries of 8- to 10-week-old Leghorn cockerels in vitro and in vivo. The incorporation of [35S]methionine into immunoprecipitable GH (T3, 100 µg/kg per day for 10 days) in vivo did not significantly alter the accumulation of [35S]GH in vitro but did block the release of [35S]GH in response to a GH secretagogue (thyrotrophin-releasing hormone; exposure to 280 nmol/l for 30 min) and reduced immunoprecipitable pituitary GH content. Pretreatment of glands from euthyroid birds with T3 (100 nmol/l) in vitro (for 20 h) reduced the basal accumulation of [35S]GH as well as that induced by another GH secretagogue (GH-releasing factor; 100 nmol/l) during a 6-h labelling period. These results show that, unlike the generally stimulatory action of thyroid hormone in mammals, in birds, T3 exerts a direct inhibitory effect on the accumulation of newly synthesized pituitary GH.

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INTRODUCTION

It is well established that thyroid hormone is required for pituitary growth hormone (GH) secretion in man and several other mammalian species. Hypothyroidism results in impaired basal and stimulated GH secretion and reduced pituitary GH content, effects overcome by the restoration of euthyroidism (Williams, Maxon, Thorner & Frohman, 1985; Dieguez, Jordan, Harris et al. 1986; Root, Shulman, Root & Diamond, 1986). Tri-iodothyronine (T3) has been shown to stimulate GH gene transcription in rats and pituitary tumour cell lines (Oppenheimer, Schwartz, Mariash et al. 1987; Samuels, Aranda, Casanova et al. 1988). However, the stimulatory effect of thyroid hormone on GH is not common to all mammalian species nor is it common to other vertebrate classes. Negative regulation of GH by thyroid hormone has been demonstrated in human (Mulchahey, DiBlasio & Jaffe, 1988) and bovine (Silverman, Kaplan, Grumbach & Miller, 1988) pituitary cells. In birds, hypothyroidism is a potent stimulator of GH secretion, in which exogenous thyroid hormone markedly suppresses GH release (Harvey, 1989). Thyroid hormone directly inhibits the release of GH from chicken pituitary glands (Harvey, 1990b) and may therefore block the accumulation of newly synthesized GH in avian species. We have investigated this possibility in the present study by determining the effect of thyroid status on the incorporation of [35S]methionine into immunoprecipitable GH in the chicken pituitary gland.
MATERIALS AND METHODS

Animals

Eight-week-old leghorn cockerels (600–700 g body weight) were maintained in battery cages on a 12 h light: 12 h darkness photoperiod and free access to water and feed; they were between 8 and 10 weeks of age when killed.

Hormones and reagents

Methionine-free Dulbecco’s modified Eagle’s medium (DMEM) was prepared from powdered DME-deficient medium (Sigma, St Louis, MO, U.S.A.) and supplemented with 4·0 mmol glutamine/l, 800 μmol leucine/l, 800 μmol lysine/l, 10 mmol Hepes/l, 0·1% (w/v) bovine serum albumin, antibiotic-antimycotic solution (Gibco, Grand Island, NY, U.S.A.) and sodium bicarbonate. Labelling media were prepared by adding [35S]methionine (New England England Ontario, Canada) to a final concentration of 30 μCi/ml. Methimazole, 3,5,3’-tri-iodothyronine (T3) and thyrotrophin-releasing hormone (TRH) were from Sigma and human growth hormone-releasing factor (GRF) was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.). Protein A (IgGisorb) was from The Enzyme Co., Malden, MA, U.S.A.

Pituitary culture

Techniques used in the incubation of whole chicken pituitary glands were similar to those described by Hall, Chadwick, Bolton & Scanes (1975) and Hall & Chadwick (1983). Animals were killed by rapid cervical dislocation and pituitaries were collected into individual wells (24-well plates) containing 1 ml ice-cold DMEM supplemented with 200 μmol methionine/l. The medium was changed to warm (39 °C) DMEM and tissues were placed in a humidified incubator under an atmosphere of 95% O2 and 5% CO2. Just before being labelled with [35S]methionine, glands were rinsed in methionine-free DMEM for two h periods to deplete intracellular methionine. All labelling with [35S]methionine was carried out in a volume of 0·5 ml. At the end of the incubation the glands were rinsed with 1 ml cold phosphate-buffered saline, and media and tissues were collected (pituitary glands were placed in 1 ml 10 mmol Tris/l plus 1% (v/v) Triton X-100 and sonicated for 30 s) and stored at −20 °C until assay. Pituitaries were thawed and refrozen three times before assay to enhance tissue disruption and hormone release. Tissue protein content was determined using the BioRad protein assay (Bio-Rad, Richmond, CA, U.S.A.).

Experiment 1

After the 2-h rinse in methionine-free DMEM, tissues (five glands per group) were incubated in the presence of [35S]methionine with or without TRH (1 μg/ml = approximately 2·8 μmol/l) for one 6-h period.

Experiment 2

Birds (n = 8 per treatment) were given daily i.p. injections for 10 days of 0·9% (w/v) NaCl(control; 1 ml/kg), methimazole (50 mg/kg) to induce hypothyroidism (Harvey, Scanes & Klandorf, 1988) or T3 (100 μg/kg) to induce hyperthyroidism (Harvey, 1983). Birds were killed 2 h after the last injection. Pituitary glands were removed, rinsed for 2 h and then labelled for 4 h. The labelling medium was collected and replaced with 0·5 ml DMEM (without [35S]methionine) containing 100 ng TRH/ml (approximately 280 nmol/l) and the incubation continued for 30 min.

Experiment 3

Tissues (seven to eight glands per group) were preincubated for 20 h with or without 100 nmol T3/l. The glands were then rinsed in methionine-free DMEM and labelled for 6 h in 100 nmol T3/l in the presence or absence of either 100 nmol TRH or human GRF/l.

Immunoprecipitation

The amount of newly synthesized GH in media and tissues was quantified by specific immunoprecipitation using a procedure based on techniques described by Platt, Karlsen, Lopez-Valdivieso et al. (1986). An aliquot of each sample (5 μl of the pituitary homogenate, 25–30 μl of the incubation media) was incubated with 10 μl chicken GH antiserum (anti-cGH; Harvey & Scanes, 1977) diluted 1:1 or 1:3 for the pituitary and medium samples respectively. Aliquots of the same samples were similarly incubated with normal rabbit serum (NRS) as a control. The incubations were at room temperature for 1 h. Samples were then precipitated with a suspension of protein A (suspended in 25 mmol Tris/l with 0·3 mol unlabelled methionine/l to reduce non-specific binding) for 30 min, layered over 1 mol sucrose/l and centrifuged for 10 min at 2000 g. The pellet was then washed twice with 25 mmol Tris/l, 5 mmol EDTA/l and 1% (v/v) Triton X-100 and resuspended in either reducing electrophoresis sample buffer (0·125 mol Tris/l, 4% (w/v) sodium dodecyl sulphate (SDS), 20% (v/v) glycerol and 10% (v/v) mercaptoethanol) or scintillation cocktail for direct counting. The amounts of sample and antiserum used were derived empirically by titration to ensure maximal precipitation of labelled GH. This was done by adding a trace amount of 125I-labelled GH to media/tissue samples or buffer before immunoprecipitation and counting the precipitates in a gamma counter. Total newly synthesized protein was determined by trichloro-
acetic acid (TCA) precipitation as described by Ramey, Highsmith, Wilfinger & Baldwin (1987). The percentage of newly synthesized protein that was GH ranged from 11 to 24%.

**Electrophoresis**

Polyacrylamide gel electrophoresis (PAGE) was employed in the purification of immunoprecipitates before counting. SDS-PAGE was performed under denaturing, reducing conditions with an acrylamide concentration of 12.5% in the resolving gels and 4% in the stacking gels. Pellets from the immunoprecipitation were dissolved in electrophoresis sample buffer and incubated at room temperature for 1 h, boiled for 15 min and then centrifuged for 2 min in a tabletop centrifuge. After electrophoresis, gels were either prepared for fluorography (see below) or sliced into 0.5 cm to 1 cm sections for scintillation counting. Gel slices were solubilized by placing them in counting vials with 0.25 ml 30% (v/v) H$_2$O$_2$ and incubating them overnight at 55°C. Scintillation cocktail (5 ml) was then added and the vials were counted with an LKB scintillation counter. The region of the gel where the labelled GH ran was determined in pilot studies by sectioning the entire lane and counting individual slices (this was corroborated by fluorography; see below). In later tests, only the region of the gel containing the labelled GH was cut out and counted.

The importance of separating the immunoprecipitates on PAGE vs direct counting was evaluated using samples from experiment 1. This analysis revealed a strong correlation ($r = 0.92$, $P < 0.001$) between results obtained with both methods and so direct counting of the immunoprecipitates was used in later tests.

The contribution of non-specific binding as determined by NRS precipitation was also evaluated. Media and tissue homogenates from experiment 1 were precipitated with both anti-cGH and NRS and counted (both directly and after separation on PAGE). This revealed no significant correlation ($r = 0.047$) between the amount of radioactivity in the two precipitates; i.e. the counts obtained with the NRS precipitates were all similar, having a coefficient of variation (C.V.) which averaged 18% (C.V. = 28% for the immunoprecipitates), and so subtracting the NRS background did not alter the results obtained with the anti-cGH alone. Therefore, for economy, the NRS precipitation was omitted from later experiments.

**Fluorography**

Fluorography of gels was carried out as described by Laskey & Mills (1975). Gels were dehydrated in dimethyl sulphoxide (DMSO), impregnated with 2,5-diphenyloxazole (PPO) (both from Sigma), dried and exposed with an intensifying screen at −70°C for 1–2 weeks using Kodak X-OMAT film.

**Chicken GH radioimmunoassay (RIA)**

The concentrations of GH in plasma and in incubation media and pituitary glands were estimated using an homologous chicken GH RIA as described by Harvey & Scanes (1977) and Harvey (1990b).

**Statistical analyses**

Results were analysed using the SOLO statistical computer program (BMDP statistical software, Los Angeles, CA, U.S.A.). One-way analysis of variance (ANOVA), linear regression and unpaired $t$-test were done on log-transformed data. A non-parametric test (Mann–Whitney U test) was used in place of the $t$-test when the group variances were heterogeneous. Since there were no significant correlations between hormone content (both newly synthesized and radioimmunoassayable) and protein content in any of the experiments, the amount of newly synthesized hormone is expressed as c.p.m. per pituitary gland rather than as a function of tissue protein content.

**RESULTS**

**Experiment 1: Effects of in-vitro TRH treatment on the accumulation of [$^{35}$S]GH by chicken pituitary glands**

Immunoprecipitation of pituitary incubation medium with anti-cGH followed by PAGE and fluorography produced a single band with a molecular weight which corresponded to that reported for chicken GH (23 500; see Houston & Goddard, 1988) (Fig 1). The identity of this band as GH was corroborated by coelectrophoresis of radioiodinated chicken GH. Incubation of medium with NRS did not produce any detectable bands on PAGE. Immunoprecipitation of the pituitary homogenate produced a distinct band corresponding to GH and several minor bands which also showed up with NRS.

Exposure of chicken pituitary glands to TRH (1 μg/ml) in vitro induced an approximately twofold increase in the release of both immunoadsorbable GH (iGH, as measured by RIA; control, 2.17 ±0.09 μg/ml; TRH, 4.0 ±0.22 μg/ml; $P < 0.001$, $t$-test) and newly synthesized GH (see Fig. 2; $P < 0.009$, Mann–Whitney U-test). TRH also produced a significant ($P < 0.036$, $t$-test) increase in the release of newly synthesized (TCA-precipitable) protein (data not shown). However, there was no significant effect of TRH on pituitary content of, or total (pituitary plus secreted), newly synthesized GH or protein.
FIGURE 1. Fluorogram of a 12.5% polyacrylamide sodium dodecyl sulphate-reducing gel run with immunoprecipitates of medium and tissue homogenate collected from a chicken pituitary gland incubated with \[^{[35S]}\]methionine. Lanes 1 and 3 are respectively medium and pituitary homogenate that were immunoprecipitated with antiserum to chicken GH. Lanes 2 and 4 are aliquots of the same samples immuno-precipitated with normal rabbit serum. The molecular weights of the major bands (GH) in both medium and tissue were identical and corresponded to the reported molecular weight of chicken GH (23 000).

FIGURE 2. Production of newly synthesized GH by chicken pituitary glands in vitro. Glands were labelled with \[^{[35S]}\]methionine for 6 h in the presence (solid bars) or absence (cross-hatched bars) of TRH (1 μg/ml = 2.8 μmol/l). Values are means ± S.E.M. (n = 5/group). *P < 0.01 (Mann–Whitney U test) compared with control.
TABLE 1. Effects of treatment with methimazole or tri-iodothyronine (T₃) on circulating GH levels in leghorn cockerels. Values are means ± S.E. (n = 8)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GH (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.65 ± 2.54</td>
</tr>
<tr>
<td>Methimazole</td>
<td>33.24 ± 5.21</td>
</tr>
<tr>
<td>T₃</td>
<td>3.47 ± 0.22</td>
</tr>
</tbody>
</table>

Birds were injected daily with 0-9% (w/v) saline, methimazole (50 mg/kg) or T₃ (100 µg/kg) for 10 days.

Experiment 2: Effects of daily injections of methimazole or T₃ on accumulation of [³⁵S]GH by chicken pituitaries in vitro

Treatment with methimazole (50 mg/kg per day for 10 days) increased, and T₃ (100 µg/kg per day) decreased, circulating concentrations of GH (Table 1; F(2,18) = 51.15, P < 0.001, ANOVA). Pituitary glands incubated in vitro did not differ in their basal iGH output or in their basal release of newly synthesized GH (Fig. 3). The in-vitro release of iGH in response to TRH was significantly increased (F(2,19) = 25.04, P < 0.001) by methimazole and reduced by T₃ injections and this pattern of response was paralleled by the newly synthesized GH (F(2,18) = 29.83, P < 0.001). Treatment with T₃ did not alter, but methimazole significantly increased, pituitary (F(2,18) = 5.39, P = 0.016) and total (secreted plus pituitary; F(2,17) = 5.94 P = 0.012) newly synthesized GH, whereas pituitary and total iGH were significantly decreased (F(2,18) = 10.87, P < 0.001 and F(2,17) = 53.96, P < 0.001 respectively) by T₃ but were unaffected by methimazole.

Experiment 3: Effects of in-vitro treatment with T₃ and neuropeptides on pituitary [³⁵S]GH accumulation

The release of (F(5,37) = 22.85, P < 0.001, ANOVA), pituitary content of (F(5,37) = 17.93, P < 0.001) and total (media + pituitary; F(5,37) = 35.58, P < 0.001) [³⁵S]GH were all significantly altered by the in-vitro treatments (Fig. 4). When pituitary glands were pre-incubated with 100 nmol T₃/l for 20 h, the secretion of [³⁵S]GH was significantly reduced (P < 0.05, Duncan’s multiple-range test). The pituitary content of [³⁵S]GH after the in-vitro T₃ treatment averaged less than control glands but was not statistically significant; however, the total (media plus pituitary) [³⁵S]GH was significantly (P < 0.05) reduced, suggesting a direct inhibitory effect of T₃ on the accumulation of newly synthesized GH in the pituitary. Exposure to 100 nmol hGRF/l produced a large increase in [³⁵S]GH (both secreted and pituitary content) and this effect was blocked by T₃. TRH treatment (100 nmol/l) had no significant effect on the release of [³⁵S]GH, nor did it affect pituitary [³⁵S]GH content. Glands exposed to TRH plus T₃ accumulated [³⁵S]GH in amounts that were comparable with glands that received T₃ alone, i.e. significantly reduced accumulation of newly synthesized GH compared with control glands. Changes in newly synthesized protein (TCA-precipitable) matched those of newly synthesized GH (see Table 2; P < 0.001 for all).

The release of iGH, but not the pituitary content or total (media plus pituitary), was altered by in-vitro exposure to human GRF (Table 3; F(5,35) = 6.17, P < 0.001, ANOVA); none of the other treatments had any significant effect on iGH.

DISCUSSION

We have shown that in the domestic fowl, T₃ not only inhibits GH release (for review see Harvey, 1989), but it can also block the accumulation of newly synthesized GH through a direct action on the chicken pituitary. This is in contrast to humans and rodents in which thyroid hormone is required for maintaining normal circulating GH levels (Peake, Birge & Daughaday, 1973; Hervas, Escobar & Escobar del Ray, 1975; Root et al. 1986). Thyroid hormone stimulates pituitary GH production in tumour-derived and normal rat pituitary cells by directly increasing GH gene transcription (see Oppenheimer et al. 1987; Samuels et al. 1988). However, in humans, despite being required for normal circulating GH levels, there is evidence that T₃ can directly inhibit transcription of the GH gene. For instance, T₃ blocks human GH gene transcription when it is transfected into rat pituitary cells (Cattini, Anderson, Baxter et al. 1985) and recent work using constructs of the human GH gene transfected into rat pituitary tumour (GC) cells suggests that the human GH gene may possess elements in the promoter region which can be negatively regulated by thyroid hormone, and elements within the gene that respond positively to thyroid hormone (Morin, Louette, Voz et al. 1990). Furthermore, T₃ has been shown to inhibit in-vitro GH release from human fetal pituitary cells (Mulchahey et al. 1988). Thus, although the predominant role of thyroid hormone in GH regulation in humans may be stimulatory (i.e. maintenance of normal GH levels in the euthyroid state), thyroid hormone can also exert an inhibitory effect on the GH gene. On the other hand, birds exhibit only negative thyroidal regulation of GH.

Another notable exception to the rodent paradigm is the bovine pituitary, in which T₃ reduces GH mRNA levels (Silverman et al. 1988). These results led Silverman et al. (1988) to conclude that ‘hormonal
responses of somatotropes vary significantly among mammalian species. The turtle is another vertebrate species which shows negative regulation of GH by thyroid hormone (Denver & Licht, 1988a,b). Thus, the results obtained in cattle and humans (Mammalia), chicken (Aves) and turtles (Reptilia) suggest that a more generalized vertebrate pattern of thyroidal regulation of pituitary GH may be one of inhibition.
TABLE 2. Effects of in-vitro treatment of chicken pituitary glands with tri-iodothyronine (T₃) and neuropeptides (TRH and human growth hormone-releasing factor; GRF) on trichloroacetic acid (TCA)-precipitable newly synthesized protein. Values are means ± S.E.M. for the numbers of experiments given in parentheses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Secreted (c.p.m./gland × 10⁻⁶)</th>
<th>Pituitary</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>1.8 ± 0.2b</td>
<td>11.7 ± 0.5c</td>
<td>13.4 ± 0.5bc</td>
</tr>
<tr>
<td>T₃ (7)</td>
<td>0.8 ± 0.1c</td>
<td>10.3 ± 1.1c</td>
<td>11.2 ± 1.2d</td>
</tr>
<tr>
<td>TRH (7)</td>
<td>1.7 ± 0.1b</td>
<td>14.9 ± 1.2b</td>
<td>16.6 ± 1.2b</td>
</tr>
<tr>
<td>TRH + T₃ (7)</td>
<td>0.9 ± 0.1c</td>
<td>10.5 ± 0.4c</td>
<td>11.4 ± 0.4cd</td>
</tr>
<tr>
<td>GRF (7)</td>
<td>3.5 ± 0.3a</td>
<td>24.0 ± 0.5a</td>
<td>27.5 ± 0.6a</td>
</tr>
<tr>
<td>GRF + T₃ (7)</td>
<td>0.9 ± 0.1c</td>
<td>7.4 ± 0.6a</td>
<td>8.3 ± 0.7e</td>
</tr>
</tbody>
</table>

Means within columns with the same letter are not significantly (P < 0.05) different (Duncan's multiple-range test).

Pituitary glands were preincubated with or without 100 nmol T₃/l for 20 h before labelling for 4 h with [³⁵S]methionine, in the presence of neuropeptides (both TRH and human GRF were given at a dose of 100 nmol/l); T₃ was present in the medium during the labelling period.

 hyperthyroidism decreases circulating concentrations of GH (for review see Harvey, 1989). In the present study, methimazole treatment (hypothyroidism) resulted in an increase in the in-vitro release of [³⁵S]GH in response to TRH and an increase in the total accumulation of newly synthesized GH (secreted + pituitary; see Fig. 4); these changes were coincident with an increase in the serum GH concentration (see Table 1). The effects of thyroid status on the accumulation of [³⁵S]GH could represent either an increase in the rate of biosynthesis or a decrease in the rate of degradation or both. In support of our results, Hoshino & Yamamoto (1977) reported that hypothyroid dwarf chickens, which have increased circulating GH levels (Scanes, Marsh, Decuypere & Rudas, 1983; Scanes, Denver & Bowen, 1986), demonstrate increased amounts of newly synthesized GH in the pituitary. The increase in newly synthesized GH in the present study was not, however, reflected in an increase in pituitary stores of iGH. Because of the presumed increase in the endogenous release rate, hypothyroidism may increase the biosynthetic rate (or decrease the degradation rate) of GH without resulting in an increase in pituitary stores of iGH (see Fig. 3). Although the metabolic clearance rate of GH has been shown to change with thyroidal state (Harvey, 1989), the increase in plasma GH levels seen in hypothyroid birds probably results primarily from increased pituitary secretion due to a combination of (1) increased responsiveness to TRH through up-regulation of TRH receptors (Harvey & Baidwan, 1990), (2) reduced hypothalamic inhibitory tone (i.e. decreased somatostatin production; see Harvey, 1989), (3) increased hypothalamic stimulation (Harvey, 1989)

Role of thyroid hormone in regulating circulating levels of GH in birds

A role for the thyroid in regulating plasma GH levels in birds seems likely since hypothyroidism increases and

FIGURE 4. Effects of a 20-h preincubation with 100 nmol tri-iodothyronine (T₃)/l on the in-vitro GH biosynthetic response to TRH and human growth hormone-releasing factor (GRF) by pituitary glands of 10-week-old leghorn cockerels. After preincubation, glands were labelled with [³⁵S]methionine for 6 h. The neuropeptides were given at a dose of 100 nmol/l for each; T₃ was present in the incubation medium during exposure to the neuropeptides. The secreted (top), pituitary content (middle) and total (bottom) newly synthesized GH was determined by specific immunoprecipitation. Values are means ± S.E.M. (n = 7–8/group). Means with the same letter are not significantly different (P < 0.05) (Duncan's multiple-range test).
Table 3. Changes in immunoassayable GH following in-vitro treatment of chicken pituitary glands with tri-iodothyronine (T₃) and neuropeptides (TRH and human growth hormone-releasing factor; GRF). Values are means ± S.E.M. for the number of experiments given in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Secreted GH (µg/l)</th>
<th>Pituitary GH (µg/gland)</th>
<th>Total GH (µg/gland)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>21.6 ± 3.1b</td>
<td>105.9 ± 20.8a</td>
<td>127.5 ± 23.2a</td>
</tr>
<tr>
<td>T₃ (7)</td>
<td>24.9 ± 2.3b</td>
<td>97.5 ± 7.5a</td>
<td>122.6 ± 8.7a</td>
</tr>
<tr>
<td>TRH (7)</td>
<td>25.3 ± 3.2b</td>
<td>87.9 ± 7.4a</td>
<td>113.3 ± 6.7a</td>
</tr>
<tr>
<td>TRH + T₃ (7)</td>
<td>19.0 ± 0.9b</td>
<td>80.1 ± 3.8a</td>
<td>99.0 ± 4.0a</td>
</tr>
<tr>
<td>GRF (7)</td>
<td>35.9 ± 8.0a</td>
<td>78.0 ± 6.3a</td>
<td>128.8 ± 8.9a</td>
</tr>
<tr>
<td>GRF + T₃ (7)</td>
<td>30.5 ± 5.8a</td>
<td>75.7 ± 8.6a</td>
<td>106.3 ± 13.1a</td>
</tr>
</tbody>
</table>

Means within a column with the same letter are not significantly (P < 0.05) different (Duncan’s multiple-range test).

See Table 2 for in-vitro culture methods.

and (4) increased GH biosynthesis and/or reduced degradation.

Chronic hyperthyroidism reduced circulating GH levels and decreased pituitary stores of iGH, suggesting that GH biosynthesis was reduced (see Fig. 3). However, this was not reflected in a reduced accumulation of [³⁵S]GH in vitro. Incubating the glands in vitro without thyroid hormone may, however, have allowed recovery of the basal synthetic rate or perhaps a reduction in the rate of degradation. Metabolic labelling in vivo or inclusion of T₃ in the incubation medium of glands from hyperthyroid birds may address this question. The reduction in the release of both iGH and newly synthesized GH in response to TRH by glands from hyperthyroid birds (experiment 2) may have resulted from a reduction in the releasable pool of hormone and from down-regulation of TRH receptors (Harvey & Baidwan, 1990). Thus, the depressive effects of hyperthyroidism on plasma GH levels may be operating through both reduced availability of releasable GH and reduced responsiveness to neuropeptides.

Site of action of thyroid hormone on accumulation of newly synthesized GH

Several sites of action for thyroid hormone in GH regulation have been postulated: hypothalamus, pituitary and tissue(s) involved in GH clearance. That thyroid hormone can act directly on the chicken pituitary to inhibit secretagogue-stimulated GH release has been shown through in-vitro studies (Scanes, Klandorf, Carsia & Perez, 1986; Donoghue, Perez, Diamante et al. 1990; Harvey, 1990b). One possible site of action for this effect may be neuropeptide receptors on the somatotroph membrane, since in-vitro exposure to T₃ reduced binding of [³H]Me-TRH to chicken pituitary membranes (Harvey & Baidwan, 1990). The present data provide evidence for another site of action for thyroid hormone distal to neuropeptide receptors in chicken pituitary cells since T₃ was effective in vitro in reducing the basal (unstimulated) release of the total newly synthesized GH (see Fig. 4). This site may be at the level of the GH gene, but other possible sites for thyroid hormone action include alterations in intracellular GH turnover, mRNA stability and effects on components of the secretory apparatus (see Oppenheimer et al. 1987, Samuels et al. 1988).

Role of neuropeptides in regulating pituitary GH

Both TRH and GRF have been shown to be effective in stimulating GH release in birds both in vivo and in vitro (for reviews see Hall, Harvey & Scanes, 1986), and there exists considerable evidence for a physiological role for TRH in the control of GH in avian species (see Harvey, 1990a). The present results confirm earlier observations on the effects of neuropeptides on the release of iGH in vitro, except for TRH in experiment 3 where there was not significant stimulation. This lack of response may have been due to a differential loss of TRH responsiveness following prolonged in-vitro culture. Reduced GH responses to TRH by chicken hemipituitaries have been observed in prolonged perfusion (Harvey, 1990b), and chicken pituitary cells maintained in primary culture for 48 hours before testing did not release GH in response to TRH, but did respond to GRF (Perez, Malamed & Scanes, 1987). The limited comparisons between TRH and GRF that can be made from the present study suggest that the two secretagogues may exert different effects on GH release and synthesis. The human GRF was highly stimulatory in releasing GH and dramatically increased the total amount of newly synthesized GH (see Fig. 4), whereas TRH stimulated release of newly synthesized GH, but it did not alter the total amount of [³⁵S]GH (see Fig. 2). Thus, while TRH may act primarily

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on hormone release, GRF may act to stimulate release and to initiate GH synthesis or reduce degradation.

The stimulatory effect of human GRF on GH release and the accumulation of newly synthesized GH in the bird pituitary is consistent with the effects of this secretagogue in mammals (for review see Guillemin, 1986). GRF has been shown to increase rat GH mRNA levels by increasing the rate of GH gene transcription (Barinaga, Yamamoto, Rivier et al. 1983; Barinaga, Bilezikjian, Vale et al. 1985; Samuels et al. 1988). Furthermore, Barinaga et al. (1985) showed that the induction of GH gene transcription by GRF was not dependent on GH release (i.e. not a mass action effect involving repletion of the hormone pool following release of stored hormone). Although we observed an increase in [35S]GH after human GRF treatment, Aramburo, Donoghue, Montiel et al. (1990) did not find enhancement of [32P]phosphate incorporation into GH by GRF in primary cultures of chicken pituitary cells. However, phosphorylated GH may represent only a small amount of the total pool of hormone. Incorporation of [35S]methionine in the present study is a more accurate indicator of total newly synthesized hormone, since this amino acid should be incorporated into all forms of GH.

In contrast to mammals, the GH response to GRF by the bird pituitary is inhibited by T3. Thyroid hormone enhances the responsiveness of human and rat pituitaries to GRF (Dieguez, Foord, Peters et al. 1985; Williams et al. 1985; Dieguez et al. 1986; Root et al. 1986). On the other hand, GRF mRNA content in the rat hypothalamus is increased in hypothyroidism and decreased in hyperthyroidism (Jones, Burrin, Ghaetei et al. 1990). In the chicken, the stimulation by human GRF of release and accumulation of [35S]GH was dramatically reduced by in-vitro exposure to T3 (see Fig. 4). Thus, in the bird, T3 not only blocks the release of hormone induced by human GRF but can also block GRF-initiated accumulation of newly synthesized GH. It is not known whether thyroid status regulates hypothalamic GRF mRNA or pituitary GRF receptors in the chicken; however, it is unlikely that the thyroidal inhibition of [35S]GH accumulation induced by human GRF in vitro is due entirely to down-regulation of GRF receptors, since the amount of [35S]GH in glands exposed to T3 and human GRF was reduced below control (untreated glands) levels, suggesting that the T3 was either blocking transcription or translation directly or increasing GH turnover.

In summary, our results show that thyroid status can alter the capacity of the chicken pituitary gland to accumulate newly synthesized GH in a manner that is directly opposite to that observed in some mammals. Whether the effect of T3 is operating at the level of transcription, translation or hormone turnover remains to be determined. Finally, our data predict a role for GRF in modulating the accumulation of newly synthesized GH in the bird pituitary, an effect that is blocked by thyroid hormone.

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