Differential effects of T₄ and T₃ on TRH- and GRF-induced GH secretion in the domestic fowl

S Harvey ¹*, E Decuypere ², VM Darras ³, L Berghman ⁴

¹ Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7;
² Laboratory for Physiology of Domestic Animals, Katholieke Universiteit Leuven;
³ Laboratory for Comparative Endocrinology, Katholieke Universiteit Leuven;
⁴ Neuroendocrinology and Immunological Biotechnology, Katholieke Universiteit Leuven, B-3030 Leuven, Belgium

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Summary — The in vivo growth hormone (GH) response of immature domestic fowl to thyrotrophin-releasing hormone (TRH) and GH-releasing factor (GRF) was suppressed in birds fed diets supplemented (1 ppm) with triiodothyronine (T₃) or given bolus intraperitoneal (ip) injections (100 µg/kg for 10 d) of T₃. Supplementation (ppm) of the diet with T₄ had no effect on secretagogue-induced GH release. Exogenous T₃ or T₄ suppressed basal, TRH- and GRF-induced GH release 2 h after daily ip administration (100 µg/kg for 10 d). 24 h after the last injection, only T₃ was effective in inhibiting basal and stimulated GH secretion in vivo. The systemic administration of T₃ was followed 2 h and 24 h later by a downregulation of pituitary TRH binding sites. T₄ administration had no effect on pituitary TRH binding. When chicken pituitary glands were incubated in vitro, basal GH release was unaffected by the addition of 10⁻⁶–10⁻⁵ M T₃ or T₄ to the incubation media. The in vitro GH response to TRH (10⁻⁶ M) or GRF (10⁻⁶ M) challenge was, however, suppressed in a dose-related manner by T₃ but was unaffected by the coincubation of T₄. These results demonstrate inhibitory effects of T₃ and T₄ on basal and secretagogue-induced GH secretion in fowl. T₄ is less active than T₃ and probably exerts some of its effects via T₃-independent mechanisms.

chicken / GH / T₃ / T₄ / TRH / GRF

Résumé — Effets différents de T₄ et T₃ sur la sécrétion de GH induite par TRH ou GRF chez le poulet. La sécrétion de l'hormone de croissance (GH) est stimulée par une injection de TRH ou de GRF. L'intensité de cette stimulation est réduite chez les poulets immatures alimentés avec une ration supplémentée en triiodothyronine (T₃; 1 ppm) ou recevant des injections intrapéritonéales (ip) quotidiennes de T₃ (100 µg/kg/j pendant 10 jours). Le supplément de thyroxine dans la ration (T₄; 1 ppm) n'a pas d'effet sur la sécrétion de GH induite par les neuropeptides. Les taux de base de GH ainsi que la réponse à TRH ou GRF sont diminués 2 h après l'administration ip quotidienne de T₃ ou T₄ (100 µg/kg/j pendant 10 jours). Vingt-quatre h après la dernière injection, la réduction du taux de base ou du taux stimulé de GH n'est plus observée que pour T₃. L'administration systémique de T₃ est suivie, 2 h et 24 h plus tard, par une réduction importante (downregulation) des récepteurs hypothypophysaires de TRH alors que l'administration de T₄ est sans effet sur ces mêmes récepteurs. L'incubation in vitro d'hypophyses de poulets en présence de 10⁻⁶–10⁻⁵ M de T₃ ou T₄, ne modifie pas la

* Correspondence and reprints
sécrétion de base de GH. Par contre, la réponse in vitro de GH induite par TRH (10^{-6} M) ou par GRF (10^{-8}) est supprimée par T_{3} d’une façon reliée à la dose de T_{3} mais elle ne l’est pas par T_{4}. Ces résultats démontrent les effets inhibiteurs de T_{3} et T_{4} sur la sécrétion de base de GH et sur la sécrétion de GH induite par les neuroleptiques chez le poulet. T_{4} est moins active que T_{3} et exerce probablement certains de ses effets par des mécanismes indépendants de T_{3}.

poulet / GH / hormones thyroïdiennes / TRH / GRF

INTRODUCTION

Thyroid status modulates growth hormone (GH) secretion in birds. Basal circulating GH concentrations are elevated in birds fed or injected with goitrogens (Leung et al, 1985a; Scanes et al, 1986a; Harvey et al, 1988), genetically deficient in triiodothyronine (T_{3}) (Scanes et al, 1983, 1986a; Harvey et al, 1984; Huybrechts et al, 1985), suffering from autoimmune thyroiditis (Scanes et al, 1976), or which have been surgically thyroidectomized (Harvey et al, 1983, 1988). In contrast, exogenous T_{3} suppresses basal and thyrotrophin releasing hormone (TRH)-induced GH secretion in euthyroid (Harvey, 1983; Marsh et al, 1984a; Scanes and Harvey, 1989) and hypothyroid (Leung et al, 1984; Scanes et al, 1986a) birds. Similarly, thyroxine (T_{4}), which can be monodeiodinated by peripheral tissues to T_{3} (Lam and Harvey, 1986; Decuypere and Kühn, 1988), can also inhibit basal and TRH-induced GH secretion, although it is less effective than T_{3} (Harvey, 1983; Leung et al, 1984; Scanes et al, 1986a) or even ineffective in some experiments (Marsh et al, 1984b; Lauterio and Scanes, 1988; Lazarus and Scanes, 1988).

Since GH increases circulating T_{3} concentrations by stimulation of peripheral 5'-monodeiodination (Kühn et al, 1987, 1988), T_{3} and T_{4} may, therefore, provide feedback regulation in the control of GH release (Harvey, 1990a). GH secretion is regulated primarily by hypothalamic releasing factors, of which TRH and a putative GH releasing factor (GRF) stimulate GH release (Harvey, 1990a). The possibility that T_{4} may inhibit GRF-induced GH secretion in vivo and in vitro has therefore been assessed in the present study and compared with the effects of T_{3}.

MATERIALS AND METHODS

**Experiment 1**

One-day-old domestic fowl of a broiler strain (Hybro, from Euribrid) were reared from hatch until 7 wk of age and were fed a commercial diet, supplemented with either T_{3} or T_{4}, at concentrations of 1 ppm (Decuypere et al, 1987). For comparative purposes, another group was fed a diet supplemented (0.1 %) with the goitrogen methimazole (MMI, 2-mercapto-5-methylimidazole, Janssens Pharmaceuticals). A fourth group of controls was fed unsupplemented diet. At 3 and 7 wk of age, birds from each group were intravenously injected with TRH (1 μg/kg) and at 5 wk with human pancreatic GRF_{1-44} NH_{2} (10 μg/kg). The peptides were obtained from Peninsula Laboratories (Belmont, CA) and were used at doses maximally effective in stimulating GH release in fowl (Harvey and Scanes, 1984). A control group was injected with the 0.9% NaCl vehicle (1 ml/kg). Heparinized venous blood samples were collected by venipuncture from the brachial vein before and at intervals after the injection of test substances. Following centrifugation and separation, the plasma was stored at -20 °C prior to GH analysis by a homologous radiomunoassay (Berghman et al, 1989) which used a murine monoclonal antibody directed against
affinity-purified chicken GH. This antibody does not cross-react with other pituitary hormones and the assay has a sensitivity of 2 ng/ml and an intrassay coefficient of variation of 4%.

**Experiment 2**

Six-wk-old white Leghorn cockerels were injected ip with T₃ (100 µg/kg), T₄ (100 µg/kg) or with MMI (50 mg/kg), once a day for 10 d. Controls were injected with 0.9% NaCl (1 ml/kg). Two h or 24 h after the last intraperitoneal injection, birds from each group were injected iv with either TRH (1 µg/kg), GRF (10 µg/kg) or the 0.9% NaCl vehicle. Heparinized venous blood samples were collected before and 10 min after injections of the secretagogues, at the time of maximal GH responses (Harvey and Scanes, 1984). Plasma GH concentrations were determined by a homologous radioimmunoassay (Harvey and Scanes, 1977) which used a polyclonal antibody raised in a rabbit against chicken GH isolated by gel filtration and ion-exchange chromatography. This antibody is specific for chicken GH and the assay has a sensitivity of <0.5 ng/ml and an intrassay coefficient of variation of <5.0%. Further birds from each group were killed 2 h or 24 h after the last ip injection of T₃, T₄ or MMI and their anterior pituitary glands rapidly dissected out and collected on ice-cold 20 mM phosphate buffer, pH 7.0. The caudal lobes containing the GH-secreting cells (Malamed et al, 1985), were separated and plasma membranes isolated after homogenization in phosphate buffer (pH 7.4), and after centrifugation and ultracentrifugation (Harvey and Baidwan, 1989). The specific binding of TRH to these membranes was determined with [3H]3-methyl-histidine²-TRH ([3H]MeTRH; 80 Ci/mmol, New England Nuclear, Missisauga, Ontario) as radioligand, as previously described (Harvey and Baidwan, 1989). Briefly, the plasma membranes (at a concentration of 50 mg wet wt of pituitary tissue/ml; 5 mg/tube) were incubated with [3H] Me-TRH for 60 min at 4 °C, alone or in the presence of 10 µM Me-TRH to determine non-specific binding. Bound and free radioactivity was separated by filtration through Whatman GF/B filters, and then counted in a liquid scintillation cocktail in a beta counter.

**Experiment 3**

Heads from freshly-killed broiler fowl were obtained from a local slaughterhouse and the anterior pituitary glands were collected on ice-cold medium 199 (M199; Gibco Laboratories, Grand Island, NY). The glands were then bisected and following a 60-min preincubation period the hemipituitaries were incubated for 4 h at 39 °C in a shaking water bath in freshly gassed (95% O₂/5% CO₂) M199 containing test substances, as detailed elsewhere (Hall et al, 1985). Contra-lateral hemipituitary glands were incubated in the absence or presence of 10⁻⁶ M TRH or 10⁻⁶ M GRF to stimulate GH release (Hall et al, 1985; Perez et al, 1987), in control media or media containing 10⁻⁵–10⁻⁹ M T₃ or T₄. Following incubation, the media were aspirated and stored at −20 °C prior to GH analysis (Harvey and Scanes, 1977).

Statistical differences in the results were determined by analysis of variance or Student's t-test where appropriate.

**RESULTS**

**Experiment 1**

Basal plasma GH concentrations were unaffected by the addition of 1 ppm T₃ or T₄ to the diet, but were elevated (P < 0.001) at 3, 5 and 7 wk of age by 0.1% MMI fig 1; data for wk 3 not shown). At 7 wk of age and in all groups, the administration of TRH was followed 10 min later by increased (P < 0.01) GH concentrations. The GH response to TRH in the birds fed 1 ppm T₃ was, however, significantly (P < 0.05) reduced in comparison with the corresponding untreated controls. Dietary T₄ supplementation did not impair the GH response to TRH, which was augmented and prolonged by MMI feeding. Similar effects of T₃, T₄ and MMI on TRH-induced GH secretion were also observed at 3 wk of age.
At 5 wk of age, the administration of GRF induced (P < 0.01) GH release in the controls and in birds fed 0.1% MMI or 1 ppm T4 (fig 1). In each case, the GH response to GRF was of comparable magnitude. Dietary supplementation with 1 ppm T3 completely suppressed GH response to GRF challenge.

The administration of the 0.9% NaCl vehicle to each treatment group at all ages had no significant effect on circulating GH concentrations (data not shown). At 5 wk of age, the administration of GRF induced (P < 0.01) GH release in the controls and in birds fed 0.1% MMI or 1 ppm T4 (fig 1). In each case, the GH response to GRF was of comparable magnitude. Dietary supplementation with 1 ppm T3 completely suppressed GH response to GRF challenge.

The administration of the 0.9% NaCl vehicle to each treatment group at all ages had no significant effect on circulating GH concentrations (data not shown).

**Experiment 2**

In birds injected for 10 d with MMI, the basal circulating GH concentrations 2 h and 24 h after the last injection were higher (P < 0.01) than those in the vehicle-injected controls (fig 2). In contrast, the basal GH concentration was decreased 2 h (P < 0.001) and 24 h (P < 0.05) after the last injection of T3, whereas resting GH concentrations were reduced (P < 0.001) 2 h but not 24 h after the last injection of T4. The administration of TRH or GRF increased (P < 0.05) the circulating GH concentrations in each group. The magnitude of the GH response to TRH or GRF challenge in birds injected with MMI 2 h or 24 h previously was greater (P < 0.05) than that in controls. In contrast, the GH response to TRH and GRF was consistently decreased (P < 0.01) 2 h and 24 h after T3 administration.

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Fig 1. Growth hormone (GH) concentrations in the plasma of control domestic fowl and birds given dietary supplements of methimazole (MMI, 0.1%), triiodothyronine (T3, 1 ppm) or thyroxine (T4, 1 ppm) from hatch. The birds were given a bolus intravenous injection of growth hormone releasing factor (GRF, 10 μg/kg) at 5 wk of age and an intravenous injection of thyrotrophin-releasing factor (TRH, 1 μg/kg) at 7 wk of age. Means ± SEM (n = 10). The asterisks indicate values significantly different (P < 0.05) from those of controls.

Fig 2. Growth hormone (GH) concentrations in the plasma of immature domestic fowl intraperitoneally (ip) injected with methimazole (50 mg/kg), triiodothyronine (T3, 100 μg/kg), thyroxine (T4, 100 μg/kg) or with the 0.9% NaCl vehicle (1 ml/kg) once a day for 10 d. The data indicate the basal GH concentrations 2 and 24 h after the last ip injection and the GH response (measured 10 min later) to intravenous thyrotrophin-releasing hormone (TRH, 1 μg/kg) or growth hormone releasing factor (GRF, 10 μg/kg) challenge. Means ± SEM (n = 10). The asterisks indicate values significantly different (P < 0.05 at least) from controls.
tion. However, while the GH response to TRH or GRF was reduced 2 h after the last injection of T₄, it was similar to that in the controls 24 h afterwards.

In each group, the injection of 0.9% NaCl instead of TRH or GRF had no significant effect on circulating GH concentrations (data not shown).

Two h and 24 h after the last injection of MMI, the relative specific binding of [³H]MeTRH to the pituitary caudal lobe was greater (P < 0.05) than that in the controls (fig 3). The binding of [³H]MeTRH was suppressed (P < 0.01) 2 h and 24 h after T₃ administration, but was unaffected by exogenous T₄.

Experiment 3

Basal GH release from chicken hemipituitary glands in vitro was not affected by the addition of 10⁻⁹-10⁻⁵ M T₃ or T₄ to the incubation media (data not shown). In the presence of 10⁻⁶ M TRH or 10⁻⁶ M GRF, GH release was significantly increased (P < 0.001 in both cases) (fig 4). The co-incubation of TRH with 10⁻⁸-10⁻⁵ M T₃ suppressed the GH response in a dose-related manner. At a concentration of 10⁻⁵ M, the GH response to TRH stimulation was completely suppressed by exogenous T₃. T₃ at concentrations of 10⁻⁷-10⁻⁵ M also suppressed (P < 0.01) the GH response to GRF. In contrast, the addition of 10⁻⁹-10⁻⁵ M T₄ to the incubation media had no significant effect on TRH- or GRF-induced GH secretion.

Fig 3. Specific binding of [³H]-3-methylhistidine-2TRH ([³H]MeTRH) to the caudal lobe membranes of birds killed 2 h or 24 h after treatment with methimazole (50 mg/kg per day for 10 d), triiodothyronine (T₃, 100 μg/kg for 10 days) or thyroxine (T₄, 100 μg/kg). Means ± SEM (n = 8). The asterisks indicate values significantly different (P < 0.05) from those in the corresponding controls. Total binding of the tracer to pituitary membranes from both control groups was 1 550 ± 48 cpm, whereas non-specific binding was 169 ± 8 cpm.

Fig 4. Release of growth hormone (GH) from chicken pituitary glands in response to thyrotropin-releasing hormone (TRH, 10⁻⁶ M) or growth hormone releasing factor (GRF, 10⁻⁶ M), in the presence or absence of 10⁻⁵-10⁻⁹ M triiodothyronine (T₃) or 10⁻⁵-10⁻⁹ M thyroxine (T₄). Means ± SEM (n = 10). The asterisks indicate values significantly different (P < 0.05) from those in corresponding controls. The basal release of GH in the absence of thyroid hormones, TRH or GRF was 0.85 ± 0.09 μg/mg pituitary/ml.
DISCUSSION AND CONCLUSION

These results provide further evidence that the hypothalamo–pituitary–thyroid axis plays a role in the regulation of GH secretion in domestic fowl. In the present study, T₃ suppressed basal and TRH-induced GH secretion, in agreement with a number of previous studies (Harvey, 1990a). Although dietary T₃ failed to inhibit basal GH secretion in this study, other studies using the same dose of T₃ (1 ppm) did report a suppression of basal GH concentrations (Scanes et al, 1986a; Lauterio and Scanes, 1988). This difference may therefore be due to strain differences in the sensitivity of the birds to exogenous T₃ and may also indicate that basal and stimulated GH secretion are mediated by different mechanisms, or that they have different thresholds for T₃ inhibition.

In the present study, T₃ not only suppressed TRH-induced GH secretion, but also impaired the in vivo GH response to GRF. This latter finding is consistent with in vivo studies on anaesthetized chickens (Scanes and Harvey, 1989) and in vitro studies on perfused chicken pituitary cells (Scanes et al, 1986b), and indicates a pituitary site of T₃ action. This finding is, however, in marked contrast to mammalian studies, in which T₃ potentiates GRF-induced GH release (Vale et al, 1983; Dieguez et al, 1985) and increases GH synthesis by direct effects on gene transcription (Spindler et al, 1982; Samuels et al, 1988).

While T₃ may act at a level subsequent to TRH or GRF binding to its membrane receptor to inhibit GH secretion, the present results indicate that T₃ is able to reduce TRH binding to the caudal lobe, which is predominately composed of somatotroph cells and is devoid of thyrotroph and lactotroph cells (Mikami and Takahashi, 1987). Such an effect would likely impair the in vivo and in vitro GH response to TRH challenge, by analogy with the inhibitory effect of thyroid hormones on TRH receptors and thyrotropin secretion in mammalian pituitary glands (see Harvey and Baidwan, 1990). Moreover, since the in vivo GH response to GRF is potentiated by TRH (Harvey and Scanes, 1985; Leung et al, 1985b; Buonomo and Baile, 1986; Scanes and Harvey, 1986; Taylor et al, 1986), the blunting of the GH secretory response to GRF stimulation in T₃-treated birds could also be partly due to a down-regulation of TRH receptors and possibly to inhibition of TRH release in vivo (Hinkle and Goh, 1982; Mori and Yamada, 1987; De los Frailes et al, 1988; Dyess et al, 1988). Since TRH may stimulate GH secretion in birds by action at extrapituitary sites, it is less potent in vivo than in vitro (Harvey, 1990b). Thus while TRH is a more effective GH secretagogue than GRF in vivo it is approximately equipotent with GRF in vitro, as observed in the present study.

Although T₃ is derived from T₄ by monodeiodination in peripheral tissues, including the pituitary gland (Lam, 1986), T₄ had no effect on GH release from pituitary tissue in vitro and had no long-term effect on in vivo GH secretion. The failure of dietary T₄ to suppress stimulated GH secretion probably reflects its lower biological potency and the dosage used, since dietary T₄ supplements that increase circulating T₃ concentrations suppress GH secretion (Leung et al, 1984). The ability of the injected dose of T₄ to acutely inhibit basal and secretagogue-induced GH release, in agreement with other studies (Harvey, 1983; Harvey et al, 1988), in the absence of long-lasting effects, is consistent with this view.

However, the inhibition of GH secretion induced by T₄ is probably not simply due to its conversion to T₃. This conclusion is
based on the inability of T4 to directly suppress in vitro GH secretion, even at high (10^{-5} M) dose levels. The acute suppression of basal GH concentrations in T4 treated birds in vivo also occurred without inducing a down-regulation of TRH binding sites, as would be expected if the effect was due to T4 to T3 conversion. Basal GH secretion is similarly transiently suppressed by exogenous T4 in birds in which peripheral and intrapituitary T4 to T3 conversion is blocked by iopanoic acid (Harvey et al., 1990), further suggesting T3-independent effects of T4 on GH secretion. Moreover, while T4 is rapidly converted to T3 in hypothyroid birds, it is mainly converted to reverse T3 (rT3) in euthyroid birds (Decuypere et al., 1987; Decuypere and Kühn, 1988), possibly to prevent the accumulation of toxic T3 concentrations. The activity of intrathyroidal type III deiodinase, responsible for the conversion of T4 to T3, is greater in birds than in mammals (Decuypere and Kühn, 1988), and since rT3 antagonizes the effects of T3 (Lynch et al., 1985; Han et al., 1986), the inhibitory effects of T4 on GH secretion are unlikely to be mediated by T3.

In summary, these results show differential effects of T4 and T3 on GH secretion in fowl and suggest they act at different sites in the hypothalamo-pituitary axis.

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