Effects of hypothyroidism, tri-iodothyronine and glucocorticoids on growth hormone responses to growth hormone-releasing hormone and His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂

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ABSTRACT

The aim of this study was to investigate the role of thyroid hormones and glucocorticoids on GH secretion. Secretion of GH in response to GH-releasing hormone (GHRH) (5 μg/kg) was markedly (P < 0.001) decreased in hypothyroid rats in vivo (peak GH responses to GHRH, 635 ± 88 μg/l in euthyroid rats vs 46 ± 15 μg/l in hypothyroid rats). Following treatment with tri-iodothyronine (T₃; 20 μg/day s.c. daily for 2 weeks) or cortisol (100 μg/day s.c. for 2 weeks) or T₃ plus cortisol, a marked (P < 0.01) increase in GH responses to GHRH was observed in hypothyroid rats (peak GH responses, 326 ± 29 μg/l after T₃ vs 133 ± 19 μg/l after cortisol vs 283 ± 35 μg/l after cortisol plus T₃). In contrast, none of these treatments modified GH responses to GHRH in euthyroid animals. Hypothyroidism was also associated with impaired GH responses to the GH secretagogue, His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ (GHRP-6). Secretion of GH in response to GHRP-6 in vivo was reduced (P < 0.01) in hypothyroid rats (peak GH responses, 508 ± 177 μg/l in euthyroid rats vs 203 ± 15 μg/l in hypothyroid rats). In-vitro studies carried out using monolayer cultures of rat anterior pituitary cells derived from euthyroid and hypothyroid rats showed a marked impairment of somatotroph responsiveness to both GHRP-6 and somatostatin in cultures derived from hypothyroid rats. In summary, our data suggest that thyroid hormones and glucocorticoids influence GH secretion by modulating somatotroph responsiveness to different GH secretagogues.


INTRODUCTION

In addition to growth hormone-releasing hormone (GHRH) and somatostatin, several other neuro peptides interact with a variety of peripheral feedback signals to regulate growth hormone (GH) secretion by the anterior pituitary gland. Synthesis and secretion of GH are dependent upon thyroid hormones both in vitro and in vivo. Administration of tri-iodothyronine (T₃) increases the rate of GH gene transcription GH mRNA levels and GH synthesis and secretion (Nyborg, Nguyen & Spindler, 1984; Yaffe & Samuels, 1984; Dieguez, Foord, Peters et al. 1985; Franklyn, Lynam, Docherty et al. 1986). Furthermore, primary hypothyroidism is associated with a reduced content of GH in the anterior pituitary gland, reduced circulating GH levels and reduced GH responses to GHRH (Root, Duckett, Sweetland et al. 1985; Dieguez, Jordan, Harris et al. 1986; Katakami, Downs & Frohman, 1986).

Glucocorticoids also have a marked stimulatory effect on the rate of GH gene transcription and GH mRNA levels (Martial, Seeburg, Guenzi et al. 1977; Evans, Birnberg & Rosenfeld, 1982; Nyborg et al. 1984). Similarly, in-vitro administration of glucocorticoids to anterior pituitary cultures derived from euthyroid rats increases GHRH receptor number and GH responses to GHRH (Vale, Vaughan, Yamamoto et al. 1983; Webb, Szabo & Frohman, 1983; Ceda & Hoffman, 1985; Seifert, Perrin, Rivier & Vale, 1985). Interestingly, while T₃ and glucocorticoids added together to anterior pituitary monolayer cultures...
from euthyroid rats act synergistically to increase somatotroph sensitivity to GHRH (Vale et al. 1983), it has been reported recently that dexamethasone reduces the stimulatory effect of T₃ administration in vivo on GH mRNA levels in hypothyroid rats (Franklyn, Ahlquist, Balfour et al. 1987).

The aims of this study were to investigate the effects of T₃, cortisol and cortisol together with T₃ on GH responses to GHRH in vivo in both euthyroid and hypothyroid rats. Furthermore, we have studied the effect of hypothyroidism on the GH responses to GHRP-6 both in vivo and in vitro. This is a synthetic hexapeptide (His-d-Trp-Ala-Trp-d-Phe-Lys-NH₂) developed from combined application of conformational energy calculations, synthesis and testing of biological activity. This compound may well be related to an endogenous peptide of similar structure since it releases GH in a dose-related and specific manner both in vivo and in vitro in several species, and its effect is independent of GHRH since their combined effects on GH release are additive at maximal concentrations (Bowers, Momany, Reynolds & Hong, 1984; Momany, Bowers, Reynolds et al. 1984; McCormick, Millard, Badger et al. 1985; Sartor, Bowers & Change, 1985a; Sartor, Bowers, Reynolds & Momany, 1985b).

MATERIALS AND METHODS

In-vivo studies

Adult male Wistar rats (weight 200–250 g) were rendered hypothyroid by administration of 0.1% (w/v) aminotriazole (Sigma, Poole, Dorset, U.K.) in the drinking water for a period of 3 weeks as described previously (Dieguez et al. 1985, 1986). Untreated euthyroid and hypothyroid rats were divided into four groups and treated for 2 weeks (days 7 to 21) with either physiological saline, T₃ (20 μg s.c. daily; Sigma), cortisol (100 μg s.c. daily; Sigma) or with cortisol plus T₃ (100 μg and 20 μg daily respectively). On the day of the experiment, rats were anaesthetised by i.p. injection of sodium pentobarbitone (80 mg/kg; May & Baker Ltd, Dagenham, Essex, U.K.). Forty-five minutes later, blood was sampled (0.3 ml) from the exposed jugular vein for the determination of basal GH levels. Immediately thereafter, GHRH(1–44) (5 μg/kg) (Sanofi, Manchester, U.K.) or GHRP-6 (10 μg/kg) (Cambridge Research Biochemicals, Cambridge, Cambs, U.K.) dissolved in 0.3 ml saline was injected into the jugular vein and additional blood samples obtained 5, 10, 15 and 30 min later from the same jugular vein. Blood samples were centrifuged immediately and the plasma separated and kept frozen at −20°C until assayed. Radioimmunoassay of plasma GH levels was performed using hypophysectomized rat serum (Charles River, Margate, Kent, U.K.) in the standard curve, and a second antibody separation method using materials from the NIADDK (Baltimore, MD, U.S.A.). Analysis of the data was performed by Student’s unpaired t-test.

In-vitro studies

Monolayer cultures of rat anterior pituitary cells derived from both euthyroid and hypothyroid rats were prepared as described previously (Dieguez et al. 1985). In brief, cells were isolated from anterior pituitary glands using a collagenase-based method and were plated at a density of approximately 10⁴/ml per well. Linbro 96-well multiwell plates were used, each well having a growth area of 1.7 cm² (Flow Laboratories, Rockville, MD, U.S.A.). The culture medium was semisynthetic and supplemented with 2% (v/v) fetal calf serum (Dieguez et al. 1985). After 4 days in culture at 37°C and 5% CO₂, the cells were washed four times with Earle’s balanced salts solution (Gibco Europe, Paisley, Strathclyde, U.K.) and subsequently incubated for 3 h with the appropriate concentrations of GHRP-6 or somatostatin (Cambridge Research Biochemicals). GH in the medium was measured by radioimmunoassay as described previously (Dieguez et al. 1985).

RESULTS

No significant modification of the GH response to GHRH in euthyroid animals was produced by T₃, cortisol or T₃ plus cortisol (Fig. 1). Basal GH levels were reduced in saline-treated hypothyroid rats (1.7 ± 0.7 μg/l) compared with saline-treated euthyroid rats (5.7 ± 1.1 μg/l) (P<0.01). Following treatment, GH levels increased significantly in hypothyroid rats treated with T₃ (3.75 ± 0.7 μg/l) or cortisol (5.9 ± 1.1 μg/l) but not in those treated with cortisol plus T₃ (3.7 ± 0.8 μg/l). Hypothyroid rats treated with either cortisol or T₃ showed a marked increase in GH responses to GHRH compared with untreated rats at 5, 10 and 15 min (P<0.005) (Fig. 2). Similar increases in plasma GH levels after administration of GHRH were observed in hypothyroid rats treated with either T₃ alone or T₃ plus cortisol.

In addition to markedly reduced GH responses to GHRH (Figs 1 and 2) in vivo, GH responses to GHRP-6 were also reduced and delayed in untreated hypothyroid compared with euthyroid rats (Fig. 3). Peak GH levels in euthyroid rats were 508 ± 177 μg/l at 5 min compared with 203 ± 15 μg/l at 15 min after administration of GHRH-6 in hypothyroid rats (P<0.001).

Figure 4 shows that GHRP-6 releases GH in a dose-related manner in cultures derived from
DISCUSSION

In agreement with previous reports, these findings indicate that GH responses to GHRH are markedly reduced in hypothyroid rats (Root et al. 1985; Dieguez et al. 1986; Katakami et al. 1986). They also agree with previous findings showing that T₃ treatment greatly enhances GH responses to GHRH in hypothyroid but not in euthyroid animals (Root et al. 1985; Dieguez et al. 1986). This provides further support for the well-established view that thyroid hormones play an important stimulatory role in GH secretion and GH responses to GHRH.

Our data also indicate that treatment with glucocorticoids enhances GH responses to GHRH in vivo in hypothyroid but not in euthyroid rats. Previous in-vitro and in-vivo studies have provided conflicting findings regarding the role of glucocorticoids in GH secretion. Thus, while long-term treatment of anterior pituitary cells in vitro with glucocorticoids leads to an increase in the rate of GH gene transcription (Evans et al. 1982; Nyborg et al. 1984), GHRH receptor number (Seifert et al. 1985) and GH responses to GHRH (Vale et al. 1983; Webb et al. 1983; Ceda, Davis & Hoffman, 1987), short-term treatment with glucocorticoids causes a decrease in basal GH secretion and somatotroph responsiveness to GHRH (Ceda et al. 1987). Similarly, rats treated with glucocorticoids may show reduced basal GH secretion (Nakagawa, Ishizuka, Obara et al. 1987), reduced GH responses to insulin-induced hypoglycaemia and reduced hypothalamic GH-releasing activity (Pecile & Muller, 1966) as well as increased GH responses to GHRH (Wehrenberg, Baird & Ling, 1983). While these discrepancies may be due in part to methodological differences (dose of glucocorticoids, duration of treatment), they also suggest that glucocorticoids may have opposing actions on GH control at the level of the hypothalamus or pituitary. Hence the dose and/or duration of treatment may be critical in determining whether stimulatory or inhibitory effects predominate. This assumption is also supported by recent data obtained in normal human subjects, showing that...
while short-term administration of glucocorticoids increased GH responses to GHRH, a longer treatment period completely abolished GH responses (Casanueva, Burguera, Tome et al. 1988). This could also explain why we found no change in GH responses to GHRH after treatment with glucocorticoids for 2 weeks, whereas others have shown that treatment for 1 week increases GH responses to GHRH (Wehrenberg et al. 1983).

It has been suggested by others (Nakagawa et al. 1987) that glucocorticoids influence GH secretion by a direct stimulatory effect at the pituitary level as well as by stimulating somatostatin release from the hypothalamus. In consequence, it is possible that the reduced sensitivity of hypothroid rat anterior pituitary cells to inhibition by somatostatin permits a net stimulatory effect of glucocorticoids on GH secretion in hypothyroid animals. However, since it has also been shown that glucocorticoids also may exert a direct inhibitory effect at the pituitary level (Ceda et al. 1987) this explanation may be over-simplistic and further work is needed before firm conclusions can be reached.

We did not observe any significant differences in GH responses to GHRH between euthyroid rats, euthyroid rats treated with T3 or glucocorticoids, or hypothyroid rats treated with T3 or glucocorticoids. In-vitro studies carried out using monolayer cultures of rat anterior pituitary cells derived from euthyroid rats have shown clearly that T3 increases maximal GH responses to GHRH (Vale et al. 1983; Dieguez et al. 1985). However, it has also been reported that T3 stimulates somatostatin release from rat hypothalamic fragments in vitro (Berelowitz, Maeda, Harris & Frohman, 1980). Thus it is possible, that in the in-vivo situation, T3 exerts two opposing functional effects on GH secretion—a direct stimulatory action at the pituitary level as well as an increase in hypothalamic somatostatinergic tone. This could result in the lack of any net effect of T3 on GH secretion in euthyroid animals. In hypothyroidism, however, not only is somatotroph responsiveness to GHRH markedly blunted but also hypothyroid rat anterior pituitary cells show impaired GH inhibition in response to somatostatin compared with euthyroid rat cells.

Based on previous data obtained in vitro, our findings of similar GH responses to GHRH in rats
treated with T<sub>3</sub> or T<sub>3</sub> plus cortisol were not unexpected. Thus, while somatotroph sensitivity to GHRH as assessed by the dose giving 50% maximum stimulation is markedly increased in cultures treated with T<sub>3</sub> plus dexamethasone as compared with when these substances are added independently, maximal GH responses to GHRH have been reported to be identical in cultures treated with either T<sub>3</sub> alone or T<sub>3</sub> plus dexamethasone (Vale et al. 1983).

In agreement with previous studies, we have found that GHRP-6 is a potent stimulator of GH secretion both in vivo and in vitro (Bowers et al. 1984; Sartor et al. 1985a,b). Interestingly, maximal peak GH responses to GHRH and GHRP-6 were similar, although GHRP-6 was given at a much higher dose. This contrasts with thyrotrophin-releasing hormone (TRH)-induced GH secretion which is markedly enhanced in hypothyroid rats both in vivo and in vitro (Szabo, Stachura, Paleologos et al. 1984; Szabo, Ruestow & Kramer, 1985). This latter finding probably reflects increased expression of TRH receptors on the somatotroph membranes as a consequence of the absence of thyroid hormones (Gershengorn, 1978; Perrone & Hinkle, 1978). Whether reduced GH responses to GHRP-6 in hypothyroidism are due to a receptor or post-receptor phenomenon remains to be established. We have also demonstrated that, in addition to GHRH, somatotroph responsiveness to GHRP-6 is reduced in hypothyroid rats both in vivo and in vitro.

In summary, our data indicate that thyroid hormones facilitate somatotroph responsiveness to GHRH, GHRP-6 and somatostatin. Based on in-vitro findings (Vale et al. 1983) it has been suggested that thyroid hormones and glucocorticoid exert a synergistic stimulatory effect on GH secretion. In vivo, however, the direct stimulatory effects of T<sub>3</sub> and glucocorticoids on the somatotroph are counterbalanced by direct hypothalamic actions of these hormones which may well involve activation of hypothalamic somatostatinergic neurones.

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REFERENCES


