Desensitization of thyrotrophin-releasing hormone (TRH)-induced growth hormone secretion in chickens: coincident down-regulation of TRH binding to pituitary membranes

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ABSTRACT

Release of GH is stimulated by TRH in chickens. However, for 60 min following a priming injection of TRH, a second injection of TRH is unable to provoke further GH release. TRH binds to the plasma membranes of the pituitary caudal lobe, in which somatotrophs predominate, although the magnitude of \(^{3}\text{H}\)-methyl-histidine\(2\)-TRH binding was reduced (by 25–50\%) 30 min after the i.v. administration of a dose of TRH (5 \(\mu\text{g/kg}\)) maximally effective in provoking GH release. A significant reduction in \(^{3}\text{H}\)-Me-TRH binding to caudal lobe membranes was also observed within 15 min of TRH administration and was maintained for at least 60 min. Control levels of \(^{3}\text{H}\)-Me-TRH binding were restored 2 h after TRH injection, coincident with the restoration of GH responsiveness to TRH challenge. The suppression of \(^{3}\text{H}\)-Me-TRH binding to pituitary membranes 30 min after in-vivo TRH administration was dose related, whereas the maximal (10 min) GH response to TRH was biphasic. The suppression of \(^{3}\text{H}\)-Me-TRH binding to chicken pituitary membranes was due to direct pituitary actions of TRH and could be induced by a 30-min exposure to 100 nm TRH in vitro.

These results demonstrate that avian pituitary TRH-binding sites differ greatly from mammalian ones in the timing of the onset and duration of down-regulation. Pituitary TRH-binding sites in birds are rapidly and transiently down-regulated following TRH administration in vivo, coincident with the period of GH refractoriness to TRH challenge.

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INTRODUCTION

Thyrotrophin-releasing hormone (TRH) is a physiological growth hormone (GH)-releasing factor (GRF) in birds (Harvey, 1983; Klandorf, Harvey & Fraser, 1985). The GH response to TRH rapidly diminishes, however, upon repeated stimulation (Harvey, 1983; Harvey, Scanes & Phillips, 1986; Scanes & Harvey, 1988). This refractoriness to TRH challenge is not due to pituitary GH depletion, since mammalian GRF is able to induce GH release during the period of GH unresponsiveness to TRH stimulation (Scanes & Harvey, 1986). The mechanism involved in GH refractoriness is unknown, although a down-regulation of pituitary TRH receptors (Hinkle & Tashjian, 1975; Gershengorn, 1978; Nemeroff, Bissette, Martin et al., 1980; Banerji & Prasad, 1982) may be involved.

Release of GH from chicken pituitary tissue in vitro is stimulated by TRH (Harvey, Scanes, Chadwick & Bolton, 1978; Leung & Taylor, 1983; Perez, Malamed & Scanes, 1987), presumably via TRH-binding sites on the plasma membranes of the caudal lobe (Harvey & Baidwan, 1989), in which somatotrophs predominate (Malamed, Gibney, Loesser & Scanes, 1985). This possibility is supported by the correlation between the number of pituitary TRH-binding sites and the circulating GH level in fed and fasted birds and in young and old fowl (Harvey & Baidwan, 1989). The possibility that rapid changes in the number or affinity of caudal lobe binding sites for TRH may participate in the desensitization of the GH response to TRH challenge has therefore been examined in the present study.


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MATERIALS AND METHODS

Influence of TRH on TRH-induced GH secretion in vivo

A maximally effective dose of TRH (5 μg/kg) (Harvey & Scanes, 1984) was administered i.v. to groups of 8- to 10-week-old cockerels (White-Leghorn) at intervals of 15, 30, 60 or 120 min after a priming injection (5 μg/kg) had been similarly injected. Heparinized venous blood samples were collected from each bird before and 10 min after each TRH injection, at the time of the maximal GH response to TRH stimulation (Harvey & Scanes, 1984). Following separation, the plasma samples were stored at −20 °C, and GH concentrations were subsequently determined by specific, homologous radioimmunoassay (Harvey & Scanes, 1977).

Influence of TRH on TRH binding in vitro

Heads from freshly killed 8-week-old chickens were collected at a local slaughterhouse (Lillydale, Edmonton) and rapidly transported, on ice, to the laboratory. The anterior pituitary glands were dissected out and collected into ice-cold, gassed (95% O2:5% CO2) Medium 199 (M199; Gibco Laboratories, Grand Island, NY, U.S.A.) and then preincubated for 60 min in a shaking water bath at 39 °C. The glands were then incubated (ten pituitaries/ml) for 30, 60 or 120 min in 20 ml glass scintillation vials, in fresh media or in media containing 100 nM TRH (36 ng TRH/ml). This dose of TRH has been shown to down-regulate TRH receptors on mammalian pituitary membranes in vitro (e.g. Gershengorn, 1978) and to stimulate GH release from the chicken pituitary in vitro (e.g. Leung & Taylor, 1983) and in vivo (Harvey & Scanes, 1984). At the end of the incubation periods, the glands were collected and washed in ice-cold 20 mM sodium phosphate buffer to dissociate any unlabelled TRH from their membrane binding sites (Harvey & Baidwan, 1989). The glands were then stored overnight at −20 °C, before membrane preparation and analysis of TRH-binding sites.

Intact glands were used in these studies, as a model of the in-situ pituitary gland. Dispersed chicken pituitary cells were not used, since the somatotrophs are poorly responsive or lack responsiveness to TRH stimulation following cell dispersion and culture (Harvey et al., 1978; Leung & Taylor, 1983; Perez et al., 1987; Perez, Malamed & Scanes, 1989).

TRH binding assay

The pituitary glands were thawed and the caudal lobes separated from the cephalic lobes before homogenization in 20 mM phosphate buffer (pH 7.4) as described previously (Harvey & Baidwan, 1989). The plasma membranes were collected following centrifugation (1100g at 4 °C for 15 min) and ultracentrifugation (30 000g at 4 °C for 30 min) and then incubated with [3H]3-methyl-histidine2-TRH ([3H]Me-TRH; 70 Ci mmol; New England Nuclear, Mississauga, Ontario, Canada) alone or in the presence of 10 μM Me-TRH (Peninsula Laboratories, Belmont, CA, U.S.A.) to determine non-specific binding. Bound and free radioactivity were separated by filtration through Whatman GF/B filters, which were then counted in water-accepting fluor in an LKB 1219 beta scintillation counter.

Influence of TRH on the number of TRH-binding sites in vivo

TRH was administered i.v., at doses of 0.05–500 μg/kg, to groups (n=25) of 8- to 10-week-old chickens and the birds were killed by cervical dislocation at intervals thereafter. Anterior pituitary glands were collected and the number of caudal lobe TRH-binding sites was determined as above. The glands were collected into ice-cold 20 mM phosphate buffer and separated into caudal and cephalic lobes. Since this procedure took 30–60 min, it should have resulted in complete dissociation of any TRH bound to the plasma membranes. Under these conditions, 50% dissociation of TRH occurs within the first minute and dissociation is complete within 20–30 min (Harvey & Baidwan, 1989). Tissue was pooled for each group, in view of the low abundance of TRH-binding sites and the amount of tissue (50 mg wet weight/ml) required for TRH-binding analysis (Harvey & Baidwan, 1989). Following TRH administration, blood samples were collected by brachial venepuncture, in order to monitor plasma GH concentrations determined by radioimmunoassay (Harvey & Scanes, 1977).

Influence of TRH on the affinity of TRH-binding sites in vivo

TRH (5 μg/kg) or vehicle (0-9%, w/v, NaCl; 1 ml/kg) was administered i.v. to 8- to 10-week-old chickens (n=50 in both groups) which were then killed 30 min later. The pituitary glands were collected and the binding of [3H]Me-TRH in the presence of different doses (2.5–200 nM) of unlabelled Me-TRH was determined, as described previously (Harvey & Baidwan, 1989). Data on [3H]Me-TRH binding were analysed using LIGAND software (Elsevier-Biosoft, Cambridge, Cambs, U.K.). Statistical differences in the results were determined by analysis of variance or Student’s t-test, wherever appropriate.
RESULTS

Influence of TRH on TRH-induced GH secretion in vivo

Plasma GH concentrations were markedly increased (11-fold; \(P<0.001\)) 10 min after TRH challenge, but had returned to pretreatment values within 60 min of injections (Fig. 1). A second challenge with TRH, 15 or 30 min after the first, failed to induce GH release, and the concentrations of plasma GH declined \((P<0.001\) in both cases) 10 min after TRH challenge (Fig. 1), as observed in birds that only received the priming TRH injection (authors’ unpublished observations). A second TRH injection 60 min after the first was not followed by a decline in the GH concentration. A second injection of TRH 120 min after the priming injection resulted in a GH response (12-fold; \(P<0.001\)) comparable to that observed after the priming injection (Fig. 1).

![Graph showing plasma GH concentrations](image)

**FIGURE 1.** (a) Plasma GH concentrations in immature chickens at intervals after the i.v. injection of TRH (5 \(\mu g/kg\)), and (b) the subsequent GH response to a second injection of TRH administered 15, 30, 60 or 120 min after the first. The GH response to the second TRH injection indicates the change in the GH concentration measured 10 min afterwards. Values are means \(\pm\) S.E.M. \((n=10)\).

Influence of TRH on TRH-binding in vitro

Specific binding of \([^{3}H]\)Me-TRH to pituitary caudal lobe membranes was not affected by the duration (30–120 min) of incubation in M199 before membrane preparation, and so the control data have been combined (Fig. 2). In contrast, incubation in 10 nM TRH for 30 min reduced \((P<0.05)\) by 24% the amount of \([^{3}H]\)Me-TRH binding in comparison with the controls. A further reduction in binding (to 41% of that in the controls; \(P<0.01\)) occurred after 60 min of incubation in 100 nM TRH. Incubation in 100 nM TRH for 120 min was accompanied by a 23% reduction \((P<0.05)\) in \([^{3}H]\)Me-TRH binding. Similar data were also observed in a subsequent experiment (data not shown).

Influence of TRH on the number of TRH-binding sites in vivo

A reduction (45%; \(P<0.01\)) in the number of \([^{3}H]\)Me-TRH-binding sites on caudal lobe membranes was observed 30 min after administration of TRH (Fig. 3). The number of \([^{3}H]\)Me-TRH-binding sites was also reduced (35%; \(P<0.01\)) 60 min after TRH administration, although not after
120 min. In each group the maximal (10 min) GH response following TRH challenge did not differ significantly (436±20 (s.e.m.) μg/l, n = 25, in the birds killed 30 min after TRH injection; 458±17 μg/l, n = 25, in the birds killed after 60 min; and 449±24 μg/l, n = 25, in the birds killed after 120 min), indicating that each group received a similar TRH challenge.

In a subsequent study, inhibition (16%; P<0.05) of [3H]Me-TRH binding was observed within 15 min of TRH administration, although further reductions (33, 43 and 45% of control binding; P<0.01 in each case) were observed after 20, 25 and 30 min respectively (Fig. 4).

When the same dose of TRH (5 μg/kg) was administered in a further study, a significant (P<0.05) inhibition of [3H]Me-TRH binding (26%) was again observed 30 min later (Fig. 5). Higher dose of TRH (50 and 500 μg/kg) also inhibited binding (by 46 and 50% respectively; P<0.01 in both cases), although lower doses (0.05 and 0.5 μg/kg) had no significant effect, despite increasing (P<0.05) plasma GH concentrations 10 min after administration (Fig. 5). TRH, at doses of 5, 50 and 500 μg/kg, increased (P<0.001) plasma GH concentrations by comparable amounts, although 5 μg TRH/kg was more effective (P<0.05) than 500 μg TRH/kg.

When pituitary caudal lobes from control birds were incubated with [3H]Me-TRH in the presence of different concentrations of unlabelled Me-TRH, two classes of binding sites were discerned (Fig. 6), with maximal binding capacities (B_max) of 11.0 and 40.7 pmol/g pituitary tissue respectively, and with binding affinities (K_d) of 18.80 and 98.67 M. The pituitary membranes of birds given a TRH (5 μg/kg) injection 30 min before death had similar [3H]Me-TRH-binding sites, although the B_max (7.5 pmol/g)
Since autoregulation of TRH binding to the chicken caudal lobe occurred in vitro, the in-vivo effect of TRH on TRH binding is likely to occur directly. Although at least 40% of the caudal lobe endocrine cells are somatotrophs (Malamed et al. 1985), GH itself had no direct effect on TRH binding (Harvey & Baidwan, 1990a), supporting this conclusion. The autoregulation of TRH-binding sites is similarly unlikely to be mediated by prolactin or thyrotrophin (TSH) (Harvey & Baidwan, 1990a), located outside the caudal lobe (Malamed et al. 1985), nor by pituitary gonadotrophins, since TRH has little, if any, effect on gonadotrophin secretion in fowl (e.g. Harvey, Sterling & Klandorf, 1983).

However, since the time-course for the down-regulation of TRH-binding sites in vitro was slightly longer than that in vivo, it is possible that this may be due to extra-pituitary effects of TRH in vivo that induce heterologous regulation of TRH-binding sites. This time-delay in vitro, may, however, reflect the imperfections of an in-vitro model, possible due to the slower penetration of the ligand into the pituitary cells of the incubated glands.

Autoregulation of TRH-binding sites also occur in mammalian pituitary tissue (e.g. Hinkle & Tashjian, 1975; Gershengorn, 1978; Banerji & Prasad, 1982), although the time-course of the response differs. The down-regulation of TRH binding in the present study occurred within 30 min of a bolus injection of TRH in vivo or exposure to TRH in vitro. Binding sites for TRH were also rapidly down-regulated (within 2 h) following exposure of the chicken pituitary to tri-iodothyronine in vivo or in vitro (Harvey & Baidwan, 1990b). In contrast, comparable reductions in TRH binding occur in mammalian systems only after several days of TRH treatment in vivo (e.g. Gershengorn, 1978; Simasko & Horiita, 1985) or after 12–24 h of TRH exposure in vitro (e.g. Hinkle & Tashjian, 1975; Gershengorn & Straub, 1990). The time-course for the down-regulation of TRH-binding sites in mammals and birds, nevertheless, correlates with temporal changes in the ability of TRH to stimulate the synthesis and release of TSH or prolactin in mammalian cell lines (Hinkle, 1984), or of GH release in birds (Harvey et al. 1986). The influence of TRH receptor down-regulation on the release of GH from mammalian pituitary glands or tumorous cell lines has not been determined previously.

The mechanism involved in the down-regulation of TRH-binding sites in mammals is thought to involve a reduction in TRH receptor mRNA, which is reduced within 3–6 h of TRH exposure and remains suppressed for at least 12 h (Gershengorn & Straub, 1990). This mechanism is, however, unlikely to account for the rapid down-regulation of

**DISCUSSION**

These results clearly demonstrate TRH-induced down-regulation of TRH binding to chicken pituitary membranes.
TRH-binding sites in the chicken pituitary gland, which may result from a rapid phosphorylation of the receptor, as observed for adrenergic binding sites (Leeb-Lundberg, Cotecchia, DeBlasi et al. 1987; Sibley, Benovic, Caron & Leffkowitz, 1988). Following TRH binding, a cascade of intracellular events, resulting in protein phosphorylation, is rapidly induced (Gershengorn, 1986; Gershengorn & Straub, 1990) and $[^3\text{H}]$TRH binding to $\text{GH}_4\text{C}_1$ cells is impaired by phorbol esters (Osborne & Tashjian, 1982) that activate signal-transduction pathways.

The binding of TRH to its binding sites has also been shown, in mammals, to result in changes in the dissociation kinetics of the receptor (Hawkins & Engel, 1987). Ligand-induced conversion of the receptor from one complex, with fast dissociation kinetics, to another, with slow dissociation kinetics, was therefore cited as a biochemical explanation for the rapidly developing refractoriness of neuronal tissue to TRH stimulation (Hawkins & Engel, 1987).

A rapid physiochemical change in the chicken pituitary TRH-binding site may also occur following TRH binding and contribute to its down-regulation, without affecting the affinity of TRH binding. Down-regulation of TRH binding in the absence of changes in TRH-binding affinity has similarly been reported in mammals (e.g. Hinkle & Tashjian, 1975; Hinkle, 1984). Since birds have a higher body temperature (39 °C) than mammals (37 °C) and ligand–receptor interactions are temperature dependent (Hinkle, 1984), it is also possible that the kinetics of their ligand–receptor interactions occur at a faster rate than those in mammalian species.

In mammalian systems, a rapid (within 20 min) loss (by 90%) of binding sites for another peptide, epidermal growth factor, has been documented (Halpern & Hinkle, 1984). In this case, down-regulation of the binding sites was thought to coincide with the internalization of the hormone–receptor complex. Down-regulation of TRH-bind-

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**FIGURE 6.** Scatchard analysis for the binding of $[^3\text{H}]$3-methyl-histidine$^2$-TRH ($[^3\text{H}]$Me-TRH) to the caudal lobe membranes of chicken pituitary glands incubated in the presence of competing doses of unlabelled Me-TRH. The tissues were obtained from birds 30 min after the i.v. administration of either 0.9% (w/v) NaCl (1 ml/kg; ○) or TRH (5 μg/kg; △). Each value is the mean ± s.e.m. of quadruplicate determinations.

$B_{\text{max}} = 11.0 \text{ pmol/g tissue}$  
$K_d = 18.80 \text{ nM}$

$B_{\text{max}} = 7.5 \text{ pmol/g tissue}$  
$K_d = 18.18 \text{ nM}$

$B_{\text{max}} = 40.7 \text{ pmol/g tissue}$  
$K_d = 98.67 \text{ nM}$

$B_{\text{max}} = 34.0 \text{ pmol/g tissue}$  
$K_d = 117.24 \text{ nM}$
The present study shows that TRH challenge may induce more GH secretion compared to the control condition, but this effect is not seen in pituitary membranes exposed to TRH-binding sites in vitro. The results indicate that TRH-induced GH secretion is mediated through a receptor-mediated mechanism, and this response is not inhibited by somatostatin. The study also shows that TRH-induced GH secretion is not affected by pretreatment with a somatostatin analog.

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REFERENCES


