Agonist-dependent up-regulation of thyrotrophin-releasing hormone receptor protein

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INTRODUCTION

TRH (thyrotrophin-releasing hormone) receptor (TRHR) is a calcium-mobilizing G-protein-coupled receptor expressed in the anterior pituitary gland, where it regulates thyrotrophin and prolactin secretion, and in other tissues, where its function is less understood. In response to agonist binding, TRHR undergoes rapid and extensive internalization, which results from receptor phosphorylation, β-arrestin binding and targeting to clathrin-coated pits [1–4]. In pituitary cell lines expressing endogenous receptors, TRH binding leads to internalization of approx. 80 % of surface-binding sites, whereas TRH removal is followed within a few hours by the reappearance of most of the binding activity on the membrane [5]. However, recycling of the receptor may not be complete, since the sustained agonist occupation of receptor for 24–48 h results in a loss of 50–75 % of the receptor-binding sites, termed down-regulation [6,7]. One possible explanation for these findings is that a fraction of the internalized receptor enters a degradative pathway, leading to a gradual loss of receptor over time [8]. In addition, TRH decreases the transcription of the endogenous TRHR gene and destabilizes the TRHR mRNA, which would contribute to down-regulation [9–11]. TRHRs are found both on the plasma membrane and in intracellular pools [12]. Although TRH effects on surface TRH-binding sites have been documented, no information is available about how an agonist regulates the overall concentration of TRHR protein. In the present study, we report that the TRHR protein concentration is increased in response to an agonist.

EXPERIMENTAL

Development of cell lines

Antibodies against many G-protein-coupled receptors, including TRHR, are not satisfactory for immunoprecipitation or immunoblotting. For this reason, we utilized cells expressing epitope-tagged receptors. HEK-293 cell lines (human embryonic kidney 293 cell lines), stably expressing either full-length or C-terminal-truncated forms of the rat TRHR tagged with two HA (haemagglutinin) epitopes at the N-terminus, have been described previously [4]. HEK-293 cells stably expressing GFP (green fluorescent protein)-tagged TRHRs or FLAG-tagged β2-adrenergic receptors were prepared by transfecting cells with the plasmids encoding receptors cloned in pcDNA3 using LIPOFECTAMINETM (Invitrogen) according to the manufacturer’s instructions. The cells expressing receptors were selected in 0.5 mg/ml G418. The plasmid encoding FLAG-tagged β2-adrenergic receptor was provided by Dr Michel Bouvier (Universite de Montreal, Montreal, Canada). The plasmid encoding GFP–TRHR was obtained from Dr Karin Eidne (University of Western Australia, Perth, Australia). Cells were grown in DMEM (Dulbecco’s modified Eagle’s medium) or DMEM/F12 supplemented with 7.5 % (v/v) foetal bovine serum, in some

Abbreviations used: GFP, green fluorescent protein; HA, haemagglutinin; HEK-293 cells, human embryonic kidney 293 cells; TRH, thyrotrophin-releasing hormone; MeTRH, [N3-methyl-His]TRH; TRHR, TRH receptor.
cases with 50 μg/ml gentamicin and 2.5 μg/ml amphotericin B, and experiments were performed in this media unless indicated otherwise.

**Immunoprecipitation and immunoblotting**

For immunoblotting, cells cultured in 35 mm dishes were rinsed twice with 2 ml of saline and lysed in 500 μl of ice-cold lysis buffer [150 mM NaCl/50 mM Tris, pH 8.0/1% (w/v) Triton X-100/1 mM EDTA/1:100 Protease Inhibitor Cocktail Set III (Calbiochem)]. Lysates were centrifuged for 10 min at 16,000 g in an Eppendorf microcentrifuge at 4 °C. The pellets from control and TRH-treated cultures did not contain significant amounts of immunoreactive TRHR. The supernatants were diluted directly in sample buffer or immunoprecipitated either with an anti-HA antibody (1:5000; Covance) before SDS/PAGE and immunoblotting for TRHR or with an anti-FLAG antibody (1:5000; Sigma) for β2-adrenergic receptors [4]. Either the immunoprecipitates or the lysates were run on SDS/polyacrylamide gels and blotted with an antibody directed to HA, FLAG or β-actin (Sigma). To compare the amounts of receptor by densitometry, samples from untreated and treated cultures were serially diluted, run on SDS/polyacrylamide gels and immunoblotted. Where histograms and graphs represent results from multiple experiments, films of blots from individual experiments were scanned and, after background subtraction, the average intensity of receptor bands was plotted in arbitrary units relative to the intensity of receptor in control lanes. Since the intensity of receptor bands in lanes from stimulated cells were often above the linear range, the TRH- and PMA-induced increases in receptor levels, measured by scanning blots from multiple experiments, were found to be generally lower than those obtained when samples from an individual experiment were serially diluted, run on Western blots and compared. The significance of differences between pairs was analysed by unpaired Student’s t test.

**Immunocytochemistry**

For immunocytochemistry, cells grown on 25 mm coverslips were rinsed with PBS, fixed in 1 ml of 4% (w/v) paraformaldehyde in PBS for 20 min at room temperature (20 °C) and incubated for 20 min in a blocking buffer, namely PBS containing 5% (v/v) goat serum. A mouse monoclonal anti-HA antibody was added at a dilution of 1:1000 in the same buffer for 1–3 h and the coverslips were washed, incubated with TRITC (tetramethylrhodamine β-isothiocyanate)-conjugated anti-mouse secondary antibody (1:50; American Qualex, San Clemente, CA, U.S.A.) and then washed and mounted [13].

**Real-time PCR analysis**

Total RNA was isolated from cells in 100 mm dishes using RNeasy (Qiagen). Total RNA (10 μg) was digested with DNase, and cDNA was generated from 1.6 μg using the RETROscript kit (Ambion, Austin, TX, U.S.A.) with random primers. For control reactions, reverse transcriptase was omitted. Primers to the rat TRHR sequence were designed to amplify a 290 bp sequence corresponding to amino acids Leu 164 to Ser 260. The primers were 5′-GGGAAGACCGTCTGTTGAACA-3′ and 5′-GGAA-GAAACGGTGCTGTTGAAACA-3′. Real-time analysis was performed using a plasmid encoding the rat TRHR (10 fg–100 pg) to establish a standard curve. PCRs amplifying the TRHR sequence contained 125 ng of primers, 5 μl of inactivated reverse transcription reaction and 25 μl of SYBRTaq Master Mix in 50 μl. PCR products were normalized to β-actin sequence amplifications from the same reverse transcription reaction using primers obtained from Biosource (Camarilla, CA, U.S.A.). Amplification of the reactions lacking reverse transcriptase served as controls for DNA contamination. Reactions were performed in a Bio-Rad iCycler after an initial 8 min 30 s denaturation step using 95° denaturation for 30 s, 45° annealing for 30 s and 72° extension for 30 s for 40 cycles. A melt curve was drawn at the end of the reaction to verify the absence of primer–primer dimers and non-specific PCR products. Changes in TRHR mRNA levels were determined by the 2−ΔΔCT method [14] using β-actin mRNA as a control.

**FACS analysis**

Control HEK-293 cells or HEK-293 cells stably expressing TRHR–GFP were either treated with 10 nM PMA or 1 μM TRH or left untreated overnight. The next day, cells from 10 cm dishes or a 75 cm² flask were washed twice with 10 ml of Hanks balanced salt solution containing 15 mM Hepes, and then pelleted in a IEC tabletop centrifuge on setting 3 for 5 min. Cells were resuspended in the same buffer and used to analyse fluorescence using a BD FACSCalibur System (BD Biosciences). The GFP was excited at 488 nM and its emission was measured at 530 nM.

**Fluorimetric analysis**

Control HEK-293 cells or HEK-293 cells stably expressing TRHR–GFP were treated with drugs for 18–20 h. For each point, cells on a 6 cm dish were rinsed twice with balanced salt solution and suspended in 2 ml of Hanks balanced salt solution containing 15 mM Hepes (pH 7.4), and placed in a cuvette and subjected to stirring. Fluorescence was measured on a PerkinElmer LS5 fluorimeter with excitation and emission at 480 and 515 nm respectively, using slit widths of 5 and 10 nm respectively. The fluorescence obtained with HEK-293 cells was approx. 25% of that measured in an equivalent concentration of cells expressing TRHR–GFP, and has been subtracted.

**RESULTS**

In all the experiments described in this paper, TRHRs were expressed at 0.5–2 pmol/mg of protein, similar to the expression levels of endogenous receptors, namely 1.1 pmol/mg of protein [15]. A TRHR tagged with an N-terminal HA epitope was stably expressed in HEK-293 cells. To determine whether ligand binding altered the concentration of receptors, we incubated HA–TRHR HEK-293 cells for 18 h with either TRH or the inverse agonist chlordiazepoxide and we followed the receptor concentration by immunoblotting with anti-HA antibodies. As shown in previous studies, the TRHR runs in broad monomer and dimer bands typical of glycosylated G-protein-coupled receptors and undergoes an upshift due to phosphorylation in response to TRH [4] (Figure 1A). After 18 h, TRH caused a large increase in the level of receptor protein, whereas chlordiazepoxide had little effect. Other experiments showed that 100 μM chlordiazepoxide was capable of blocking the induction of receptor protein by 100 nM TRH (results not shown). The amount of receptor protein did not increase significantly until TRH had been present for approx. 8 h and continued to increase for 48 h (Figure 1B). The increase in receptor protein, measured after an 18 h incubation, was half-maximal at 1 nM and maximal at 100 nM TRH, consistent with the receptor $K_d$ of 10 nM (Figure 1C). Titration of the samples...
Thyrotrophin-releasing hormone (TRH) regulation of TRH receptor protein

Figure 1 Effects of TRH agonist and antagonist on TRHR concentration

HA–TRHR HEK-293 cells were incubated as described, lysed, and 20 µl of the sample was subjected to SDS/PAGE and immunoblotted using anti-HA antibody. Total protein concentrations were not affected by the treatments. (A) TRH (1 µM) or 100 µM chlordiazepoxide (CDE) for 1 or 18 h; (B) TRH (1 µM) for 0–48 h; (C) TRH (0–1 µM TRH) for 18 h; (D) cells were incubated for 18 h with or without 1 µM TRH. On the following day, one set of dishes was harvested immediately (0 h). The remaining dishes were washed and incubated in TRH-free medium for the indicated time periods before collection. Results were similar for a washout experiment where TRH was added at different time points and cells were harvested simultaneously. In the graphs below the blots in (A, C and D), results are expressed as the means ± range of the intensity of receptor bands for two independent experiments, whereas the graph in (B) shows individual points from two experiments. Results are normalized to receptor levels in the far left lanes. Increases due to TRH were significant (P < 0.01).

Figure 2(A). The protein kinase C activator PMA also increased the amount of receptor monomer and dimer. These differences were not due to the movement of receptors to or from a detergent-insoluble compartment, since detergent supernates contained essentially all of the immunoreactive receptor in all cases. TRH sometimes increased the receptor protein in transiently transfected cells, but the changes were small and inconsistent.

To ascertain whether this TRH response occurred in other cell lines, we tested a different clone of stably transfected HEK-293 cells, a pool of stably transfected HEK-293 cells and a pool of stably transfected Chinese-hamster ovary cells, all expressing the full-length TRHR. In each case, TRH increased the amount of TRHR detected in Western blots; results with the pool are shown in Figure 2(A).

In addition, we stably expressed a truncated TRHR (Δ335–412 HA–TRHR) in HEK-293 cells and measured the receptor concentration after 18 h exposure to TRH, phorbol ester or no drug. This receptor mutant, which lacks two putative palmitoylation sites and the distal region of the cytoplasmic tail, signals normally but does not undergo ligand-dependent endocytosis [3]. TRH significantly increased receptor concentration, and PMA...
produced an even larger effect (Figure 2B). The finding that TRH increases the amount of Δ335–412 HA–TRHR protein proves that receptor endocytosis is not required.

TRHR couples via Gq/11 with phospholipase C; TRH stimulates the production of inositol trisphosphate, which releases intracellular calcium, and diacylglycerol, which activates protein kinase C. To probe the mechanism of the TRH-dependent increase in receptor protein concentration, we exposed cells to drugs that activate protein kinase C (the phorbol ester PMA) or increase intracellular calcium (the ionophore ionomycin) to mimic hormone action. PMA increased TRHR protein as effectively as TRH, whereas ionomycin did nothing by itself and did not alter the TRH response (Figures 2 and 3A). The drug U73122 partially blocks the activation of phospholipase C by TRH, and it partially inhibited the TRH response. GF109203X, a protein kinase C inhibitor, and thapsigargin, an inhibitor of the calcium pump that maintains intracellular calcium stores, both decreased the TRH effect. The combination of GF109203X and thapsigargin, which should block both arms of the TRH signalling pathway, completely abrogated the ability of TRH to increase the receptor protein concentration. These results are corroborated by binding studies that revealed changes in radioligand binding similar to changes in receptor protein (Figure 3B). Since [3H]MeTRH (where MeTRH stands for [N3-methyl-His]TRH) is membrane-impermeable, these differences reflect an increase in receptor at the plasma membrane. TRH was washed out before the addition of [3H]MeTRH to allow internalized receptors to cycle to the cell surface. Nonetheless, the increases in ligand binding were always smaller than the increases in total receptor protein. To explore further whether surface receptors increased, we immunostained cells from control, TRH- and PMA-treated cells without permeabilization (Figure 4). As shown by the more intense staining in the middle and right panels, TRH and PMA both increased the density of receptors at the plasma membrane.

To investigate the specificity of the TRH effect on receptors, we prepared a stable cell line expressing a TRHR fused to GFP at its cytoplasmic C-terminal tail and analysed GFP fluorescence using a fluorimeter to estimate receptor concentration. In agreement with results obtained by immunoblotting, PMA increased the
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Figure 8 Effects of protein, RNA synthesis and proteasome inhibitors

HA–TRHR HEK-293 cells were incubated for 18 h with or without 100 nM TRH in the presence of 10 µg/ml cycloheximide (Chx), 1 µg/ml actinomycin D (ActD) or MG132 (1 h with 1 µM and 18 h with 50 nM). Cells were used for immunoblotting with (A) anti-HA or (B) anti-β-actin antibody. The inhibitors reduced the total cell protein and β-actin and blunted the up-regulation of receptor protein normally caused by TRH. (C) The histogram represents the means ± S.E.M. of receptor intensity of blots for three independent experiments. The increase due to TRH was significant (P < 0.01) only in untreated cells.

Figure 9 Effects of agonist and protein kinase C activation on β2-adrenergic receptor protein

HEK-293 cells stably expressing FLAG-tagged β2-adrenergic receptors were incubated for 18 h with either vehicle, 100 µM isoprenaline (Iso) or 10 nM PMA. Lysates from 6 cm dishes were immunoprecipitated (IP) and immunoblotted with (A) anti-FLAG or (B) anti-β-actin antibody as described in Figure 1. (C) The histogram shows the means ± S.E.M. of receptor intensities for four experiments; one point is omitted in which PMA caused a 14-fold increase in intensity.

Previous studies reported a decrease in TRHR mRNA with prolonged agonist exposure, which is supposed to depend on regulatory elements in the 3'-untranslated region of the mRNA that were not present in the receptor construct used in our experiments [9–12]. It is possible to envision a mechanism in which TRH or protein kinase C activation facilitates folding or post-translational modification of the receptor. Alternatively, MG132 may have affected other proteins or pathways required for up-regulation. (iii) TRH may increase the amount of newly synthesized receptor, and MG132 may have affected other proteins or pathways required for up-regulation. Alternatively, the mechanism(s) responsible for receptor up-regulation may depend on a labile protein.

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Previous studies reported a decrease in TRHR mRNA with prolonged agonist exposure, which is supposed to depend on regulatory elements in the 3'-untranslated region of the mRNA that were not present in the receptor construct used in our experiments [9–11]. In pituitary tumour cell lines, TRH causes a decrease in TRH binding sites at the plasma membrane, indicating that the competing influences of TRH on receptor mRNA and receptor protein result in a net decrease in surface receptors. Additional work will be required to understand how hormonal activation of the TRHR affects receptor concentrations in more physiological settings.

In addition to TRHR, a few other G-protein-coupled receptors, including the SST1 (somatostatin receptor 1) [21,22], dopamine DA2L receptor [23], dopamine DA1, angiotensin II [24], GnRH (gonadotropin-releasing hormone) [25] and 5HT2A receptors, are up-regulated by agonists. In most cases, effects on the density of binding sites but not receptor protein have been documented. An agonist probably causes stabilization of the SST1 receptor and synthesis of GnRH, 5HT2A and DA2L receptors. In contrast, most G-protein-coupled receptors undergo homologous down-regulation [8], and there are numerous examples of receptors whose concentrations decrease rapidly after agonist exposure in heterologous expression systems similar to the one used here [16]. In some cases, this down-regulation occurs when the agonist-occupied receptor undergoes endocytosis and sorting to lysosomes. In others, down-regulation occurs even though receptors either do not internalize or internalize and recycle without degradation.

The results of the present study indicate that regulation of TRHRs is more complex than previously appreciated. Not only is the density of surface-binding sites controlled by agonist, but also the concentration of receptor protein. The pool of receptors potentially available for signalling appears to expand significantly in response to the agonist. Future studies will be necessary to appreciate the physiological significance of this TRH-mediated, signal-dependent regulation.

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REFERENCES

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