HYDROCORTISONE INCREASES THE NUMBER OF RECEPTORS FOR THYROTROPIN-RELEASING HORMONE ON PITUITARY CELLS IN CULTURE

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SUMMARY

Hydrocortisone (cortisol) increased the binding of thyrotropin-releasing hormone (TRH) to specific membrane receptors in 4 clonal strains of rat pituitary cells. At the highest effective cortisol concentration ($3\text{-}5 \times 10^{-6}$ M), the increase was observed within 6-8 hr and became maximal (140 to 160% of control binding) by 18-24 hr. Half-maximum stimulation occurred in serum-containing medium at 9×10^{-8} M cortisol, and a significant increase in TRH binding was seen at 3×10^{-8} M. Equilibrium binding studies showed that enhanced TRH binding was explained by an increase in receptor number with no change in affinity. Similar effects were seen with Dexamethasone, but no increase in TRH binding was noted when testosterone, methyltestosterone, progesterone, estradiol or the antiestrogen Lilly 88571 were added to the culture medium. Cortisol treatment did not cause the appearance of specific TRH binding sites in cell strains previously shown to lack receptors for the tripeptide (F_4C_1, GH_12C_1 and R_5 cells). When added cortisol was removed from medium, receptor number decayed to control values with a $T_{\frac{1}{2}}$ of about 30 hr. Previous studies have shown that TRH receptors in GH-cells can be down-modulated by TRH and thyroid hormones; the present findings demonstrate that glucocorticoid hormones can increase the number of TRH receptors in GH-cells.

INTRODUCTION

Membrane receptors for thyrotropin-releasing hormone (TRH) on prolactin- and growth hormone-producing cells in culture are not fixed in number. TRH decreases the number, but not the affinity, of its own receptors in the clonal GH-strains of rat pituitary cells (1,2). Thyroid hormones (thyroxine and triiodothyronine) have a similar effect on TRH receptors, but they appear to act by a different mechanism (3). Thyroxine treatment of donor rats also decreased TRH binding in crude pituitary membrane preparations (4), and a similar effect was seen in primary mixed cultures of normal pituitary cells

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(5). Hydrocortisone stimulates the synthesis of growth hormone and inhibits prolactin production by GH-cells (6-8). We have therefore examined the effects of hydrocortisone on TRH receptors in GH-cells and find that the glucocorticoid produces a 40 to 80% increase in the number of specific TRH binding sites in these cells.

METHODS AND MATERIALS

Cell culture. Methods of cell culture have been described (9). In brief, cells were grown in 35 mm dishes at 37°C in 1.5 ml/dish of Ham's F 10 medium supplemented with 15% horse serum and 2.5% fetal calf serum (complete medium). Compounds to be added to the cultures were dissolved in dH_2O or ethanol, diluted in sterile medium, and added in a volume of 10-100 µl. The highest concentration of ethanol in the final medium was 0.3%, an amount that had no effect on the outcome of the experiments described. Control dishes received ethanol alone. The pituitary cell strains used were GH_1, GH_3, GH_12C_1 (9); GH_4C_1 (10); F_4C_1 (11); and GC (8). Control rat fibroblasts were the R_5 line (12). Cell protein was determined by the method Lowry et al. (13). In the experiments presented, none of the steroid hormones used altered the total cell protein per dish.

Measurement of TRH receptors. Cells were cultured without or with added steroid hormones or other drugs as described in the figure legends. Medium was removed, cells were washed once with complete medium, then 1.0 ml of fresh prequilibrated complete medium was added followed immediately by [^{3}H]-TRH (0.5 to 30 nM) alone or with excess unlabeled TRH (20-30 µM). Steroids or drugs were not present during the binding assay; at the concentrations used their presence or absence did not affect binding. The dishes were incubated 60-65 min at 37°C in the cell culture incubator. Dishes were then washed rapidly 5 times in a large volume of cold saline, the cells lysed in 1.5 ml of 10% Triton X-100 and radioactivity determined by liquid scintillation counting at an efficiency of 25-30% (12). This procedure is similar to that previously described in detail (1,12). Specific TRH binding is defined as total [^{3}H]TRH bound minus [^{3}H]TRH bound in the presence of 20-25 µM unlabeled TRH (1,12). Each binding point was determined in duplicate or triplicate dishes.

Materials. [^{3}H]TRH (L-[2,3-^{3}H]proline), 20-40 Ci/mmole was from New England Nuclear. Hydrocortisone sodium succinate (Solu-Cortef) was from the Upjohn Co. and is referred to in the text and figures as cortisol. Dexamethasone, 17β-estradiol, testosterone, 17α-methyltestosterone and progesterone were from Sigma Chemical Co. The antiestrogen, compound 88571, was a gift from Eli Lilly Research Labs. (14).

RESULTS

At a concentration of 3×10^{-6} M, cortisol increased the binding of TRH to specific receptors on GH_4C_1 cells within 6-8 hr (Fig. 1A). The effect reached a maximum (about 140 to 150% of control binding) by 18-24 hr and persisted for at least an additional 24 hr (Fig. 1A). The dose-response

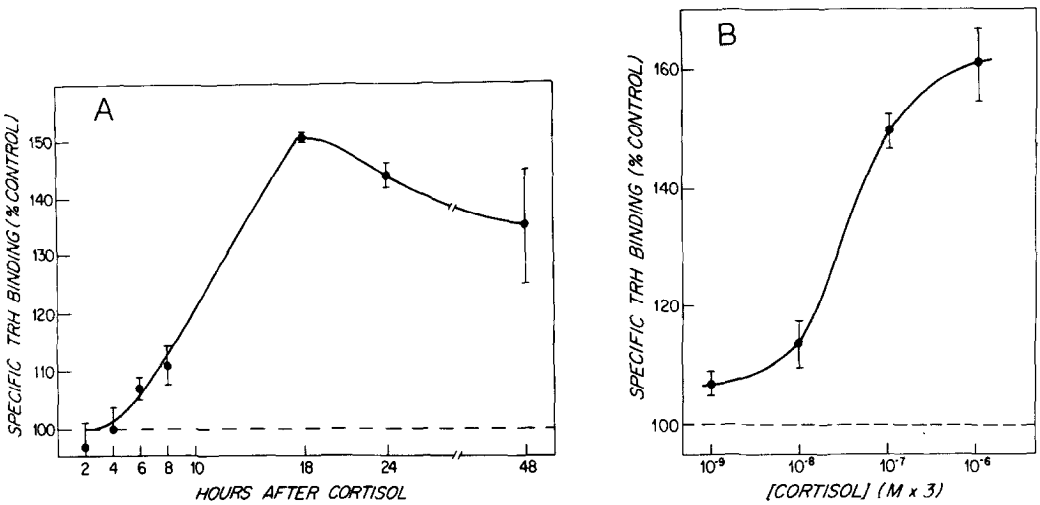


Fig. 1A. Time-course of effect of cortisol (3×10^{-6} M) on specific TRH binding to GH_4C_1 cells. At each time interval, control and cortisol-treated cultures were harvested for binding assay using 25 nM [^{3}H]TRH. Each point gives the mean value of duplicate dishes and the bars give the ranges. Control specific binding (100% control) ranged from 4.1 to 5.3 x 10^3 cpm/mg protein over the 48 hr experiment. Fig. 1B. Effect of various concentrations of cortisol (3×10^{-9} to 3×10^{-6} M) on specific TRH binding to GH_4C_1 cells. Treatment with cortisol was for 22.5 hr. Each point gives the mean of duplicate dishes and the bars give the ranges. Control specific binding (100% control) was 2.6×10^3 cpm/mg protein.

curve for cortisol, measured after 22.5 hr of treatment, is shown in Fig. 1B. Concentrations of cortisol higher than 5×10^{-6} M did not produce greater effects, and the half-maximum increase in TRH binding occurred at about 9×10^{-8} M cortisol. The results of equilibrium binding studies indicate that the increase in TRH binding was due to an increase in the number of binding sites per pituitary cell and not to an increase in the affinity of receptors for TRH (Fig. 2). After 49 hr of treatment with cortisol, maximum TRH binding was increased about 75% with no significant change in the apparent K_d. There were 121,000 binding sites per cell in cultures treated with cortisol as compared to 69,000 for control cells, while the apparent K_d of the TRH-receptor complex was 7.55 and 7.25 nM for cortisol-treated and control cells, respectively (Fig. 2). In each of the above experiments, specific TRH binding was measured using intact cells. In additional experi-

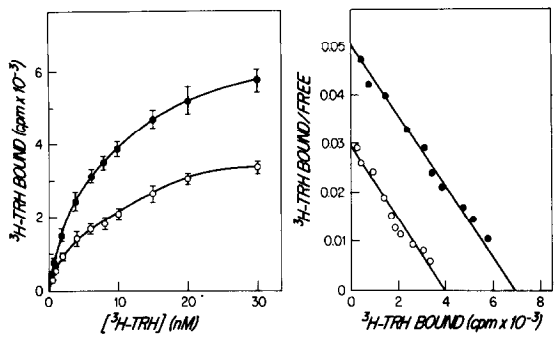
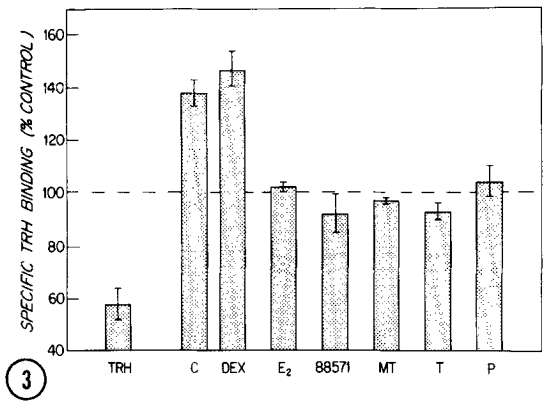


Fig. 2. Binding studies on GH_4C_1 cells incubated without or with cortisol (3×10^{-6} M) for 49 hr. Left panel. Specific [^{3}H]TRH (0.5 to 30 nM) binding was measured as described in Methods in control (o—o) and cortisol-treated (●—●) cells. Each point is the mean of triplicate dishes and the bars give the SD. Right panel. Scatchard plot of the binding data shown in the left panel for control (o—o) and cortisol-treated cells (●—●). The K_a values were 1.38×10^8 and 1.32×10^8 M^{-1} for control and steroid-treated cells, respectively. The values for apparent K_d and total receptors per cell are given in the text and indicate that the increase in TRH binding in cortisol-treated cells is due solely to an increase in receptor number. In calculating receptors/cell, the value of 10^6 cells = 210 µg protein was used.

ments, crude membrane fractions were prepared from control and cortisol-treated cells and specific TRH binding was measured (12). Membranes from GH_4C_1 cells treated with cortisol (3×10^{-6} M, 42-48 hr) bound 1.5- to 2.3 fold more TRH at saturation than did membranes from control cells.

The steroid specificity of TRH receptor modulation was examined (Fig.3). At the dose levels used and under the conditions tested, only the glucocorticoids, cortisol and Dexamethasone, increased TRH binding. Estradiol, the anti-estrogen Lilly 88571 which enhances prolactin production by GH_4C_1 cells (14), testosterone, methyltestosterone and progesterone were without effects on TRH binding. TRH produced its characteristic effect (1), namely a decrease in specific TRH binding (Fig. 3). In results not shown, cells incubated simultaneously with TRH (10 nM) and cortisol (3×10^{-6} M) showed no down-modulation of receptors by TRH after 50 hr of treatment. Thus, the effects of cortisol alone (a 40% increase) and TRH alone (a 40% decrease) antagonized each other and resulted in TRH binding equal to that measured in control cells.



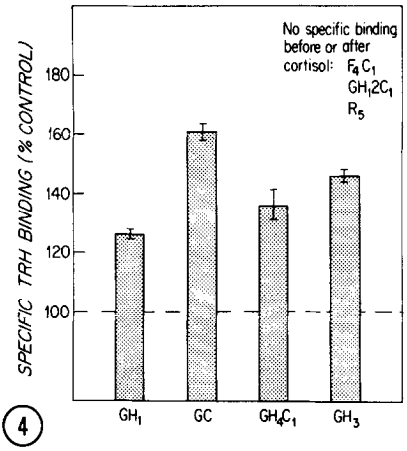


Fig. 3. Effects of various hormones and drugs on specific TRH binding to GH_4C_1 cells. Each treatment was for 42 hr. The concentrations used were TRH (10 nM), cortisol (C, 3 x 10^{-6} M), Dexamethasone (Dex, 3 x 10^{-7} M), and 3 x 10^{-6} M for each of the following estradiol (E_2), Lilly 88571 (88571), methyltestosterone (MT), testosterone (T) and progesterone (P). Each bar gives the mean of duplicate dishes and the brackets give the range. Control specific binding (100% control) was 2.2 x 10^3 cpm/mg protein.

Fig. 4. Effects of cortisol (3 x 10^{-6} M, 48 hr) on specific TRH binding to 4 cell strains that have receptors for the tripeptide (GH_1, GC, GH_4C_1 and GH_3) and 3 lines that do not (F_4C_1, GH_12C_1 and R_5). Each bar gives the mean of duplicate dishes and the brackets give the range. Control specific binding (100% control) ranged from 1.0 to 4.2 x 10^3 cpm/mg protein for receptor-bearing cells and <50 cpm/mg protein for the other lines.

The cell strain specificity of TRH receptor modulation by cortisol was investigated (Fig. 4). Four pituitary cell strains that possess TRH receptors each responded with an increase of TRH binding to 125 to 160% of control. Two pituitary strains which lack TRH receptors (F_4C_1 and GH_12C_1), and a fibroblast line (R_5), showed no specific binding either before or after treatment with cortisol.

The results in Fig. 5 show the time-course of the decrease in TRH receptors to control values after removal of cortisol. No change in the 65% increase in TRH binding was observed within the first 6 hr after removal of cortisol from the medium. Thereafter, TRH binding decreased to control values with a half-time of 24-36 hr. The results in this experiment also show that in the continued presence of cortisol, the increased number of TRH receptors remains high for at least 120 hr.

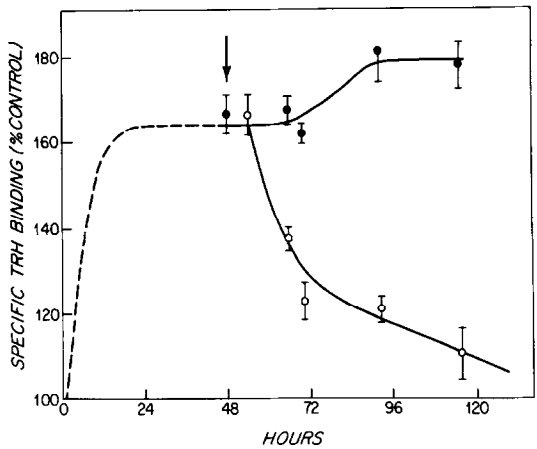


Fig. 5. Effect of cortisol withdrawal on specific TRH binding to GH_4C_1 cells. Replicate cultures were untreated or treated with 3 x 10^{-6} M cortisol for 48 hr (arrow). At this time there was a 67% increase in specific binding. Thereafter, the cortisol-treated cells were divided into 2 groups; one group continued to receive 3 x 10^{-6} M cortisol (●—●) while the other received control medium alone (o—o). Specific binding was monitored periodically and compared to concomitant control cultures that had never received cortisol. All dishes were washed twice with fresh complete medium at 48 hr (arrow). Each point gives the mean value of duplicate dishes and the bars give the range.

DISCUSSION

Results presented in this communication show that cortisol (3 x 10^{-8} to 3 x 10^{-6} M) increases the number of plasma membrane receptors for TRH without altering their affinity. The effect occurs only in cell strains which have a measurable number of specific binding sites in the absence of added steroid. Thus the effect may be due either to an enhanced rate of new receptor synthesis, processing or insertion into plasma membrane, or to a stabilization of preexisting TRH receptors. Our data do not permit discrimination between these two classes of mechanism. Our findings indicate, however, that cortisol does not cause cells previously lacking TRH receptors to acquire them in a measurable form.

It must be emphasized that our experiments were performed in medium containing serum, and therefore that there was a finite background concentration of glucocorticoid hormones (as well as other serum hormones and factors)

present which we supplemented with exogenous steroid additions. We did not measure the endogenous glucocorticoid hormone concentration of the medium. If glucocorticoid-free medium had been used, it is likely that the effects we observed would be detected at substantially lower concentrations of added cortisol. Furthermore, the lack of effect of other steroid hormones and drugs (Fig. 3) could be due to maximum effects produced by endogenous hormones already present in the medium or to masking of the effects by other serum factors, rather than to no effect of estrogen, androgen or progestin on TRH receptors. Striking examples of this phenomenon in GH-cells are the marked effects of added thyroid hormones on growth hormone synthesis (15,16) and TRH receptor modulation (3) if the experiments are performed in medium lacking thyroid hormones, whereas little or no effect of added hormone is detected in medium containing undepleted serum.

It appears possible that the effect of cortisol on TRH receptors is mediated via a classical cytoplasmic glucocorticoid receptor for 1. such a receptor is present in GH-cells (E. B. Thompson, P. S. Dannies and A. H. Tashjian, Jr., unpublished data), and 2. cortexolone, a glucocorticoid antagonist, reduced the effect of Dexamethasone on TRH receptors by 50% when coincubated at a 15-fold molar excess (3×10^{-6} M) with the glucocorticoid.

Because cortisol affects prolactin and growth hormone synthesis in GH-cells independently of effects on TRH receptors (6-8), it has not yet been possible to demonstrate unambiguously whether the increase in TRH receptors that follows cortisol treatment renders the cells more responsive to TRH.

In addition to the steroid (Fig. 3) and cell (Fig. 4) specificities shown, it has been possible to examine the effect of cortisol on somatostatin receptors which are also present on GH_4C_1 cells (17). Such experiments are analogous to those performed with insulin and growth hormone receptors which can be monitored and modulated independently in lymphocytes (18). In experiments in which cortisol increased TRH binding by about 40%, there was no change in specific somatostatin binding to the same cells (A. Schonbrunn and

A. H. Tashjian, Jr., to be published). These findings collectively indicate that the number of TRH receptors on GH-cells can be modulated differentially by several specific hormonal signals and that other receptors on the same cells behave differently. Further mechanistic studies in this cell system should give new insights into how cells control the numbers of specific recognition molecules on their plasma membranes.

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