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THE SYNTHESIS OF β-ADRENERGIC RECEPTORS IN CULTURED HUMAN LUNG CELLS: INDUCTION BY GLUCOCORTICOIDS

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Summary

The rate of β-adrenergic receptor synthesis and incorporation into plasma membranes is measured in human lung (VA_2) cells following irreversible blockade of existing receptors with N-[2-hydroxy-3-1-(naphthoxy)-propyl]-N-bromoacetylethylenediamine, a covalent β-receptor antagonist. New receptor incorporation is measurable for approximately 24 hours in these cultured cells and the rate of synthesis is on the order of 2% of the total (initial) receptor concentration per hour. Hydrocortisone induces a dose dependent increase in β-receptor concentration in VA_2 cells, VA_4 cells and WI38 cells. Maximum increases are observed with 0.1 μM hydrocortisone within 24 hours and represent a 100% increase in β-receptor number. A hydrocortisone stimulated doubling in the rate of β-adrenergic receptor synthesis and incorporation appears to be responsible for this increase in β-receptor concentration in the affected cells.

Introduction

Membrane proteins are known to undergo continuous turnover with a mean half-life of 30-60 hours (1). There is evidence, however, that the physiological state of a tissue can influence the rate at which specific membrane proteins undergo turnover. In the case of membrane-bound receptors, various factors have been shown to affect receptor concentration. For example, turnover of the nicotinic acetylcholine receptor is increased by muscle denervation (2) and this effect can be blocked by direct electrical stimulation of the muscle (3). In addition, antibodies directed against the acetylcholine receptor can accelerate receptor degradation (4).

Hormones are also known to regulate the concentration of certain membrane receptors. Low levels of insulin were first shown to down-regulate the concen-

Abbreviations: NHNP-NBE, N-[2-hydroxy-3-1-(naphthoxy)-propyl]-N-bromoacetylethylenediamine; TRH, thyrotropin releasing hormone; [^{125}I]-IHYP, [^{125}I]-iodohydroxybenzylpindolol.

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tration of insulin receptors in lymphocytes by Gavin and co-workers (5). This phenomenon of insulin regulation of insulin receptor concentration has since been observed in several species and is believed to involve an energy dependent increase in insulin receptor degradation (6). Both thyrotropin releasing hormone (TRH) (7) and thyroid hormone (8) reduce the number of thyrotropin-releasing hormone receptors in cultures of rat pituitary cells, whereas hydrocortisone increases TRH receptor concentrations (9) in these cells. Williams, Lefkowitz and co-workers (10) have demonstrated that the administration of thyroid hormone to rats results in an increased number of β-adrenergic receptors in the heart, with no effect on receptor affinity for agonists or antagonists. Regardless of the stimulus for the regulation of membrane receptor concentrations, changes in the number of receptor proteins must ultimately be due to altered rates of either receptor synthesis and/or degradation.

To date there have been no studies on the synthesis rate and turnover rate of the β-adrenergic receptor. However, a study on rat pineal gland β-receptors by Kababian and co-workers in 1975 suggested that the β-receptor is a membrane component which may undergo a rapid turnover (11). The absence of data on the turnover rate of β-receptors has been due in part to the lack of an irreversible β-receptor ligand. The development of N-[2-hydroxy-3-(1-naphthoxy)-propyl]-N-bromoacetylethylenediamine (NHNP-NBE) by Atlas and co-workers (12) has helped to overcome this deficit. A detailed characterization of the irreversible nature of this compound and its effect on β-receptor-mediated processes has been reported by this laboratory (13).

In this present study we have used the affinity ligand, NHNP-NBE, to measure the synthesis rate of the β-receptor in cultured cells. A specific induction of the synthesis of β-receptors by hydrocortisone is also documented.

Materials and Methods

Materials - Ham's F-12 media, Minimal Essential Media and fetal bovine serum were obtained from GIBCO Laboratories. [^{125}I]-iodohydroxybenzylpindolol ([^{125}I]-IHYP) was synthesized as previously described (14). [^{3}H]-leucine and [^{3}H]-methionine were obtained from New England Nuclear. Puromycin dihydrochloride, cycloheximide, (1)-isoproterenol hydrochloride and hydrocortisone were

obtained from Sigma. (d)- and (l)-propranolol were a gift from Ayerest. NHNP-NBE was synthesized by Dr. D. Triggle.

Tissue Culture - For these studies we have used WI38 cells, a line of normal diploid human embryonic lung cells and VA_2 and VA_4 cells, SV40 transformed clones of the WI38 and WI26 cell lines. The WI38 cells and VA_4 cells were obtained from American Type Culture Collection and the VA_2 cells were a gift from Dr. A.G. Gilman. Cells were grown in either Ham's F-12 media or Minimal Essential Media supplemented with 10% fetal calf serum in an atmosphere of 95% air-5% CO_2. Cells were trypsinized and seeded at appropriate densities at least 48 hours prior to any experiment. This period is adequate to allow replacement of any receptors altered by trypsin.

β-Receptor Assay - β -receptors were measured in either purified plasma membranes (14) or in intact cells (16) with [^{125}I]-IHYP, a potent β-adrenergic antagonist. Specific binding was defined as the total binding minus the binding observed in the presence of 10 μM or 0.1 μM (l)-propranolol for membranes and intact cells, respectively.

Protein was measured using fluorescamine in dioxane (17) with bovine serum albumin as the standard.

Results

β-Receptor Binding - [^{125}I]-IHYP binding to VA_2 cell membranes and VA_4 and WI38 cells is rapid, saturable and stereospecific (unpublished observations). The equilibrium dissociation constant (K_d) for the binding of [^{125}I]-IHYP to VA_2 cells and the number of specific [^{125}I]-IHYP binding sites (B_{max}) in VA_2 cells which we have determined by Scatchard analysis are in close agreement with previously published data (15).

Determination of β-Receptor Synthesis Rates - In order to estimate the synthesis rate of the β-receptor in cultured cells, the cells are incubated with NHNP-NBE for two ten minute periods and unbound ligand is washed off the cultures. This procedure results in almost total inactivation of existing β-receptors as determined by [^{125}I]-IHYP specific binding. The appearance of new β-receptors with time is followed by the appearance of [^{125}I]-IHYP specific binding sites in both intact cells and isolated membrane fractions.

In confluent cultures of VA_2 cells new β-receptors appear rapidly following irreversible ligand treatment (Figure 1). The rate of appearance of new β-receptors in VA_2 cells, as calculated from the initial slope of the curve, is on the order of 2% per hour. Assuming that each receptor has an equal probability of being removed from and replaced in the membrane,

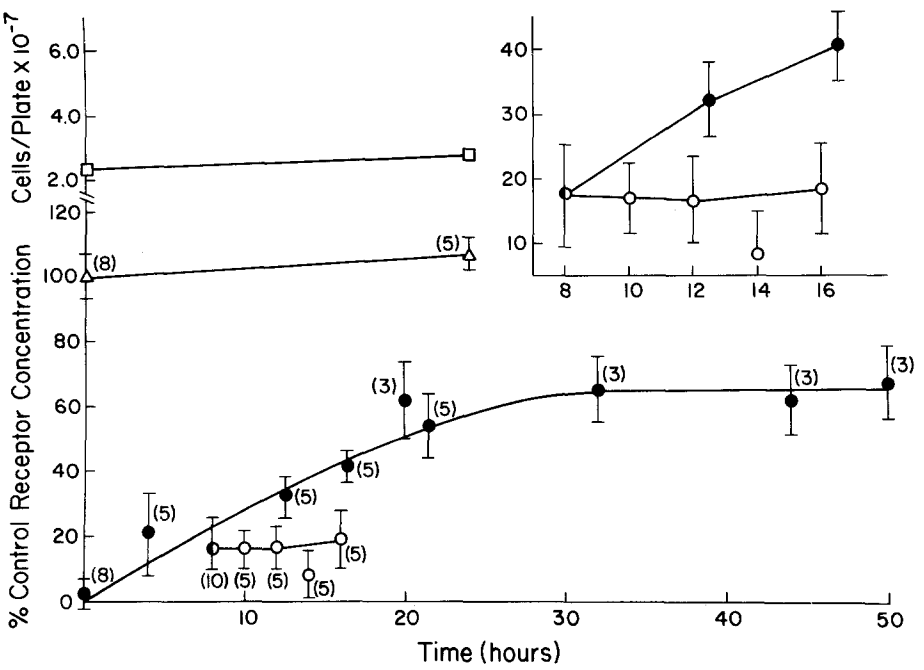


Figure 1: β-Adrenergic receptor incorporation rates into VA_2 cell membranes: Cells were incubated twice with NHNP-NBE (100 μM) to irreversibly inactivate existing β-receptors at time 0. Cells were incubated at 37°C in Minimal Essential Media with 10% fetal calf serum for the times indicated. Cells were harvested and membranes isolated as described (14). The number of VA_2 cells/plate is indicated by the open squares. β-Receptor concentrations were determined by assessing [^{125}I]-IHYP specific binding (18) using 70 μg of membrane protein and 87 pM [^{125}I]-IHYP per assay. The control synthesis rates are indicated by the open triangles. [^{125}I]-IHYP specific binding in control cultures was 16 fmol/mg protein. The closed circles represent β-receptor incorporation rates subsequent to NHNP-NBE treatment. The inset shows the β-receptor incorporation in cells treated with NHNP-NBE (closed circles) or NHNP-NBE plus puromycin (0.1 mg/ml) 8 hours subsequent to NHNP-NBE treatment (open circles).

the half-life of the β-receptors in this system can be estimated to be on the order of 25-30 hours.

The addition of puromycin to the cultures, eight hours after receptor blockade, completely inhibits protein synthesis (data not shown) and inhibits the further incorporation of β-receptors into the VA_2 cell membranes (Figure 1). These data demonstrate that active protein synthesis is required for the appearance of new β-receptors in the membranes and confirms the idea that the appearance of [^{125}I]-IHYP binding sites with time is due to new receptor synthesis and incorporation

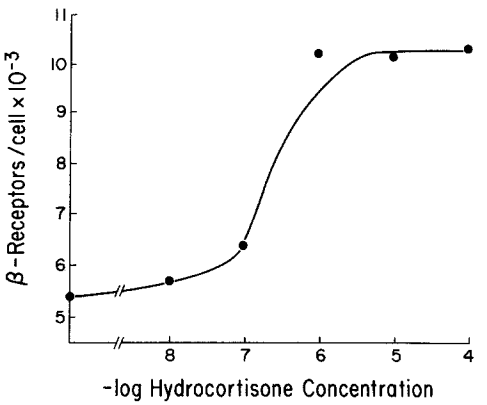
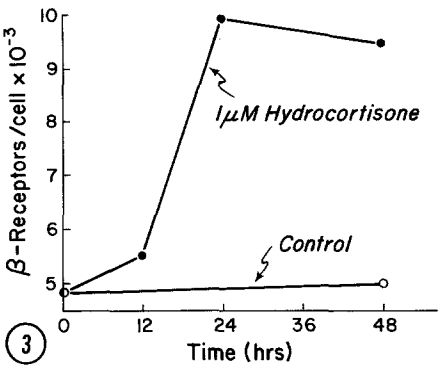


Figure 2: Log dose-response curve for hydrocortisone induced increase in [^{125}I]-IHYP specific binding in WI38 cells: WI38 cells were incubated for 48 hours in Ham's F-12 media plus various concentrations of hydrocortisone added in 70% ethanol. β-receptors were assayed on 60 mm plates as described in Figure 1 using an [^{125}I]-IHYP concentration of 130 pM. Each plate contained 4.5 x 10^5 cells at the time of the assay. Control cells received an equivalent amount of ethanol. Specific binding in control cells was 9 fmol [^{125}I]-IHYP per 10^6 cells.

and not dissociation of NHNP-NBE. Control cultures which received NHNP-NBE treatment but no puromycin continue to incorporate β-receptors at the normal rate. Puromycin inhibition of β-receptor incorporation is essentially immediate suggesting that an intracellular pool of β-receptors may not exist in the VA_2 cells.

β-Receptor Regulation by Glucocorticoids - We have obtained evidence that glucocorticoids are capable of regulating β-receptor concentrations in human lung cells. Incubation of lung cells (VA_2 or VA_4 cells) with hydrocortisone for 24 hours results in a 100% increase in [^{125}I]-IHYP specific binding. Scatchard analysis of the binding data from control cells and hydrocortisone treated cells demonstrates that the increase in [^{125}I]-IHYP specific binding observed is due to a change in receptor number with no change in receptor affinity for [^{125}I]-IHYP (not shown). WI38 cells, a line of normal diploid human lung tissue, appear to be equally sensitive to glucocorticoid stimulation. The dose-response curve for [^{125}I]-IHYP specific binding in WI38 cells incubated with hydrocortisone is illustrated in Figure 2. Hydrocortisone treated cells show an approximate doubling of [^{125}I]-IHYP specific binding within 48 hours with an ED50 for hydrocortisone of 0.3 μM. The time course of this glucocorticoid



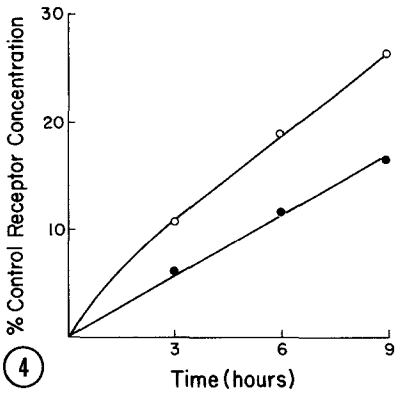


Figure 3: Time course of glucocorticoid induced increase in [^{125}I]-IHYP specific binding in WI38 cells: WI38 cells were incubated for various time periods in Ham's F-12 media containing 1 μM hydrocortisone. Hydrocortisone was dissolved in 70% ethanol and control cells received an equivalent amount of ethanol. Receptor concentrations were measured as described in Figure 1 using an [^{125}I]-IHYP concentration of 130 pM. Cell number per plate increased approximately 3-fold in both control and hydrocortisone-treated cultures during the time course of the experiment.

Figure 4: β-Adrenergic receptor incorporation rates into hydrocortisone treated VA_4 cell membranes: VA_4 cells were incubated in Ham's F-12 media containing 1 μM hydrocortisone for 18 hours. Hydrocortisone was added in 70% ethanol and control cells received an equivalent amount of ethanol (final ethanol concentration = 0.25%). At time zero, the VA_4 cell β-receptors were inactivated with NHNP-NBE, fresh Ham's F-12 media with or without 1 μM hydrocortisone was added to the cells and new receptor incorporation rates were measured in intact VA_4 cells as described in Figure 1. The concentration of [^{125}I]-IHYP added per plate was 125 pM in a final volume of one ml. The rate of receptor incorporation into control VA_4 cell membranes is indicated by the closed circles. The open circles represent the rate of receptor incorporation into hydrocortisone treated VA_4 cell membranes. Control cultures contained 6700 β-receptors/cell.

induced doubling in [^{125}I]-IHYP specific binding is illustrated in Figure 3. The maximal response is evident within 24 hours and persists for at least 48 hours in the presence of hydrocortisone.

Hydrocortisone appears to increase the rate of β-adrenergic receptor synthesis and incorporation into lung cell membranes. Preincubation of VA_4 cells with 1 μM hydrocortisone for 18 hours prior to the determination of β-receptor synthesis rates results in an approximate doubling of the rate of receptor appearance in glucocorticoid treated cells as compared to control cells (Figure 4). The receptor synthesis rates during the first nine hours of hy-

drocortisone treatment are indistinguishable from synthesis rates in control cultures (data not shown). The increase in β-receptor concentration observed in human lung cells grown in the presence of hydrocortisone therefore most likely reflects an induction of receptor synthesis.

Discussion

Our results indicate that β-receptors in cultured human lung cells appear to be subject to dynamic regulation. Cells treated with hydrocortisone possess a receptor complement two-fold greater than observed in untreated cells. The molecular mechanism responsible for this change in receptor density appears to be a glucocorticoid stimulated doubling in the rate of receptor synthesis. Although glucocorticoids may accelerate β-receptor synthesis, the concentration of β-receptors in hydrocortisone treated cells reaches a constant value within 24 hours. This observation implies that at steady state, receptor synthesis and degradation must be occurring at equal rates. The simplest explanation for a concomitant increase in receptor synthesis and degradation in these cells would involve the hypothesis of Tweto and Doyle(18) which describes degradation of the plasma membrane as a unit. If β-receptors are homogeneously distributed in the plasma membrane of the human lung cells, then an increase in receptor synthesis and insertion into the membrane would subsequently be accompanied by an increase in receptor degradation resulting in a new steady-state concentration of membrane-associated receptors proportional to the synthesis rate.

Glucocorticoids are used in the treatment of status asthmaticus even though their mechanism of action has not been clear (19,20). However, the glucocorticoid stimulated increase in β-receptor synthesis rates may be significant in terms of the therapeutic role of these agents in asthmatic patients.

Utilizing covalent β-receptor modification, the direct synthesis of β-receptors can now be monitored. This technique has provided evidence for the induction of β-receptor synthesis by glucocorticoids in lung cells by apparent control over receptor synthesis and degradation. These results provide the means for investigations into the molecular basis of receptor density regulation.

Acknowledgements

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