On the Mechanisms Involved in the Regulation of the Cell-surface Receptors for Human Choriogonadotropin and Mouse Epidermal Growth Factor in Cultured Leydig Tumor Cells

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ABSTRACT The MA-10 cells are a clonal strain of mouse Leydig tumor cells that have receptors for human choriogonadotropin (hCG) and mouse epidermal growth factor (mEGF).

Exposure of the cells to hCG results in a reduction in the number of surface hCG receptors, and little or no change in the number of surface mEGF receptors. On the other hand, exposure of the cells to mEGF results in a reduction in the number of both surface mEGF receptors and surface hCG receptors.

In order to study these phenomena, we assumed that the number of surface receptors is determined by the rate at which receptors appear at the surface and by the rate of receptor internalization. When these rates were measured, we found that hCG and mEGF reduce their respective surface receptors by increasing the rate of receptor internalization, and that mEGF reduces the surface hCG receptors by decreasing the rate of appearance of the receptor.

The MA-10 cells are a clonal strain of mouse Leydig tumor cells adapted to continuous culture in this laboratory (1). These cells have separate receptors for mouse epidermal growth factor (mEGF) and human choriogonadotropin (hCG) (2).

The binding of hCG to the cells results in increases in cAMP and steroid production (1, 3) and is followed by the internalization and degradation of the surface-bound hormone and the down-regulation of hCG binding activity (4–6). As a consequence of this, the cells lose their ability to respond to the hormone with increased steroid production. Their steroidogenic response to cholera toxin and cAMP is also reduced, but to a lesser extent (6).

The binding of mEGF to the cells also appears to be followed by the internalization and degradation of the surface-bound hormone and the down-regulation of mEGF binding activity (2). MEGF binding does not result in a stimulation of cell division, but it decreases hCG binding activity by 80–90% (2). As a consequence of this, the cells lose their ability to respond to hCG with increased steroid production. Their steroidogenic response to cholera toxin and cAMP remains essentially unchanged (2).

The MA-10 cells are appropriately suited to study the mechanisms involved in the regulation of hormone receptors, since (*a*) they have distinct receptors for at least two different hormones (mEGF and hCG), (*b*) both of these hormones down-regulate their homologous receptors, (*c*) the activity of one receptor (i.e., hCG) can be assessed not only by hormone binding but also by its associated biological responses, and (*d*) one of the hormones, mEGF, also down-regulates the receptor for a heterologous hormone (hCG).

The studies presented herein were aimed at elucidating the mechanisms by which mEGF and hCG down-regulate their respective receptors (homologous down-regulation) and the mechanisms by which mEGF down-regulates hCG receptors (heterologous down-regulation). In order to study these phenomena, we have employed a model recently developed by Wiley and Cunningham (7, 8) to describe the interaction of mEGF with human fibroblast. We show that this model can also be used to describe the interaction of mEGF and hCG with the MA-10 cells, and the mechanisms involved in the homologous and heterologous down-regulation of these hormone receptors. Our data show that mEGF and hCG down-regulate their homologous surface receptors by increasing the rate of receptor internalization, and that mEGF down-regulates

the surface hCG receptors by decreasing the rate of appearance of hCG receptors at the cell surface.

MATERIALS AND METHODS

Hormones and Supplies: HCG (Batch CR-123) was obtained from the National Institute of Child Health and Human Development and iodinated as described elsewhere (9). Under the conditions used, all the iodine is localized in the α-subunit (5, 10, 11). MEGF was a generous gift of Professor Stanley Cohen of this institution and was iodinated as described by Carpenter and Cohen (12). The specific activities of ^{125}I-hCG and ^{125}I-mEGF were 5–7 × 10^4 cpm/ng and 2–3 × 10^5 cpm/ng, respectively. All tissue culture supplies were obtained from Gibco Laboratories (Grand Island Biological Co., Grand Island, NY). Crude hCG (3000 IU/mg) was obtained from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (Cohn Fraction V) was from Miles Laboratories, Inc. (Elkhart, IN).

Cell Culture: The origin and handling of the MA-10 cells has been described (1). Experimental cultures were plated into 6-cm culture dishes containing 5 ml of Waymouth's MB752/1 modified to contain 20 mM HEPES, 1.12 g/l $NaHCO_3$, 40 μg/ml Gentamycin, and 15% horse serum (pH 7.4). All experiments were started 3–4 d after plating. At the end of the experiment, the dishes contained 80–100 μg of DNA (3.6–4.5 × 10^6 cells). All experiments were done using a single batch of serum.

Analysis of ^{125}I-hCG Binding: Cells were plated on day 0. On day 1 the medium was replaced, and half of the cultures received 5 ng/ml mEGF (2). On day 3 the experiments were started by replacing the medium with 4 ml of warm medium, and the same concentration of mEGF was added to the appropriate dishes. The desired concentrations of ^{125}I-hCG were then added, and the cells were incubated at 37°C. For each point shown in the figures, three dishes were used. Two dishes received ^{125}I-hCG only, and the third dish also received 25 IU/ml of crude hCG (to correct for nonspecific binding). At the appropriate times, the dishes were placed on ice and an aliquot of the medium was saved. The dishes were then washed five times with 2 ml of ice-cold Hanks' balanced salt solution containing 1 mg/ml bovine serum albumin. The total washing time was about 1 min. Surface-bound ^{125}I-hCG was removed by adding 1.5 ml of ice-cold 50 mM glycine, 100 mM NaCl pH 3.0 (5). After a 4-min incubation on ice, this solution was removed, and the dishes were washed once with 1 ml of the same solution. The radioactivity present in the combined solutions was measured in a Beckman 4000 Gamma Counter (Beckman Instruments, Inc., Palo Alto, CA). Intracellular ^{125}I-hCG was determined after dissolving the acid-treated cells in 2 ml of 0.5 M NaOH. Nonspecific binding accounted for 2–30% of the total binding (depending on experimental conditions). All data presented were corrected accordingly.

Analysis of ^{125}I-mEGF Binding: For these experiments the cells were subcultured on day 0, and the medium was replaced on days 1 or 2. Experiments were started on day 3 by replacing the medium with 4 ml of warm medium and adding the desired concentrations of ^{125}I-mEGF. In this instance nonspecific binding was determined in the presence of an excess of mEGF (2.5–5.0 μg/ml). The surface-bound and internalized radioactivity were determined as described above. Nonspecific binding accounted for 2–20% of the total binding (depending on experimental conditions). All data were corrected accordingly.

Analysis of Binding Data: All data presented were corrected for the fraction of surface-bound hormone that is not removed by the acid treatment and for the fraction of internalized hormone that is removed by the acid treatment, using the algorithm described by Wiley and Cunningham (8). Using the methods described by Wiley and Cunningham (8), we determined that our acid treatment removes 88% of the surface-bound hormone and 1% of the internalized hormone (^{125}I-hCG or ^{125}I-mEGF).[1] Thus, this acid treatment is comparable to the one reported by Haigler et al. (14).

In some steady-state experiments, it was necessary to calculate the concentration of free hormone present in the medium. To do this, we precipitated duplicate 0.5-ml aliquots of the incubation medium with 10% (wt/vol) trichloroacetic acid for 30 min at 4°C. After centrifugation, the supernatants were aspirated and the pellets counted. Under these conditions, 85–90% of the intact ^{125}I-hCG or ^{125}I-mEGF can be precipitated. Acid-soluble radioactivity did not increase when the hormones were incubated alone for 18–24 h at 4° or 37°C.

All the straight lines shown in the graphs were calculated by unweighted linear regression analysis and had a correlation coefficient greater than or equal to 0.90. Statistical analysis was performed using "t" tests.

[1] It should be noted that the method used to iodinate mEGF results in minimal "spontaneous" cross-linking to the receptor (13), and that the inclusion of these correction factors should prevent this phenomenon (if present in the MA-10 cells) from affecting any of the data presented.

RESULTS

Homologous and Heterologous Down-Regulation of Surface hCG Receptors

We have previously shown that the ^{125}I-hCG binding activity of the MA-10 cells can be reduced by prolonged incubation of the cells with the homologous hormone (i.e., hCG) or a heterologous hormone such as mEGF (2, 4, 6). In those studies ^{125}I-hCG binding activity was determined at 37°C, a temperature at which not all the hormone is localized at the cell surface. Thus, we could not clearly establish that the reduction of ^{125}I-hCG binding activity was due to a loss of surface receptors. In order to overcome this caveat, we incubated cells (37°C) with mEGF or hCG under conditions that lead to a maximum reduction of ^{125}I-hCG binding activity (2, 6), and then measured the number of surface ^{125}I-hCG receptors by analyzing ^{125}I-hCG binding at 2–4°C. Under these conditions, ^{125}I-hCG internalization is inhibited, most of the hormone is localized at the cell surface, and "equilibrium" conditions are reached (4, 5). The results of a representative experiment are presented in Fig. 1, and shown that (*a*) mEGF reduced the number of surface hCG receptors/cell and increased the affinity of the cells for the hormone and (*b*) hCG reduced the number of surface hCG receptors/cell and had no effect on the affinity (cf. Table I).

A reduction in the number of surface hCG receptors can be brought about by decreasing the rate of appearance of receptors at the surface (V_R) and/or by increasing the rate constant for receptor internalization (k_t).[2] Thus, we sought to determine which of these parameters are affected when the surface hCG receptors are reduced with the homologous hormone (hCG) or a heterologous hormone (mEGF). In order to do this, we applied a model developed recently by Wiley and Cunningham (7, 8). This model was used by those authors to describe the interaction of mEGF with human fibroblasts, and is based on the observation that, upon binding to the cell surface, mEGF is internalized and degraded, and that these processes come to a steady state. By analyzing the steady-state interaction of mEGF with the cells, they were able to calculate several constants that accurately describe the surface binding, internalization, and degradation of mEGF, and the homologous reduction of mEGF receptors. Moreover, they were able to calculate the two parameters (V_R and k_t, see above) that determine the number of surface receptors before the ligand is added. Thus, we reasoned that if this model is used to analyze the interaction of ^{125}I-hCG with two groups of cells with different numbers of ^{125}I-hCG receptors (i.e., control and mEGF-treated cells), we should be able to determine not only the mechanisms involved in the homologous down-regulation of surface hCG receptors but also those involved in the heterologous down-regulation.

Application of the Steady-State Model to HCG

The steady-state model was easily adapted to the hCG system because it shares many characteristics with the EGF systems (7, 12, 14). Thus, we have previously described a

[2] The nomenclature of Wiley and Cunningham (7, 8) is used throughout this paper. K_{SS} = steady state association constant; $[R]_S$ = number of cell surface receptors; V_R = rate of receptor appearance at the surface; k_e = rate constant for internalization of occupied receptors; k_t = rate constant for internalization of unoccupied receptors; k_h = rate constant for degradation of the internalized hormone.

method that differentiates between the surface-bound and internalized radioactivity; showed that the radioactivity present in both compartments represent mainly intact hormone; and showed that the main degradation product of the labeled hormone is monoiodotyrosine, which is detectable in the medium only (4, 5). These observations satisfied three of the criteria set forth by Wiley and Cunningham for using the steady-state model (7). The fourth criterion, that the cells themselves approximate a steady state, was satisfied by doing all measurements in the same medium used to culture the cells. Moreover, all measurements done in the mEGF-treated cells were done when the mEGF-induced reduction of surface hCG receptors had attained a steady-state (i.e., after a 48-h incubation with 5 ng/ml mEGF—see reference 2). The fifth criterion is satisfied by the results presented in Fig. 2, which show that the amount of surface-bound and internalized ^{125}I-hCG approach a constant value (in both control and mEGF-treated cells) during an 18–20 h incubation with ^{125}I-hCG at 37°C.

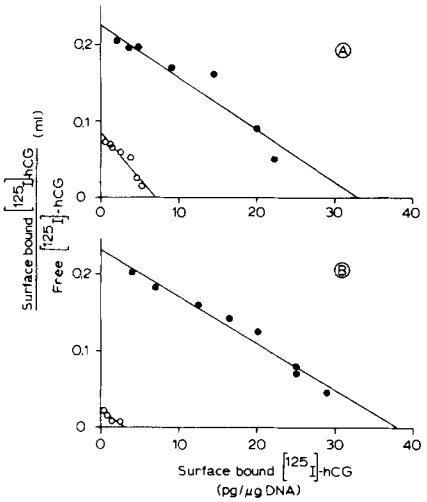

FIGURE 1 Effects of prolonged exposure to mEGF or hCG on the surface binding of ^{125}I-hCG. (*A*) Cells were plated on day 0. On day 1 the medium was replaced with the same medium containing no mEGF (●) or 5 ng/ml mEGF (○). After a 48-h incubation at 37°C, the cells were cooled at 2°–4°C for 30 min, and the medium was replaced with 2 ml of ice-cold medium without (●) or with (○) 5 ng/ml mEGF. The dishes were then incubated at 2°–4°C with increasing concentrations of ^{125}I-hCG (2–100 ng/ml) until binding equilibrium was reached (~12 h). The surface-bound radioactivity was determined as described in Materials and Methods. (*B*) Cells were plated on day 0. On day 2 the medium was replaced with medium containing no hCG (●) or 40 ng/ml hCG (○). After an 18-h incubation at 37°C, the cells were cooled at 2°–4°C for 30 min and washed five times (see Materials and Methods) to remove any residual hormone. The binding of ^{125}I-hCG to the cell surface was then determined as described above.

TABLE I

Cellular Constants for the Interaction of ^{125}I-hCG with Control and mEGF-treated MA-10 Cells *

	Control	mEGF-Treated
$[R]_S$ (receptors/cell)	12,075 ± 537	2,593 ± 114‡
K_a (M^{-1})	(1.35 ± 0.07) × 10^9	(2.0 ± 0.16) × 10^9‡
K_{SS} (M^{-1})	(1.26 ± 0.08) × 10^{10}	(2.4 ± 0.11) × 10^{10}‡
V_R (receptors/cell × min)	21 ± 1.5	4 ± 0.5‡
k_e (min^{-1})	(4.0 ± 0.001) × 10^{-2}	(3.7 ± 0.004) × 10^{-2}
k_t (min^{-1})	(1.7 ± 0.15) × 10^{-3}	(1.5 ± 0.18) × 10^{-3}
k_n (min^{-1})	(5.4 ± 0.3) × 10^{-3}	(6.0 ± 0.2) × 10^{-3}

* The different constants were measured as described in the text. Each number represents the average (± SEM) of three independent determinations.
‡ Significantly different from control values at $P < 0.01$.

Steady-State Plots

The results presented in Fig. 3 show representative steady-state plots (7) of the association of ^{125}I-hCG with the cell surface (Panel *A*) or whole cells (Panel *B*) for the control of mEGF-treated cells. The slopes of the lines from both plots should be identical, and give the value of K_{SS} (the steady-state affinity constant). In three independent experiments, the K_{SS} for the control cells was calculated to be (1.1 ± 0.06) × 10^9 M^{-1} and (1.3 ± 0.07) × 10^9 M^{-1} from the surface and total plots, respectively. For the mEGF-treated cells, K_{SS} was calculated to be (2.1 ± 0.1) × 10^9 M^{-1} and (2.4 ± 0.1) × 10^9 M^{-1} from the surface and total plots. For each group of cells, the values obtained from the total and surface plots are not statistically different. The difference between the control and mEGF-treated cells, however, is statistically different (cf. Table I). We do not have an explanation for this increase. It should be noted, however, that mEGF also increases the Ka for ^{125}I-hCG (cf. Fig. 1*A* and Table I).

The *x*-intercepts of the surface plots (Fig. 3*A*) give the values of V_R/k_e (7). This value was lower in the mEGF-treated cells than in the controls, showing that mEGF affects V_R, k_e, or both.

It should be noted that at the steady state the free concentration of ^{125}I-hCG left in the medium is lower than the initial concentration of ^{125}I-hCG. This is, of course, due to degradation of the cell-bound ^{125}I-hCG. When this is expressed as percent of the initial ^{125}I-hCG concentration, the cells degraded 10–20% of the added hormone when the initial concentration was high, and 50–60% when the initial concentration was low. When the absolute amount of hormone degraded at each concentration was divided by the amount of cell-associated radioactivity at each concentration, a constant ratio of 5–6:1 was obtained for both control and mEGF-treated cells. These data show that the amount of hormone degraded at the steady state is directly proportional to the amount of hormone bound to the cells.

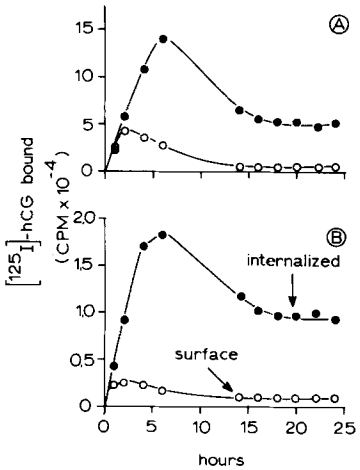

FIGURE 2 Time course of the association of ^{125}I-hCG with control (*A*) and mEGF-treated (*B*) cells. Cells were plated on day 0. On day 1 the medium was replaced, and half of the cultures (those shown in panel *B*) received 5 ng/ml mEGF. After a 48-h incubation at 37°C ($t = 0$ in the figure), the medium was replaced with 4 ml of warm medium containing 4 ng/ml ^{125}I-hCG (panel *A*) or 1 ng/ml ^{125}I-hCG and 5 ng/ml mEGF (panel *B*) and the cells were further incubated at 37°C. The surface-bound (○) and internalized (●) radioactivity were determined at the times indicated as described in Materials and Methods.

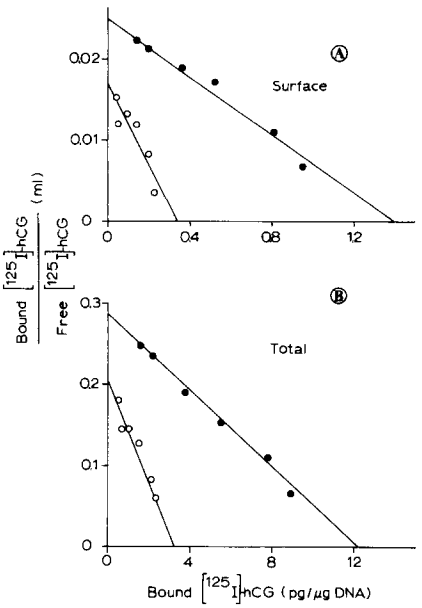

FIGURE 3 Steady-state plots of the binding of ^{125}I-hCG to control and mEGF-treated cells. Cells were preincubated without (●) or with (○) 5 ng/ml mEGF for 48 h as described in the legend to Fig. 2. At this time, the medium was replaced with 4 ml of warm medium containing increasing concentrations (1–20 ng/ml) of ^{125}I-hCG (●) or 5 ng/ml mEGF and increasing concentrations (0.5–10 ng/ml) of ^{125}I-hCG. After an 18- to 20-h incubation at 37°C, the surface-bound and internalized radioactivity were determined, and the total radioactivity was calculated by adding these two components. The concentration of free ^{125}I-hCG was also determined. Panels *A* and *B* show the steady-state plots for the surface-bound and total cellular hormone, respectively.

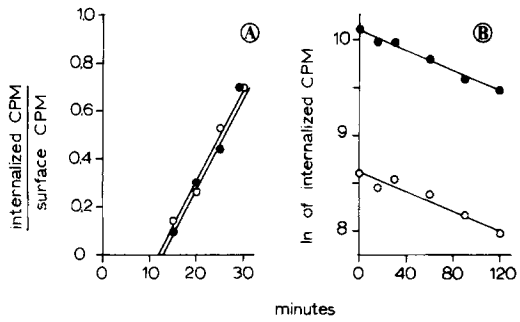

FIGURE 4 Determination of the rates of ^{125}I-hCG internalization (*A*) and degradation (*B*). Cells were preincubated without (●) or with (○) 5 ng/ml mEGF for 48 h as described in the legend to Fig. 2. (*A*) The cells were brought to the steady state by an 18-h incubation with 4 ml of medium containing 2 ng/ml hCG (●) or 1 ng/ml hCG and 5 ng/ml mEGF (○). At this time ($t = 0$ in the figure) they were placed on ice, quickly washed five times, and placed back in 4 ml of warm medium containing 1 ng/ml ^{125}I-hCG (●) or 0.5 ng/ml ^{125}I-hCG and 5 ng/ml mEGF (○). (The concentration of ^{125}I-hCG added back to the cells is lower than the initial concentration of ^{125}I-hCG added because during the initial incubation the cells degraded ~50% of the added hormone.) The cells were then incubated at 37°C, and the surface-bound and internalized radioactivity measured at the times indicated. k_e was calculated from the slopes of the lines shown (7). (*B*) The experimental protocol was as described above, except that the cells were brought to the steady-state with ^{125}I-hCG, and then switched to medium containing hCG. Control (●); mEGF-treated (○). k_n was calculated from the slopes of the lines shown (7).

Determination of the Rate Constants for Internalization of Unoccupied (k_t) and Occupied Receptors (k_e), and for Degradation of the Internalized Hormone (k_h)

The rate constants for internalization of occupied hCG receptors (k_e) and for the degradation of the internalized hCG (k_h) were measured as described by Wiley and Cunningham (7). The results of representative experiments are shown in Fig. 4.

The calculated values of k_e (3.7–4.0 × 10^{-2} min^{-1}; also see Table I) are two to three times higher than those previously calculated by measuring the rate of disappearance of ^{125}I-hCG from the cell surface under non-steady-state conditions (5). This discrepancy appears to be due to (*a*) the lack of appropriate corrections for the spillover of radioactivity from the surface and intracellular compartments (see Materials and Methods) and (*b*) using the rate of disappearance of surface-bound radioactivity to calculate k_e. If the same data presented previously (5) are analyzed by the rate of accumulation of intracellular radioactivity, as described by Schwartz et al. (15), the calculated values of k_e range from 2.4 to 4.2 × 10^{-2} min^{-1} at hCG concentrations of 5–40 ng/ml. These values are in reasonable agreement with those shown in Fig. 4 (cf. Table I).

Using the values of k_e calculated from Fig. 4 and the values for the *x*-intercept of the surface steady-state plots (cf. Fig. 3*A*), we calculated the values for V_R. The number of surface hCG receptors in control and mEGF-treated cells (cf. Fig. 1*A*) is equal to the ratio of the rate of appearance of receptors into the membrane (V_R) to the rate constant for internalization of unoccupied receptors (k_t). Thus, k_t can be calculated using the *x*-intercepts of the Scatchard plots shown in Fig. 1*A* and the values of V_R calculated above.

The different cellular constants described above were measured several times, and a summary of the values obtained is shown in Table I. From the results presented, we conclude that (*a*) mEGF reduces surface hCG receptors because it decreases the rate of appearance of receptors at the surface fivefold and (*b*) hCG reduces surface hCG receptors (in both control and mEGF-treated cells) because when the hormone binds to its receptor it increases the rate of receptor internalization 25-fold (i.e., k_e/k_t ~25). Thus, the homologous and heterologous reduction of hCG receptors occur by different mechanisms. Also, note that mEGF has no significant effects on the rate constants for internalization of the unoccupied receptors (k_t) and the occupied receptors (k_e), or the rate constant for degradation of the internalized hormone (k_h). The affinity of the receptor for hCG (K_a) and the affinity of the cells for hCG (K_{SS}), however, are somewhat higher in the mEGF-treated than in the control cells.

Analysis of the Cellular Constants for the Interaction of mEGF with the MA-10 Cells

Since the MA-10 cells also have mEGF receptors, and since they also internalize and degrade this ligand (2), we sought to determine the different cellular constants involved in this interaction and to compare them with those obtained for hCG. By doing this, we can compare how the cells handle two different ligands (i.e., mEGF and hCG). The results of those experiments are summarized in Table II and show that, as expected (7), mEGF down-regulates its surface receptors by increasing the rate of receptor internalization (k_e/k_t ~195).

TABLE II

Cellular Constants for the Interaction of ^{125}I-mEGF with the MA-10 Cells *

$[R]_S$ (receptors/cell)	134,569 ± 3,319
K_a (M^{-1})	(2.49 ± 0.06) × 10^8
K_{SS} (M^{-1})	(3.27 ± 0.12) × 10^9
V_R (receptors/cell × min)	113 ± 18
k_e (min^{-1})	(1.64 ± 0.025) × 10^{-1}
k_t (min^{-1})	(8.4 ± 0.2) × 10^{-4}
k_h (min^{-1})	2.2 × 10^{-2}

* The different constants were measured as described in the text. Each number represents the average (± range) of two independent experiments, except for k_h, which is the result of a single experiment.

Predictions from the Steady-State Model

In regard to the regulation of surface hormone receptors, the data presented show that the homologous down-regulation of hCG and mEGF receptors occurs by the hormone-induced increase in the rate of receptor internalization, while the heterologous down-regulation of hCG receptors occurs by a decrease in the rate of appearance of the surface receptor (cf. Tables I and II).

In order to test the validity of these data, we used some of the constants calculated above to predict the time courses involved in the down-regulation of receptors, and in the recovery from the down-regulated state.

This can be done by adapting the equations of Berlin and Schimke (16) to the receptor system. Thus, at a given steady-state, the number of surface receptors—$[R]_S$—is given by:

$$[R]_S = V_R/k_t \qquad (1)$$

This steady-state can be altered by changing V_R to $V_{R'}$ and/or k_t to $k_{t'}$. The new steady-state level of receptors, $[R]'_S$, will be determined by the new ratio, $V_{R'}/k_{t'}$. The time course required for this change to occur is described by the following equation (16):

$$[R]'_S = \frac{V_{R'}}{k_{t'}} - \left[\frac{V_{R'}}{k_{t'}} - R_o\right] e^{-kt' \times time} \qquad (2)$$

where R_o = initial number of receptors.

For the homologous down-regulation of receptors, we cannot calculate the time course required for this phenomenon to occur because the rates of association and dissociation of the hormones to and from the receptors are not known. We can, however, calculate the time course required for the receptors to recover from the down-regulated state once the homologous hormones are removed. This can be done since we know that mEGF and hCG reduce their receptors by increasing the rate of receptor internalization from k_t to k_e (cf. Table I, left column, and Table II). Thus, when the cells are exposed to these hormones at 37°C, the steady-state levels of the homologous receptors (R_o in equation 2) will be given by V_R/k_e. When the hormones are removed, the rate of receptor internalization decreases back to k_t, and the rate of receptor appearance (V_R) remains unchanged. These constants can then be substituted into Eq. 2, and the time course required for the receptors to recover from the down-regulated state can be predicted. As shown in Fig. 5, the predicted time courses agree reasonably well with the experimental values obtained.

For the heterologous down-regulation of hCG receptors by mEGF, Eq. 2 can be used to calculate the time course required for mEGF to reduce the hCG receptors and for the receptors to recover upon removal of mEGF (Fig. 6). The experimental points shown in Fig. 6*A* show that there is a lag of 6–8 h after the addition of mEGF before ^{125}I-hCG binding starts to decline. In order to account for this lag, we calculate the theoretical time course by using Eq. 2 and assuming that, 7.5 h after addition of mEGF, V_R (for the hCG receptors) changed from the value shown on the left column of Table I to that shown on the right column. The experimental points shown in Fig. 6*B* show that upon removal of mEGF there is also a 6–8 h lag before ^{125}I-hCG binding starts to increase. Thus, to predict this return, we also used Eq. 2, assuming that, 7.5 h after removal of mEGF, V_R (for the hCG receptors) changed from the value shown on the right column of Table I to that shown on the left column. Both predicted time courses agree well with the experimental data.

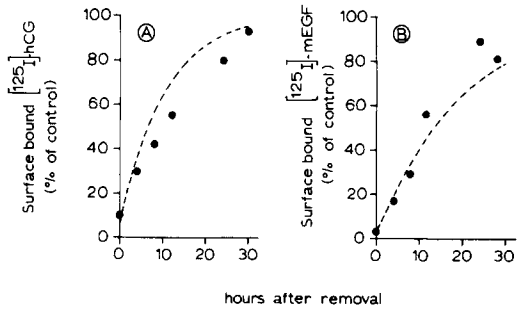

FIGURE 5 Prediction of the time course of recovery of ^{125}I-hCG (*A*) and ^{125}I-mEGF (*B*) surface receptors after homologous down-regulation. On day 2 the medium was replaced with 4 ml of medium containing 20 ng/ml hCG (*A*) or 20 ng/ml mEGF (*B*). After a 20 h (*A*) or 12 h (*B*) incubation at 37°C (t = 0 in the figure), the cells were washed five times (to remove the free hormone), placed back in 4 ml of medium without hormone, and incubated at 37°C. At the times indicated, the cells were placed on ice, washed twice with cold medium, and placed in 2 ml of cold medium containing 20 ng/ml ^{125}I-hCG (*A*) or 20 ng/ml ^{125}I-mEGF (*B*). Surface-bound radioactivity was determined after a further 2-h incubation at 2°–4°C. The solid circles show the experimental points, and the dashed lines show the theoretical curves calculated as described in the text.

The results presented in this section strengthen the validity of the cellular constants measured with the steady-state model and further support our contention that the homologous down-regulation of mEGF and hCG receptors, and the heterologous regulation of hCG receptors occur by different mechanisms.

DISCUSSION

The number of surface receptors for a given ligand is dictated by the ratio of the rate of receptor appearance (V_R) to the rate constant of receptor internalization (see Eq. 1). The value of this rate constant equals k_t if the receptors are not occupied by the homologous ligand and k_e if they are occupied. Our data on the interaction of mEGF and hCG with the MA-10 cells show that these hormones down-regulate their homologous receptors because upon binding they increase the rate constant for receptor internalization 195- and 24-fold, respectively (Tables I and II).

There is a consensus in the literature that the homologous down-regulation of surface polypeptide hormone receptors occurs by a ligand-induced increase in the rate of receptor internalization (4–7, 12, 14, 17–20). Although the internalized ligands are usually degraded (4, 5, 12, 14), the fate of the internalized receptors appears to vary with the cell type and/or hormone (12, 14, 17–23). Our data do not provide evidence

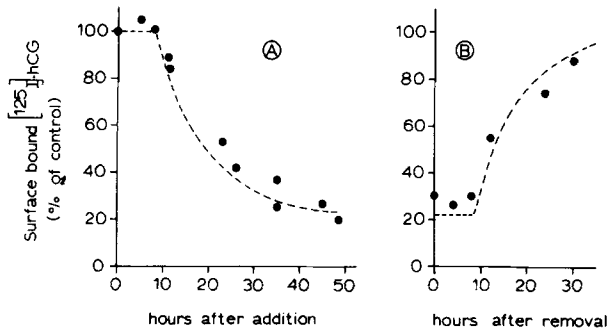

FIGURE 6 Prediction of the time course required for the heterologous down-regulation (*A*) and the recovery from the down-regulated state (*B*) of ^{125}I-hCG surface receptors. (*A*) On day 2 the medium was replaced with 4 ml of warm medium. MEGF (5 ng/ml) was added to the dishes at different times in such a way that the time during which the cells had been in contact with mEGF varied as indicated in the figure. The binding of ^{125}I-hCG was then determined at the same time in all dishes. At this time the dishes were placed on ice, washed twice, and then placed in 2 ml of cold medium containing 20 ng/ml ^{125}I-hCG. Surface-bound radioactivity was determined after a 2-h incubation at 2°-4°. The solid circles show the experimental points, and the dashed lines show theoretical curves calculated as described in the text. (*B*) On day 1 the medium was replaced with 4 ml of growth medium containing 5 ng/ml mEGF. After a 48-h incubation at 37°C ($t = 0$ in the figure), the cells were washed five times (to remove the free hormone), placed back in 4 ml of warm medium without hormone, and incubated at 37°C. At the times indicated, the binding of ^{125}I-hCG was measured as described in legend to Fig. 5. The solid circles show the experimental points, and the dashed lines show the theoretical curves calculated as described in the text.

about the fate of the mEGF and hCG receptors. In other cell types, the internalized mEGF and hCG receptors appeared to be degraded rather than recycled back to the surface (24, 25). Further experiments are needed to determine whether this is also the case in the MA-10 cells.

The surface hCG receptors of the MA-10 cells can also be reduced with mEGF. The specificity of this effect is supported by the finding that mEGF lowers the steroidogenic response of the cells to hCG but not to cholera toxin or cAMP (2), and by the lack of effect of mEGF on the binding, internalization, and degradation of low density lipoprotein in the MA-10 cells (26). Moreover, Mondschein and Schomberg (27) have also reported that mEGF prevents the induction of hCG receptors in cultured rat granulosa cells.

The data presented herein show that mEGF reduces the surface hCG receptors by reducing the rate of appearance of receptors at the cell surface (V_R). It should be pointed out that this is an operational definition that does not exactly define the locus of action of mEGF. Thus, one may view V_R as the product of the number of intracellular receptor times a rate constant for insertion; or, alternatively, V_R may be viewed as being proportional to the rate of receptor synthesis. A reduction in any of these parameters could be responsible for the effects of mEGF on V_R. Further experiments are needed to clarify this point. It is clear, however, that in the MA-10 cells the homologous reduction of mEGF and hCG receptors, and the heterologous reduction of hCG receptors, occur by different mechanisms.

Lastly, it should be pointed out that the absolute values of the cellular constants reported here are subject to change depending on experimental conditions. The relative differences mentioned, however, remain unchanged.

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