Epidermal Growth Factor Inhibits Transiently the Progression from G₂-Phase to Mitosis: A Receptor-mediated Phenomenon in Various Cells

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ABSTRACT

An immediate effect of epidermal growth factor (EGF) on the cell cycle is described. EGF, when given to replicating cells such as HeLa, A431, and D HER 14, very rapidly inhibits the transition from G₂ phase to mitosis (M) in a transient fashion. The influence of EGF (10⁻¹⁰ to 10⁻⁷ M concentrations) on the G₂-M transition of individual cells has been analyzed by time-lapse photography in cell lines carrying intact, mutated, or no EGF receptor. The G₂-M transition of cells devoid of EGF receptor or carrying an EGF receptor devoid of most of the cytoplasmic domain was not influenced by EGF. In cells carrying intact EGF receptor, EGF caused a transient and dose-dependent delay in G₂ phase which could last for >2 h. Cells were inhibited in G₂ within <10–20 min prior to prophase. A parasynchronous recovery from G₂ inhibition was observed at large EGF concentrations; the G₂-M transition rate exceeded that of the controls. The system described may represent a model for the mechanistic analysis of a ligand-induced transient restriction of the cell cycle, particularly at the G₂-M border.

INTRODUCTION

A number of peptide growth factors have been shown to be bifunctional with respect to their influence on cell proliferation. Depending on the cell type and the proliferative state of a target cell, growth factors may either stimulate cells to replicate their DNA and to divide or they may inhibit cell cycle progression. The dual effectivity has been recognized in the case of EGF,³ basic fibroblast growth factor, transforming growth factor, tumor necrosis factor, and some of the interleukins (for review see Ref. 1). The significance of the inhibition of cell growth by physiological factors is unknown. However, it is possible that the ligand-induced and receptor-mediated inhibition of proliferation observed in vitro may reflect to some extent a mode by which higher organisms restrict cell proliferation physiologically. Within many tissues, cells expressing their differentiated function may be blocked and “rest” in the G₀/G₁ phase of the cell cycle. A smaller portion of cells may be blocked in the G₂ phase from which they enter mitosis immediately after certain “stimuli” (2–4). These observations are indicative of an extracellular control by which cells monitor superimposed signals from the organism.

The control of the G₂-M transition has attracted great attention. The transition is characterized by disappearance of the nuclear membrane, chromatin condensation, and organization of the mitotic apparatus. A number of events at the molecular level which occur at and are required for the G₂-M transition have been described within eukaryotic cells (5–12) but the mechanism coupling this to extracellular control has not yet been found.

The study of the ligand-induced inhibition of cell proliferation may yield clues to the mode by which cells are restricted physiologically. Phorbol ester tumor promoters, which are powerful mitogens in a number of cellular systems (13), have also been shown to inhibit the cell cycle progression of cultured cells in various phases. When treated with TPA HeLa cells exhibit a temporary block in G₁ phase, a delayed traverse through S phase, and a transient blockage in G₂ phase (14). A detailed study of the TPA-affected inhibition of the transition from G₂ phase to mitosis by time-lapse photography and single cell analysis indicated that the inhibition was receptor mediated (15). We became interested in the mechanism of the phorbol ester-induced transient inhibition of the G₂-M transition. On the basis of preliminary metabolic studies in this model system it was hypothesized that EGF should induce a transient inhibition of the G₂-M transition as well. The action of EGF on the G₂-M transition has been characterized in detail by a time-lapse study of different cell lines including cells which carry mutated or no EGF receptors. The data presented in this paper show that EGF inhibits the G₂-M transition very rapidly and in transient fashion.

MATERIALS AND METHODS

Materials. Eagle's minimum essential medium (Gibco), DMEM (Flow Laboratorities), calf serum (Gibco), and murine EGF (Toyobo, Japan and Boehringer, Germany) were obtained from the quoted sources.

Cell Cultures. HeLa cells were routinely cultivated in Eagle's minimum essential medium supplemented with 10% calf serum. This HeLa line contained 1.4 x 10⁶ EGF receptors/cell, exhibiting low as well as high affinity-binding sites. The epidermoid carcinoma cell line A431 (a generous gift from Dr. H. zur Hausen) was cultivated in DMEM containing 10% calf serum. It contained approximately 10⁶ EGF receptors/cell, exhibiting low as well as high affinity-binding sites. In addition to the human cell lines, three mouse cell lines (a generous gift of Dr. J. Schlessinger) were studied: (a) NIH 3T3 cells (clone 2.2) without detectable endogenous EGF receptor (parental cell line), (b) D HER 14 cells; NIH 3T3 cells (clone 2.2) transfected with a plasmid containing the wild-type human EGF receptor sequence and expressing approximately 3 x 10⁶ EGF receptors/cell with high and low affinity-binding sites, (c) F plo NA8 14 cells: NIH 3T3 cells (clone 2.2) transfected with a plasmid containing a truncation mutant of the human EGF receptor sequence which contains in addition to the receptor domain the membrane spanning domain plus 7 cytoplasmic amino acid residues (i.e., including amino acid residue 653), but missing the kinase domain as well as the autophosphorylation domain (16). F plo NA8 14 cells carried more than 10⁶ EGF receptors/cell, exhibiting low affinity-binding sites only. The details of the construction of the recombinant plasmids and the transfection procedure have been described by Livneh et al. (16). The D HER 14 cell line has been extensively characterized elsewhere (17, 18). The transfected cell lines as well as the parental NIH 3T3 (clone 2.2) cell line were cultivated in DMEM with 10% calf serum.

All cells were kept in 95% air : 5% CO₂ at 95% humidity and 37°C. The cell lines were free of Mycoplasma as checked routinely.

Determination of the G₂-M Transition by Time-Lapse Analysis. For time-lapse analysis cell cultures were established in the respective
complete medium including 10% calf serum in plastic flasks at approximately $2 \times 10^4$ cells/cm$^2$ 2 days prior to the experiment in order to obtain exponential growth. EGF at desired concentrations or PBS as control was added to the culture medium while the flask was tilted in such way that the medium had no contact with the cells. The flask was turned back into its normal position, closed tightly, and placed immediately on an inverted phase contrast microscope (Leitz Diavert; equipped with a 10X lens and a 8X ocular) in an incubator at 37°C. Recording started immediately. Every 10 min one picture was taken from the same field (containing approximately 600–1200 cells) for periods indicated in “Results.” During the 10-min intervals illumination of the instrument was turned off. Analysis from enlarged prints (24 x 30.5 cm) was carried out for each individual cell division which started and mostly finished during the time course of an experiment. The start of mitosis was determined “retrospectively”; it was defined as the point when the cells started to contract (indicated as “prophase”) and the end was defined as the point when visible separation of daughter cells was seen. The exactness of the analysis is given by the 10-min recording interval.

The following parameters were acquired: (a) cell number at zero time ($N_0$); (b) number of cells entering division (prophase) at a particular time/10 min ($G_2$-M transition rate); (c) cumulative number of cells ($N$) given $N_0$ plus the sum of cell divisions within a given period of time. Cumulative countings were expressed in relative terms as the logarithm of $N/N_0$ (log $N/N_0$) as is usual in the determination of division delay (e.g., Ref. 19). Linear regression analysis of the cumulative data was done using the Hewlett Packard SD-03A program on an HP 67 calculator.

In order to compare different experiments the average number of $G_2$-M transitions/10 cells/10 min was determined for certain periods (for details see “Results”).

The $G_2$ delay was determined from plots of the relative cell accumulation log ($N/N_0$) versus time. It was defined as the distance between the X axis (time) intercepts of the control curve (PBS) and of a line parallel to the control curve drawn tangential to the experimental curve (EGF). This method was applicable since cells which were delayed in their $G_2$-M transition by EGF recovered in the presence of EGF and multiplied afterwards at the same rate as or transiently at an even larger rate than the control.

The transition point reflects the time point (before visible prophase) up to which cells are sensitive to inhibition by EGF in $G_2$ phase. After this, they become refractory to inhibition and continue to enter mitosis (see “Results”). The transition point was operationally defined as the time elapsing between addition of EGF and reaching a $G_2$-M transition rate (per 10 min) which differed clearly from the mean of the control groups and was no larger than one cell entering prophase/10 min during a 30-min period.

Each type of experiment was carried out twice.

RESULTS

EGF-induced Inhibition of the $G_2$-M Transition of HeLa Cells. Preliminary experiments concerning the action of EGF in exponentially multiplying HeLa cells have indicated that the mitogen (at $10^{-8}$ and $10^{-9}$ M in the presence of 10% calf serum) causes a decrease of the mitotic activity without any sign of cytotoxicity. After a certain time, the mitotic activity recovers. One reason for the rapid decrease of mitotic activity could be that those HeLa cells actively engaged in mitosis at the time of addition of EGF finish cell division in the presence of the mitogen but few or no cells follow from the previous phase, i.e., they are inhibited in $G_2$ phase. In order to obtain detailed insight into the events occurring at the $G_2$-M transition we have analyzed individual cells entering mitosis (i.e., prophase) in nonsynchronized undisturbed cultures using time-lapse photog-raphy as a highly selective method. The HeLa cells carried intact EGF receptors ($1.4 \times 10^4$/cell, exhibiting low and high affinity-binding sites).

A representative response of HeLa cells to EGF ($10^{-8}$ M, equivalent to 60 ng/liter) in comparison to a control is shown in Fig. 1. The cumulative number of cells which enter prophase (most of which finish division during the time course of the experiment) is shown in Fig. 1A and the $G_2$-M transition rate in Fig. 1, B and C. From Fig. 1A it is evident that EGF delays the cumulative increase of cells in prophase for >50 min, i.e., the cells are held in $G_2$. From Fig. 1C it is evident that at zero time three cells and, 10 min later, only one cell enters mitosis; for the next 30-min period no additional cells follow up from $G_2$. EGF thus appears to act rather rapidly, i.e., within <20 min prior to prophase. Cells beyond the transition point appear to pass through mitosis normally. After 50–60 min the culture recovers from division delay and exhibits an almost parasychronous increase of mitotic activity with a maximum $G_2$-M transition of 11 cells at 90 min after addition of EGF (Fig. 1C).

The steep increase of the cumulative cell number in the presence of EGF, which lasts over approximately 2 h (Fig. 1A), reflects this situation as well. After that period cells appear to multiply at a rate similar to that observed in control cultures (Fig. 1B).

The three different periods, an initial low $G_2$-M transition rate followed by a high transition rate and then by a normal $G_2$-M transition rate, were deducible from the cumulative data of a particular experiment. Comparison of duplicate experiments showed that these periods were reproducible. The average $G_2$-M transition rates of HeLa cells after treatment with $10^{-8}$ M EGF were 1.2 in the first hour, 7.8 in the second hour, and 4.4 later on, and in the parallel experiment the transition rates were 1.0, 7.9, and 5.1, respectively. The average transition rates increased >6-fold from the first to the second hour and returned subsequently to control values. (The average $G_2$-M transition rates of control cultures varied between 4.2 and 6.7.) HeLa cells in the presence of $10^{-8}$ M EGF appear to be inhibited initially in such a way that they are able to enter mitosis during recovery in a parasychronous fashion.

The onset of the delay effected by $10^{-9}$ M EGF occurs as rapidly as at the higher EGF concentration (Table 1); the average $G_2$-M transition rate was decreased to 2.5. The $G_2$ delay was smaller (Table 1). The average $G_2$-M transition rates at $10^{-10}$ M EGF after recovery did not exceed the values after resumption of normal replication. At this concentration EGF has apparently no synchronizing effect. At $10^{-10}$ M EGF can be considered ineffective.

EGF-induced Inhibition of the $G_2$-M Transition of A431 Cells. The human epidermoid carcinoma cell line A431 carries a large number of EGF receptors (approximately $10^4$/cell, with low

Fig. 1. Influence of EGF ($10^{-8}$ M) in the $G_2$-M transition of HeLa cells. HeLa cells were incubated with EGF at zero time, recorded, and analyzed as described in “Materials and Methods.” For clarity values have been plotted up to 120 min. (A) Cumulative number of cells entering prophase, 0.2% PBS (O), $10^{-8}$ M EGF (C); (B) and (C) Number of cells entering prophase at a particular time (i.e., within 10 min); (B) in the presence of 0.2% PBS (O); (C) in the presence of $10^{-6}$ EGF (C).
EGF INHIBITS THE G₂-M TRANSITION

Table 1 Summary of the responses of HeLa cells to EGF

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* For determination see "Materials and Methods."

Table 2 Summary of the responses of A431 cells to EGF

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* For determination see "Materials and Methods."

INTACT EGF RECEPTOR MEDIATES THE G₂ INHIBITION BY EGF. In addition, three mouse cell lines were analyzed: NIH 3T3 (clone 2.2) cells, used for generating transfectants, did not carry EGF receptors; D HER 14 cells, a transfectant of the former cell line, expressed intact human EGF receptors (3 × 10⁵/cell, with low and high affinity-binding sites); F plo NA8 14 cells expressed a truncated human EGF receptor (>10⁶/cell; only low affinity-binding sites) which lacks almost the entire cytoplasmic domain except 7 amino acid residues.

In these three cell lines for a period of at least 200 min the effect of 10⁻⁸ M EGF was tested in duplicate first, since this concentration caused a clear-cut inhibition of the G₂-M transition in HeLa and A431 cells. Representative data are shown in Fig. 3. It is evident from representative experiments shown in Fig. 3 that EGF inhibited the G₂-M transition in D HER 14 cells (Fig. 3B) but failed to do so in the parental NIH 3T3 (clone 2.2) line (Fig. 3A) and in F plo NA8 14 cells (Fig. 3C). EGF was consequently considered to be inactive in these two latter cell lines.

The inhibition of D HER 14 cells effected by 10⁻⁸ M EGF set in rapidly but did not last so long (approximately 40 min) as in HeLa or in A431 cells. Smaller concentrations of EGF also exhibited a clear-cut inhibition of the G₂-M transition (Table 3). At all concentrations of EGF D HER 14 cells exhibited initially an average G₂-M transition rate which was only one-third (or less) of that seen after recovery or in control conditions.
and the receptorless line NIH 3T3 clone 2.2 were not inhibited cultures. After recovery of D HER 14 cells from EGF-induced inhibition the average G2-M transition rates increased later on to values which usually exceeded that of control cultures. In this context it should be noted that treatment with EGF of NIH 3T3 (clone 2.2) cells and F plo NA8 14 cells could also result in a stimulation of the average G2-M transition rate for unknown reasons (see also Fig. 3, A and C).

Taken together, the data obtained with NIH 3T3 (clone 2.2) cells and the derived transfecteds demonstrated that only D HER 14 cells which carry intact human EGF receptors responded to EGF with an inhibition of the G2-M transition. The inhibition thus appears to depend on the presence of intact EGF receptors. The binding of EGF as such to the truncated EGF receptors seemed to be without consequence for the G2-M transition. The inability of the deletion mutant to support an inhibition of the G2 transition to mitosis may indicate that the endogenous protein kinase activity of EGF receptor is essential for effecting this inhibition.

**DISCUSSION**

Different cell lines with different EGF receptor characteristics were used and it has been shown by single cell observation that, in undisturbed nonsynchronized cultures which carry intact EGF receptors, EGF inhibited or delayed the passage of cells from G2 into mitosis without signs of cytotoxicity (i.e., cells were held in G2 phase). Typically, the cultures recovered and started to divide again after a certain length of time. The onset of the reaction could be distinguished within <10–20 min seemingly independently of concentration above a threshold concentration of EGF. Application of a wide variety of EGF concentrations to A431 cells, however, showed that the division delay was in fact within a certain range dependent on the dose of EGF. Inhibitory concentrations were in the range of those required for growth stimulation of other cells. The degree of EGF-induced delay appeared to depend to some extent on the number of receptors per cell but also on the cell type. In two epithelial derived cell lines treated with the same concentration of EGF, A431 cells with the larger number of receptors exhibited a longer delay than HeLa cells. However, the mesenchymal cell line, D HER 14, although it expresses twice as many EGF receptors as HeLa cells, was less sensitive to EGF-induced division delay. The G2-M transition rates after resumption from inhibition effected by higher EGF concentrations were larger than those observed at lower EGF concentrations or in control cultures. F plo NA8 14 cells, with an incomplete EGF receptor, and the receptorless line NIH 3T3 clone 2.2 were not inhibited in the G2-M transition by EGF. Therefore, EGF-induced inhibition of the G2-M transition in the other cell lines is apparently mediated by EGF receptors.

Since the effect of EGF on the G2-M transition described here is transient it is questionable whether it contributes to the antiproliferative action of EGF observed in A431 cells (20, 21). MacLeod et al. (22) have shown that A431 cells treated with EGF (10^{-8} M) for 24 h have accumulated to a considerable degree in the G2 phase. At this time the cells have stopped dividing. This inhibition in G2 does not appear to be reversed. EGF treatment of HeLa cells did not inhibit the increase in cell number. Therefore, the rapid and reversible inhibition of the G2-M transition observed in HeLa and A431 cells and the G2 blockage of A431 cells observed after 1 day may not be causally linked.

Inhibition of cells in the G2 phase can be effected by different treatments including ionizing radiation, DNA-cross-linking agents, inhibitors of protein synthesis, and phorbol esters. These appear to act through different mechanisms. The inhibition of cells in the G2 phase by X-rays (23–25) as well as by DNA-cross-linking agents (for reviews see Refs. 26 and 27) has been connected with DNA damage. The transition points for X-rays, as well as inhibitors of protein synthesis in the G2 phase measured in a number of cellular systems, appear to be similar if not identical (for review see Ref. 28). Due to the different cellular systems used and different methods of determining the G2 delay, the transition points cannot be directly compared with those described in this study. The transition point for the action of X-rays (1 Gy) in G2 measured in HeLa cells by the time-lapse technique was at approximately 20 min prior to prophase. In contrast to the EGF-induced G2 delay which exhibits a nonlinear relationship (Fig. 2B), that effected within the same order of magnitude by low doses of X-rays is linearly related to the dose applied (19).

The phenomenon most closely related to EGF-induced inhibition of the G2-M transition appears to be the action of the phorbol esters. This has been characterized by time-lapse analysis in HeLa cells (15) and carries characteristics of a receptor-mediated response as well: (a) phorbol esters are inhibitory at molar concentrations as low as those typical of EGF inhibition, (b) the TPA- and RPA-sensitive transition point in G2 is observed on the average within <20–30 min prior to prophase, (c) the duration of the G2 delay is related to dose within a certain range, (d) the recovery from G2 inhibition occurs in the presence of the phorbol ester. Thus, even though EGF and phorbol esters operate through different receptors, it is tempting to speculate that both induce inhibition of the G2-M transition through a comparable mechanism.

The possibility must be considered that the mechanism of the EGF-induced cell cycle delay could involve nonspecific, to a certain degree toxic, effects. However, in EGF-treated cultures no morphological signs of cytotoxicity have been observed, even though at high concentrations (10^{-7} and 3 \times 10^{-4} M EGF) temporary morphological changes appear in A431 cells which then disappear after approximately 1.5 h. No morphological changes have been seen in HeLa cells at 10^{-4} M EGF. Moreover, the cultures recover from G2 inhibition. A direct argument for the nontoxic character of the EGF-effected inhibition comes from a look at the average G2-M transition rates during recovery from the effect of high EGF concentrations in particular. These values clearly exceed those of the control cultures. Were it the

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* Table 3 Summary of the responses of D HER 14 cells to EGF

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* Due to variations of the G2-M transition rates among individual cultures the G2 delay was taken as the distance on the X axis between zero time and the regression line through the cumulative number of cells entering mitosis in the individual experiment after recovery from inhibition (these cells tended to grow in colonies probably with the consequence of topographical differences of the proliferative capacity within the colonies; the size of the colonies recorded varied among the different experiments).

* ND, not determinable.
case that the cells were somewhat damaged and another cohort of cells were responsible for the resumption of mitotic activity, a replication rate would be expected which did not exceed that of the control cultures. The large G₂-M transition rates during recovery of HeLa cells at 10⁻⁸ M EGF and of A431 cells at 10⁻⁷ M EGF are best explained by an accumulation of a certain fraction of cells in late G₂ followed by a parasynchronous recovery after disappearance of the proposed inhibitory signal. However, a stimulation cannot be excluded.

The degree as well as the duration of the division delay of a given cell in response to EGF is positively correlated with the dose of EGF applied. At low concentrations of EGF the inhibition of the G₂-M transition lasts for short periods and is incomplete. At higher concentrations of EGF the inhibition lasts longer and appears to be more complete over a certain period. These observations are consistent with the hypothesis of an EGF-induced generation of an intracellular metabolite which, depending on the concentration, mediates the inhibition of the G₂-M transition more or less efficiently. The duration of the inhibition or of the delay in G₂ depends on the period for which the proposed mediator is generated at a rate which exceeds its metabolism or catabolism.

With respect to the nature of the proposed inhibitory mediator, it may be assumed that the inhibition of cells at the entrance to mitosis reflects a certain degree of specificity regarding the molecular events to be inhibited and consequently with respect to the mediator itself. If it is further hypothesized that the other receptor-mediated inhibition of the G₂-M transition described so far, namely, that affected by certain phorbol esters (14, 15), follows a similar pathway, it seems useful to look for common mechanistic aspects.

ACKNOWLEDGMENTS

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REFERENCES

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