MOLECULAR AND CELLULAR BIOLOGY, Dec. 1987, p. 4324–4328
0270-7306/87/124324-05$02.00/0

Vol. 7, No. 12

Induction of Glucagon Sensitivity in a Transformed Kidney Cell Line by Prostaglandin E_2 and Its Inhibition by Epidermal Growth Factor

MICHAEL C. LIN,* FREDERICK J. DARFLER, AND SUZANNE K. BECKNER

Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892

Received 2 February 1987/Accepted 10 September 1987

A model system using a transformed dog kidney cell line (Madin-Darby canine kidney), has been established for studying the process of differentiation. Glucagon responsiveness can be restored to these transformed cells by various differentiation inducers, including prostaglandin E_2. Glucagon response was measured in terms of the ability of glucagon to stimulate cAMP production. Induction of glucagon sensitivity seems to be mediated by cAMP. The ability of various prostaglandin analogs to elevate the cAMP level correlates closely with their ability to induce glucagon sensitivity. In fact, 8-Br-cAMP is also a potent inducer. To define the nature of this cAMP-mediated process, we identified several inhibitors of this induction process. These differentiation inhibitors include serum, phorbol ester, and epidermal growth factor. These inhibitors do not have a direct effect on cAMP production by cells in the presence or absence of hormones. Furthermore, induction by 8-Br-cAMP is also inhibited by these agents. Therefore, the site of inhibition is located beyond the point of cAMP production. Possible interaction between cAMP- and epidermal growth factor-dependent phosphorylations is discussed.

During the process of differentiation, cells often develop specific hormone sensitivity. In many instances, the acquisition of certain hormone receptors and responsiveness represents an endpoint for a specific phase of cellular differentiation (12). For example, differentiation of granulosa cells, derived from ovarian follicles, is accompanied by the appearance of receptors for luteinizing hormone (3). Similarly, differentiation of mouse fibroblasts, 3T3-L1 cells, into adipocytes results in enhanced responsiveness to insulin, β-adrenergic hormones, and ACTH, presumably because of increased density of receptors (17).

Recently we have established a model system, using a cultured dog kidney cell line (Madin-Darby canine kidney [MDCK]), for studying the process of differentiation (1, 13). Normal MDCK cells, which exhibit several differentiated functions characteristic of kidney distal tubules, respond to glucagon with an increase in cAMP level. In contrast, a cloned line of MDCK cells transformed with Harvey murine sarcoma virus that express *ras* oncogene product p21 do not exhibit this differentiated function (13). However, glucagon response can be induced in transformed MDCK cells by various agents, including prostaglandin E_2 (PGE_2). The measurement of intracellular cAMP in response to glucagon represents a convenient method to assess the degree of differentiation. Furthermore, both parental and transformed MDCK cells grow readily in chemically defined media (11, 18); thus, they represent an ideal model system for studying the mechanism for induction of differentiation in a homogeneous cell population under well-defined conditions.

In this report, we further define conditions for induction of differentiation in transformed MDCK cells. Induction of glucagon sensitivity by PGE_2 seems to be a cAMP-mediated process. In fact, 8-Br-cAMP is also a potent inducer. Additionally, we demonstrate that the induction process can be inhibited by epidermal growth factor (EGF), a phorbol ester (phorbol 12-myristate-13-acetate [TPA]), and serum factors. These compounds do not change the level of intracellular cAMP and thus will help to define the nature of this cAMP-dependent induction of differentiation.

MATERIALS AND METHODS

Cell culture. A cloned line of MDCK cells transformed by Harvey murine sarcoma virus was established as previously described (13) and recloned for the present study. Cells were maintained as a monolayer culture at 37°C under 5% CO_2–95% air in Dulbecco modified Eagle (DME) medium containing 5% fetal bovine serum, 50 U of penicillin per ml, and 50 μg of streptomycin per ml.

cAMP assay for hormone response. Monolayer cultures in 35-mm (diameter) dishes were rinsed once with Dulbecco phosphate-buffered saline, and then DME medium containing 20 μM Ro 20-1724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone], a phosphodiesterase inhibitor, with 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.4) was added. After 30 min at 25°C, hormone solution in 10 to 20 μl was added to initiate the reaction. The final concentrations of glucagon and PGE_2 were 2 and 1 μM, respectively. After 3 min, medium was removed, 2 ml of boiling water was added, and the cells were scraped off the dish. The whole mixture was boiled for 5 min and centrifuged. This treatment served to terminate the reaction and to extract intracellular cAMP. The assay was kept to 3 min to allow linear production of intracellular cAMP without its leakage into the medium. The concentration of cAMP in the extract was estimated by a radioimmunoassay using anti-cAMP antibody and anti-rabbit immunoglobulin G as the second antibody (11).

Culture conditions for induction. MDCK cells were first plated in DME medium containing 5% fetal bovine serum. After 4 h, the cells were rinsed with Dulbecco phosphate-buffered saline and added to a chemically defined medium, DME medium–F-12 (1:1), containing 5 μg of insulin per ml,

* Corresponding author.

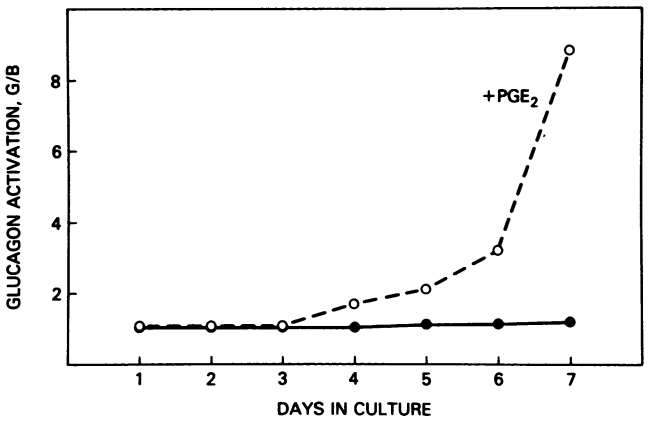

FIG. 1. Time course of induction of glucagon sensitivity by PGE_2. Transformed MDCK cells (10^5 cells per 35-mm dish) were plated in DME medium containing 5% fetal bovine serum. After 4 h at 37°C, the monolayer culture was rinsed with Dulbecco phosphate-buffered saline, and defined medium with or without PGE_2 (0.1 μM) was added. Glucagon sensitivity was assayed daily as described in the text. Hormone activation was expressed as the ratio of the cAMP obtained in the presence of glucagon over that obtained in the absence of glucagon (G/B, glucagon activation/basal activity). Each experimental point represents the average of four determinations which agreed within 5%. This experiment is representative of four others.

5 μg of transferrin per ml, 50 nM hydrocortisone, 5 pM triiodothyronine, and 10 nM selenium oxide in 20 mM HEPES buffer, pH 7.4 (11, 18). For induction of hormone sensitivity, an inducer, such as PGE_2 or 8-Br-cAMP, was included at the concentration indicated. Except as stated otherwise, induced hormone sensitivity was assayed as described above after 5 days of culture. In the experiment in which inhibition of induction was studied, an inhibitor, such as EGF or TPA, was added to the defined medium at the initiation of induction. In general, each experiment was repeated at least three times, and results representative of the experiments are shown. Because of variability of basal conditions between experiments, statistical treatment of all of the experiments performed was not feasible in most cases. As an example, the basal level of intracellular cAMP varied depending on the passage number of cells, cell density at the time of measurement, and the exact culture conditions, such as the fluctuation of the CO_2 level in the incubator due to door opening during the culture period.

Materials. Fetal bovine serum was obtained from GIBCO Laboratories, Grand Island, N.Y. Ro 20-1724 was a gift from Hoffmann-La Roche Inc., Nutley, N.J. The phorbol esters TPA and 4α-phorbol 12,13-didecanoate and prostaglandins were from Sigma Chemical Co., St. Louis, Mo. [^{125}I]monosuccinyl cAMP tyrosyl methylester, rabbit serum, and anti-rabbit immunoglobulin G second antibodies were from Meloy Laboratories, Springfield, Va. cAMP antibodies and EGF were obtained from Collaborative Research, Inc., Waltham, Mass.

RESULTS

Induction of glucagon sensitivity by PGE_2. To define the mechanism of PGE_2 action in the induction of cellular differentiation, the abilities of prostaglandins to induce glucagon sensitivity and increase cAMP production in transformed MDCK cells were examined. When transformed cells were cultured in the presence of PGE_2, cells gradually became responsive to glucagon which was not seen in the absence of PGE_2 (Fig. 1). Whereas the degree of hormone sensitivity induced varied depending on the passage number and culture age of the cells, the appearance of glucagon responsiveness was consistently seen after 4 days of incubation with PGE_2. In Fig. 2, the relationship between PGE_2 concentrations and induction of glucagon sensitivity is shown. After 5 days of culture, the concentration of PGE_2 required to achieve half-maximal induction was about 20 nM, and the maximal effect was seen at 100 nM. In contrast, the concentration required for half-maximal activation of intracellular cAMP accumulation by PGE_2 was 100 nM (data not shown). Activation of cAMP production by PGE_2 was measured during 3 min of incubation, whereas the induction process was measured after 5 days of culture. cAMP production occurred immediately and is believed to the primary effect of PGE_2. In contrast, glucagon responsiveness appeared only after several days of incubation. Therefore, even though the induction process seemed to be more sensitive to PGE_2, the difference between the two concentration requirements does not necessarily imply a change in affinity.

In addition to PGE_2, five other prostaglandins were also tested for their ability to induce glucagon sensitivity. PGE_2 remained the most potent inducer; PGE_1, PGA_2, and PGB_2 were also capable of inducing hormone sensitivity. When the activation of cAMP production by these agents was compared to their ability to induce glucagon responsiveness, a correlation became clear (Table 1). Both $PGF_{2\alpha}$ and PGB_1 did not activate cAMP production and were also ineffective in the induction of glucagon sensitivity. PGA_2 and PGB_2 elevated cAMP levels and were both capable of some degree of induction. PGE_1 is an effective inducer and a potent activator of cAMP. It appears that activation of cAMP production correlates well with the induction process. In fact, 8-Br-cAMP is also an effective inducer (Table 2); maximal induction was achieved at a concentration of 50 μM. With 100 μM 8-Br-cAMP, the glucagon sensitivity, expressed as the ratio of the cAMP level in the presence of the hormone to that in the absence of the hormone, was lower. However, there was a substantial increase in basal cAMP production which most likely was due to incomplete

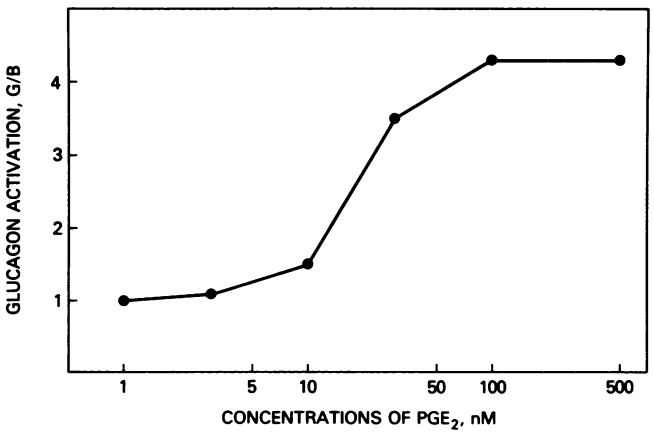

FIG. 2. Concentration dependency of PGE_2 induction of glucagon sensitivity. Transformed MDCK cells were cultured with PGE_2 at the concentration indicated. Glucagon response was assayed, as described in the text, after 5 days of culture. Each experimental point represents the average of four determinations which agreed within 5%. This experiment was repeated once.

TABLE 1. Comparison of activation of cAMP production and induction of glucagon sensitivity by prostaglandins

Addition	Ratio of cAMP production levels with/without prostaglandin[a]	cAMP induction (glucagon activation/basal activity ratio)[b]
None	1.0	1.0
PGE_1	9.6	2.8
PGE_2	10.2	6.7
$PGF_{2\alpha}$	1.0	1.0
PGA_2	3.2	1.5
PGB_1	1.0	1.0
PGB_2	6.0	2.0

[a] Transformed MDCK cells (10^5 cells per 35-mm dish) were plated in DME medium containing 5% fetal bovine serum. After 4 h at 37°C, the cells were rinsed once with Dulbecco phosphate-buffered saline, and defined medium was added. On day 3, the effect of prostaglandins (1 μM) on intracellular cAMP production was measured as described in the text.

[b] MDCK cells were plated as described in footnote *a*. Prostaglandins (0.1 μM) were included in the defined medium as indicated. After 5 days of culture, the glucagon sensitivity of induced, transformed MDCK cells was assayed as described in the text.

removal of the 8-Br-cAMP added during the induction process.

These results demonstrate that cAMP induces differentiation and suggest that induction by prostaglandin is mediated by cAMP. When PGE_2 was used as the inducer, the maximal concentration of intracellular cAMP achieved was 1 μM. However, it is clear (Table 2) that when 8-Br-cAMP was added extracellularly, a concentration higher than 10 μM was needed to cause induction. This discrepancy likely reflects the exogenous cAMP needed to achieve the effective intracellular concentration required for induction.

Inhibition of PGE_2 induction by serum and phorbol ester. The induction of glucagon sensitivity by PGE_2 can only be seen in chemically defined medium. Fetal bovine serum at 0.05% effectively inhibited induction, and at 0.2% it completely abolished the ability of PGE_2 to induce differentiation (Table 3). Preliminary studies suggest that a serum factor with a molecular mass between 50 and 100 kilodaltons is responsible for this inhibitory effect.

After testing several other agents, we found that a phorbol ester, TPA, is also a potent inhibitor of induction. At a concentration as low as 0.2 nM, TPA completely blocked the induction of glucagon sensitivity by PGE_2 (Fig. 3). In contrast, an inactive analog of phorbol ester, 4α-phorbol 12,13-didecanoate, had only a slight inhibitory effect, even at concentrations as high as 50 nM. To determine whether TPA inhibits induction by blocking hormonal activation of cAMP production, MDCK cells were treated with TPA at 1 nM for 2 h at 37°C and then assayed for hormone sensitivity in the presence of TPA. Uninduced transformed cells remained responsive to PGE_2 in the presence of 1 nM TPA (Table 4). Furthermore, the responsiveness of the induced cells to glucagon and PGE_2 only slightly decreased in the presence of TPA. These results show that TPA does not inhibit hormone-sensitive adenylate cyclase at the concentration tested and that TPA inhibition of induction is not due to a decrease in cAMP production. Therefore, the site of the TPA effect has to be subsequent to the increase of intracellular cAMP.

TABLE 2. Induction of glucagon sensitivity in transformed MDCK cells

Culture conditions	Intracellular cAMP (pmol/10^6 cells)[a]	
	No addition	Glucagon
Control	8 ± 1	8 ± 2 (1.0)
PGE_2 (0.1 μM)	5 ± 1	41 ± 3 (8.2)
8-Br-cAMP		
5 μM	9 ± 2	10 ± 3 (1.1)
20 μM	10 ± 2	17 ± 2 (1.7)
50 μM	12 ± 2	136 ± 10 (11.3)
100 μM	49 ± 5	175 ± 18 (3.6)

[a] Transformed MDCK cells were plated, and defined medium was added after 4 h at 37°C. PGE_2 or 8-Br-cAMP, at the concentration indicated, was included. After 5 days of culture, the effect of glucagon on the production of cAMP was assayed as described in the text. The values represent the average of quadruplicate samples ± the standard deviation. The values in parentheses represent the ratio of the cAMP level in the presence of hormone over that in the absence of hormone.

TABLE 3. Inhibition of PGE_2 induction by serum[a]

Culture conditions	Mean ± SD intracellular cAMP (pmol/10^6 cells)	
	No addition	Glucagon
Control	14 ± 2	14 ± 1 (1.0)
PGE_2 (0.1 μM)	9 ± 2	86 ± 5 (9.5)
PGE_2 (0.1 μM)–serum (0.05%)	15 ± 3	38 ± 2 (2.5)
PGE_2 (0.1 μM)–serum (0.2%)	18 ± 3	19 ± 2 (1.0)
PGE_2 (0.1 μM)–serum (1.0%)	20 ± 3	22 ± 3 (1.1)

[a] MDCK cells were plated, and PGE_2 was included as indicated. In addition, fetal bovine serum was added at the concentrations indicated. Glucagon sensitivity was asayed after 5 days of culture. The values in parentheses are ratios of cAMP with hormone/cAMP without hormone.

Inhibition of induction by EGF. In search of a natural inhibitor, we discovered that EGF is a potent inhibitor of differentiation. Induction of glucagon sensitivity by PGE_2 was largely inhibited in the presence of 4 nM EGF. However, in contrast to the effect of TPA, complete inhibition by EGF was not seen, even at a concentration as high as 100 nM (data not shown). The inhibitory effect of EGF seems highly specific; although the defined medium contained 1 μM insulin, its omission had no effect on the induction of

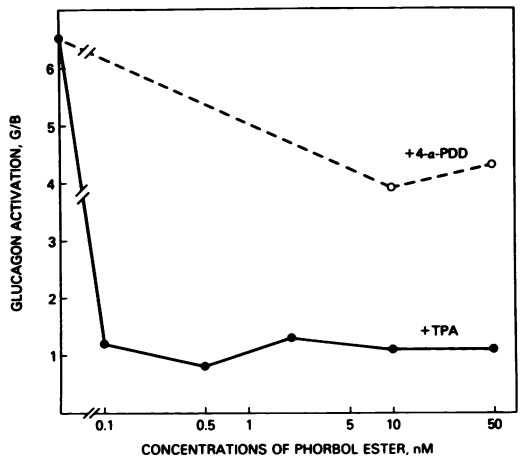

FIG. 3. Effect of phorbol esters on induction of glucagon sensitivity by PGE_2. Transformed MDCK cells were cultured in the presence of PGE_2 (0.1 μM) with TPA or 4 α-phorbol 12,13-didecanoate at the concentration indicated. Glucagon sensitivity was assayed, as described in the text, after 5 days of culture. Each experimental point represents the average of four determinations which agreed within 5%. This experiment is representative of two others.

TABLE 4. Effect of TPA on induced glucagon sensitivity

Conditions		Mean ± SD intracellular cAMP (pmol/10^6 cells)		
Culture	Assay	No addition	Glucagon	PGE_2
Control		8 ± 1	9 ± 2 (1.1)	56 ± 4 (7.0)
	TPA	9 ± 1	11 ± 3 (1.2)	50 ± 6 (5.6)
PGE_2		7 ± 3	47 ± 6 (6.7)	111 ± 9 (15.9)
	TPA	7 ± 1	39 ± 2 (5.6)	85 ± 6 (12.1)

[a] Transformed MDCK cells were cultured, as described in the text, for 5 days in defined medium with or without (control) 0.1 μM PGE_2. Before being assayed for hormone sensitivity, cells were incubated with 1 nM TPA, as indicated, at 37°C for 2 h, and hormone sensitivity was measured without removal of TPA. The values in parentheses are ratios of cAMP with hormone/cAMP without hormone.

glucagon sensitivity. Furthermore, nerve growth factor and multiplication stimulating activity also were devoid of any effect on the induction process (data not shown).

The inhibitory effect of EGF, like that of TPA, does not involve direct inhibition of glucagon stimulation of cAMP production (data not shown). In fact, EGF also inhibits the ability of 8-Br-cAMP to induce glucagon sensitivity (Table 5). Induction was carried out with 50 μM 8-Br-cAMP, and EGF at 4 and 20 nM effectively inhibited induction. Therefore, both EGF and TPA exert their inhibitory effect on the induction process downstream from the production of cAMP.

TABLE 5. Effect of EGF on induction by PGE_2 and 8-Br-cAMP[a]

Culture conditions	Intracellular cAMP (pmol/10^6 cells)	
	No addition	Glucagon
Control	29 ± 3	27 ± 4 (0.9)
PGE_2 (0.1 μM)	12 ± 2	69 ± 2 (5.8)
PGE_2 (0.1 μM)–EGF (4 nM)	23 ± 2	34 ± 3 (1.5)
PGE_2 (0.1 μM)–EGF (20 nM)	26 ± 4	34 ± 3 (1.3)
8-Br-cAMP (50 μM)	10 ± 2	87 ± 4 (8.7)
8-Br-cAMP (50 μM)–EGF (4 nM)	12 ± 1	36 ± 2 (3.0)
8-Br-cAMP (50 μM)–EGF (20 nM)	20 ± 3	28 ± 2 (1.4)

[a] Transformed MDCK cells were cultured in the presence or absence of PGE_2, 8-Br-cAMP, and EGF, at the concentrations as indicated, for 4 days. Glucagon sensitivity was assayed as described in the text. The values represent the average of triplicate determinations ± the standard deviation. The values in parentheses are ratios of cAMP with hormone/cAMP without hormone.

It is noteworthy that Levine and associates reported (9, 10) a stimulatory effect of EGF and TPA on the production of PGE_2 in normal MDCK cells. We have confirmed this observation that EGF at 5 nM increases PGE_2 production by 20-fold in parental MDCK cells cultured in defined medium. TPA was even more effective (more than a 50-fold increase) at the same concentration. In contrast, EGF has no effect on production of PGE_2 in transformed cells. However, TPA at 5 nM increased PGE_2 production in transformed MDCK cells by 10-fold and resulted in a final concentration of 0.5 nM in the culture medium. In light of the data in Fig. 2, it is apparent that this concentration of PGE_2 is not expected to induce glucagon sensitivity. The ability of EGF and TPA to increase PGE_2 production in normal MDCK cells appears to be the opposite of their ability to inhibit PGE_2 induction of glucagon sensitivity; however, this property of EGF and TPA does not seem to contribute to induction of glucagon sensitivity in transformed MDCK cells.

DISCUSSION

Cellular differentiation is a complex multistep process. During this process, cells acquire numerous specific characteristics reflective of the differentiated cells. Hormone sensitivity is one such differentiated function which accompanies cellular development. Although acquisition of hormone responsiveness represents only a finite span of the overall differentiation, an understanding of the regulation of its appearance will undoubtedly provide insight into at least part of this complex process.

The MDCK cell system has been established to allow studying of this type of regulation under well-defined conditions. We reported previously that selective loss of glucagon receptors and responsiveness occurred when MDCK cells were transformed by Harvey murine sarcoma virus (13). We have further shown that glucagon sensitivity could be restored to the transformed line by differentiation inducers such as PGE_2. We have now demonstrated that induction by prostaglandin is a cAMP-mediated process. In fact, 8-Br-cAMP is also a potent inducer. The cellular environment required for induction of differentiation is well defined in terms of the factors present in the culture medium. However, the biochemical events subsequent to cAMP production which lead to the appearance of glucagon sensitivity are completely unknown.

cAMP-dependent phosphorylation has been implicated in the induction of luteinizing hormone receptors (7) and alkaline phosphatase (4) and in stimulation of syntheses of prolactin, growth hormone (20), and other proteins (21). While activation of cAMP-dependent protein kinase is the first step shared by all systems, the subsequent events which more specifically account for induction differ from system to system. Induction of translatable mRNA for alkaline phosphatase in cells exposed to dibutyryl-cAMP seems to account for the induction of enzymatic activity in mouse L cells (4); if so, a nuclear event is likely to be involved and to precede induction. On the other hand, during stimulation of thymocytes by PGE_1, phosphorylation of a specific ribosomal protein has been shown to be responsible for increased protein synthesis (21). Therefore, it is obvious that, while activation of protein kinase by cAMP is a common first step, the subsequent events and target proteins vary considerably.

In the present study, one of the ultimate goals was to define the events leading to the appearance of glucagon sensitivity during the induction process. Our discovery that induction by PGE_2 can be inhibited by serum, phorbol ester, and EGF helps to define the nature of this cAMP-dependent process. None of these inhibitors changed the level of intracellular cAMP with or without PGE_2 in the medium; therefore, their ability to exert an inhibitory effect must reside at a site beyond the production of cAMP. The nature of the inhibitory factor in serum is unknown; however, the biochemical mechanisms responsible for the actions of EGF and phorbol ester have been extensively studied. EGF has been shown to induce phosphorylation of tyrosine residues on its receptors, an event thought to initiate the cellular effect of EGF (2). TPA is also capable of phosphorylation by activation of Ca^{2+}-dependent protein kinase, which phosphorylates serine and threonine residues (14). Recently, TPA has also been shown to activate, either direct or indirectly, a tyrosine kinase which phosphorylates a 42-

kilodalton protein also known to be modified by EGF-dependent kinase (5). Whether modification of this protein by TPA or EGF relates to their inhibitory effect on the induction of glucagon sensitivity remains to be established. In the MDCK system, the ultimate question is how both EGF- and cAMP-dependent phosphorylations interact in such a way as to account for the inhibitory effect of EGF on the induction of glucagon sensitivity by PGE_2.

Possible interaction between cAMP and TPA or EGF has been shown in several cases. Electrical communication between cultured human amniotic membrane epithelial cells can be reversibly inhibited by TPA, and this inhibition can be prevented by cAMP when added together with TPA, though cAMP did not restore the cell coupling once it had already been blocked by TPA (6). This observation suggests action of cAMP on the early membrane effects of TPA. In the case of cultured granulosa cells, induction of luteinizing hormone receptors by follicle-stimulating hormone or 8-Br-cAMP is inhibited by EGF (8). While the steady-state level of intracellular cAMP in the presence of follicle-stimulating hormone is reduced by EGF, the fact that the effect of 8-Br-cAMP is also blocked by EGF suggests an action of EGF directly on the cAMP-dependent pathway, which leads to the expression of LH receptors. Furthermore, in rat adipocytes, β-adrenergic hormones or cAMP reversibly inhibits EGF binding because of a reduction in the affinity of binding sites (15). In fact, phosphorylation of EGF receptors by cAMP-dependent protein kinase has been demonstrated in isolated membranes from rat liver (16). Whether induction of glucagon response in transformed MDCK cells by a cAMP-dependent process relates to phosphorylation of EGF receptors by cAMP-activated protein kinase remains to be established. Conversely, EGF has been shown to phosphorylate a 35-kilodalton protein which resembles the β subunit of the guanine nucleotide regulatory component in adenylate cyclase (19). Clearly, cellular differentiation potentially involves interaction of multiple phosphorylating systems. Whether inhibition of induction of differentiation by EGF is due to the protective effect of EGF against phosphorylation of its receptors by cAMP also needs to be examined. Our preliminary results show that treatment of transformed MDCK cells with PGE_2 leads to loss of EGF binding, suggesting desensitization of EGF receptors during induction of differentiation. Therefore, it is tempting to speculate that the dedifferentiation of transformed cells is in part due to overexpression of EGF receptors, which leads to unregulated growth. The ability of PGE_2, via cAMP, to desensitize EGF receptors may be responsible for the turnoff of unregulated growth and result in induction of differentiation.

Whereas the present results were obtained with a virus-transformed model kidney cell line, it is tempting to extrapolate these results to kidney development. In the hormonal milieu of the fetal kidney, a partially differentiated, proliferating phenotype may exist under regulation by EGF. With further development, these cells become exposed to the effect of locally produced prostaglandins, which leads to subsequent elaboration of differentiated functions, such as glucagon receptors and responsiveness. It may be possible to verify these hypotheses in fetal kidney cells.

LITERATURE CITED

1. **Beckner, S. K., F. J. Darfler, and M. C. Lin.** 1985. Induction of glucagon responsiveness in transformed MDCK cells unresponsive to glucagon. Methods Enzymol. **109**:356–360.
2. **Cohen, S., G. Carpenter, and L. King, Jr.** 1980. Epidermal growth factor-receptor-protein kinase interactions. J. Biol. Chem. **255**:4834–4842.
3. **Erickson, G. F., C. Wang, and A. J. W. Hsueh.** 1979. FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium. Nature (London) **279**:336–338.
4. **Firestone, G. L., and E. C. Heath.** 1981. The cyclic AMP-mediated induction of alkaline phosphatase in mouse L-cells. J. Biol. Chem. **256**:1396–1403.
5. **Gilmore, T., and G. S. Martin.** 1983. Phorbol ester and diacylglycerol induce protein phosphorylation at tyrosine. Nature (London) **306**:487–490.
6. **Kanno, Y., T. Enomoto, Y. Shiba, and H. Yamasaki.** 1984. Protective effect of cAMP on tumor promoter-mediated inhibition of cell-cell communication. Exp. Cell Res. **152**:31–37.
7. **Knecht, M., and K. J. Catt.** 1982. Induction of luteinizing hormone receptors by adenosine 3′,5′-monophosphate in cultured granulosa cells. Endocrinology **111**:1192–1200.
8. **Knecht, M., and K. J. Catt.** 1983. Modulation of cAMP-mediated differentiation in ovarian granulosa cells by epidermal growth factor and platelet-derived growth factor. J. Biol. Chem. **258**:2789–2794.
9. **Levine, L., and A. Hassid.** 1977. Epidermal growth factor stimulates prostaglandin biosynthesis by canine kidney (MDCK) cells. Biochem. Biophys. Res. Commun. **76**:1181–1187.
10. **Levine, L., and K. Ohuchi.** 1978. Retinoids as well as tumour promoters enhance deacylation of cellular lipids and prostaglandin production in MDCK cells. Nature (London) **276**:274–275.
11. **Lin, M. C., S. K. Beckner, and F. J. Darfler.** 1985. Characterization of hormone-sensitive Madin-Darby canine kidney cells. Methods Enzymol. **109**:360–365.
12. **Lin, M. C., and S. K. Beckner.** 1983. Induction of hormone receptors and responsiveness during cellular differentiation. Curr. Top. Membr. Transp. **18**:287–315.
13. **Lin, M. C., S.-W. M. Koh, D. D. Dykman, S. K. Beckner, and T. Y. Shih.** 1982. Loss and restoration of glucagon receptors and responsiveness in a transformed kidney cell line. Exp. Cell Res. **142**:181–189.
14. **Niedel, J. E., L. J. Kuhn, and G. R. Vandenbark.** 1983. Phorbol diester receptor copurifies with protein kinase C. Proc. Natl. Acad. Sci. USA **80**:36–40.
15. **Pessin, J. E., W. Gitomer, Y. Oka, C. L. Oppenheimer, and M. P. Czech.** 1983. β-Adrenergic regulation of insulin and epidermal growth factor receptors in rat adipocytes. J. Biol. Chem. **258**:7386–7394.
16. **Rackoff, W. R., R. A. Rubin, and H. S. Earp.** 1984. Phosphorylation of the hepatic EGF receptor with cAMP-dependent protein kinase. Mol. Cell. Endocrinol. **34**:113–119.
17. **Rubin, C. S., E. Lai, and O. M. Rosen.** 1977. Acquisition of increased hormone sensitivity during in vitro adipocyte development. J. Biol. Chem. **252**:3554–3557.
18. **Taub, M., L. Chuman, M. H. Saier, Jr., and G. Sato.** 1979. Growth of Madin-Darby canine kidney epithelial cell line in hormone-supplemented, serum-free medium. Proc. Natl. Acad. Sci. USA **76**:3338–3342.
19. **Valentine-Braun, K. A., J. K. Northup, and M. D. Hollenberg.** 1986. Epidermal growth factor (urogastrone)-mediated phosphorylation of a 53-KDa substrate in human placenta membranes. Proc. Natl. Acad. Sci. USA **83**:236–240.
20. **Waterman, M., G. H. Murdoch, R. M. Evans, and M. G. Rosenfeld.** 1985. Cyclic AMP regulation of eukaryotic gene transcription by two discrete molecular mechanisms. Science **229**:267–269.
21. **Wettenhall, R. E. H., and G. J. Howlett.** 1979. Phosphorylation of a specific ribosomal protein during stimulation of thymocytes by concanavalin A and prostaglandin E_1. J. Biol. Chem. **254**: 9317–9323.