MOLECULAR PHARMACOLOGY, 20:585–591

Desensitization of Adenylate Cyclase to Prostaglandin E_1 or 2-Chloroadenosine

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Received February 20, 1981; Accepted June 8, 1981

SUMMARY

The hypothesis was examined that prolonged activation of adenylate cyclase can result in a decrease in the specific activity of the enzyme, much as prolonged inhibition of adenylate cyclase gradually leads to an increase in the specific activity of the enzyme. Activation of adenylate cyclase of NG108-15 neuroblastoma-glioma hybrid cells by prostaglandin E_1 resulted in the gradual loss of basal adenylate cyclase activity as well as enzyme activity stimulated by prostaglandin E_1, 2-chloroadenosine, NaF, or both prostaglandin E_1 and guanyl-5′-yl imidodiphosphate. Exposure of NG108-15 cells to 8-Br cyclic AMP also resulted in the loss of basal, prostaglandin E_1-stimulated, and 2-chloroadenosine-stimulated adenylate cyclase activities. Cyclohexamide had no effect on prostaglandin E_1-dependent desensitization of adenylate cyclase, but inhibited recovery of enzyme activity from the desensitized state. In contrast, exposure of NG108-15 cells to 2-chloroadenosine resulted in the rapid loss of response to 2-chloroadenosine with a half-life of 1.8 hr, but prostaglandin E_1-stimulated and basal enzyme activities decreased only slightly.

INTRODUCTION

NG108-15 cells possess opiate receptors (1, 2), muscarinic acetylcholine receptors (3, 4) and *alpha*-adrenergic receptors (5) which mediate inhibition of adenylate cyclase (ATP pyrophosphate lyase-cyclizing; EC 4.6.1.1). Thus, exposure of cells to morphine (1), carbamylcholine (6), or norepinephrine (7) reduces cellular cyclic AMP levels; however, exposure of cells to the receptor ligand for 10–24 hr gradually results in an increase in adenylate cyclase specific activity. Thus, cyclic AMP levels of cells slowly return to the control value. Withdrawal of the inhibitory ligand unmasks the elevated enzyme activity and results in a prolonged 4- to 10-fold increase in cellular cyclic AMP levels. Cells thus develop an apparent tolerance to and dependence upon morphine, carbamylcholine, or norepinephrine with respect to maintenance of cellular cyclic AMP levels.

The hypothesis that activation of adenylate cyclase may lead, conversely, to a reduction in adenylate cyclase activity is examined in this report. Preliminary results by Dr. Shail Sharma[2] showed that treatment of NG108-15 cells with PGE_1[3] for several days resulted in decreases in basal and PGE_1-stimulated activities. These observations are confirmed and extended in this report.

MATERIALS AND METHODS

Cell culture and homogenate preparation. Culture conditions for NG108-15 hybrid cells (subcultured 14–20 times) have been described (8); the growth medium consisted of DMEM (Grand Island Biological Company Catalogue No. 430-2100), 5–10% fetal bovine serum, 100 μM hypoxanthine, 1 μM aminopterin, and 16 μM thymidine. Each flask (75 sq cm surface area) was inoculated with 5.0–7.5 × 10^5 cells in 15 ml of growth medium. The medium was replaced on the 3rd day and each day thereafter. A confluent layer of cells (approximately 1.5 × 10^7 cells, 15 mg of cell protein per flask) was obtained on the 6th or 7th day of incubation. Desensitization experiments usually were initiated 5–6 days after cells were plated, when cell layers were approximately 80% confluent. The medium was changed at zero time as indicated in the figures and tables, and cells were incubated without further change of medium unless specified. Each monolayer of cells was washed twice with 15 ml (each wash) of isotonic salt solution (150 mM NaCl, 5.4 mM KCl, 0.17 mM Na_2HPO_4, 0.22 mM KH_2PO_4, 0.11 mM

A preliminary report of this work has been published [*Fed. Proc.* 37:1539 (1978)].

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[2] S. K. Sharma and M. Nirenberg, unpublished observations.

[3] The abbreviations used are: PGE_1, prostaglandin E_1; PGA_1, prostaglandin A_1; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; ClAdo, 2-Cl-adenosine; Bt_2 cyclic AMP, N^6,O^2-dibutyryl cyclic AMP; Bt_2 cyclic GMP, N^6,O^2-dibutyryl cyclic GMP; Ro20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; Gpp(NH)p; guanyl-5′-yl imidodiphosphate.

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$CaCl_2$, and 25 mM D-glucose, pH 7.4, 340 mOsmoles/kg). Then 10 ml of an isotonic salt solution without Ca^{2+} ions, adjusted to pH 6.6 (150 mM NaCl, 5.4 mM KCl, 0.17 mM Na_2HPO_4, 0.22 mM KH_2PO_4; and 25 mM D-glucose) were added; after 5 min at 24°, the cells were dissociated by sharply tapping the flasks. The cells were centrifuged for 4 min at 1000 × *g*, and the cell pellets were frozen in Dry Ice and stored over liquid nitrogen. The cells were thawed, suspended in a cold solution containing 25 mM Tris-HCl (pH 7.5) (approximately 7.5 mg of protein per ml), and homogenized with 10 strokes of a Dounce homogenizer with a size A pestle; 0.5-ml portions of the homogenate were frozen rapidly and stored in the vapor phase of a liquid nitrogen freezer. Homogenates were thawed immediately before use. Protein was determined by a modification of the method of Lowry *et al.* (9) with bovine serum albumin as the standard.

Adenylate cyclase assay. Adenylate cyclase activity was determined by a modification (10) of method C of Solomon *et al.* (11). Each 100-μl reaction mixture contained 50 mM Tris-HCl (pH 7.5); 5 mM $MgCl_2$; 20 mM creatine phosphate, disodium salt; 10 units (71 μg of protein) of creatine phosphokinase; 1 mM [α-^{32}P]ATP, tetrasodium salt (2 μCi); 0.5 mM cyclic AMP; *G*-^{3}H-labeled cyclic AMP (approximately 10,000 cpm); 0.5 mM Ro20-1724; 0.25% ethanol; and 50–200 μg of NG108-15 homogenate protein. Reaction mixtures were incubated for 6 min at 37° unless otherwise indicated. Under these conditions ^{32}P-labeled cyclic AMP synthesis was proportional to the time of incubation for at least 20 min in the presence or absence of PGE_1 or ClAdo. Each reaction mixture was deproteinized by the addition of 0.9 ml of cold 6% trichloroacetic acid. The tubes were centrifuged at 1800 × *g* for 20 min and each supernatant solution was added to a Dowex AG50W-X4 column. The cyclic AMP fraction from the column was eluted onto an alumina column and eluted from the alumina with 4 ml of 0.1 mM imidazole-HCl (pH 7.5) into a counting vial. Values reported are the means of duplicate or triplicate determinations; most replicate values differed by less than 10%.

RESULTS

As shown in Fig. 1, PGE_1 stimulated NG108-15 adenylate cyclase activity. However, continued exposure of NG108-15 cells to 25 μM PGE_1 gradually resulted in desensitization of adenylate cyclase to PGE_1 (Fig. 1A) and ClAdo (Fig. 1B) and also reduced the specific activity of basal adenylate cyclase (Fig. 1B). The specific activity of adenylate cyclase in homogenates prepared from untreated, control cells also decreased; however, a larger decrease was always observed when cells were treated with PGE_1.[4] Basal and PGE_1-stimulated enzyme activities, expressed as percentage of control values, decreased exponentially with half-lives of approximately 7.5 and 6 hr, and pseudo-first order rate constants for PGE_1-dependent loss of enzyme activity of 1.6×10^{-5} sec^{-1} and 3.2×10^{-5} sec^{-1}, respectively (Fig. 1B, *inset*). The decrease in ClAdo-stimulated adenylate cyclase specific activity was biphasic, suggesting that the response to ClAdo was lost by a more complex kinetic mechanism than that found for basal or PGE_1-stimulated adenylate cyclase (Fig. 1B, *inset*).

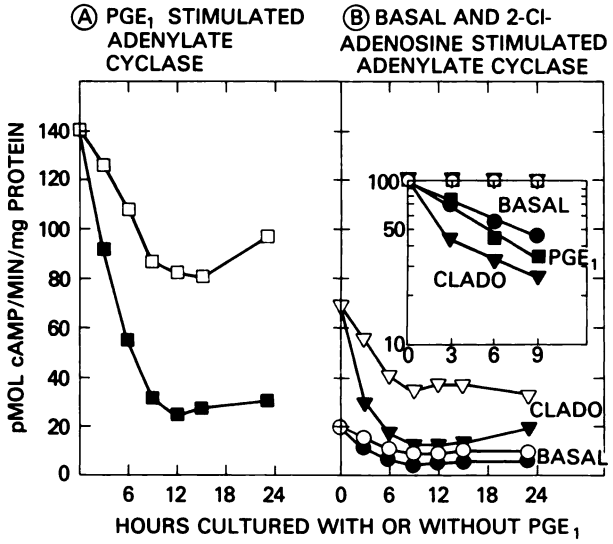

FIG. 1. *Effects of culturing NG108-15 cells in the presence or absence of PGE_1 on basal, ClAdo-stimulated, and PGE_1-stimulated adenylate cyclase activities*

Cells were cultured for 0–23 hr in the presence of 25 μM PGE_1 (●, ■, ▼) or in the absence of PGE_1 (○, □, ▽). At the times indicated the cells were harvested and frozen. The data shown in A and B are from the same experiment. Homogenates were prepared and assayed for adenylate cyclase activity in the presence of 10 μM PGE_1 (A) or 50 μM ClAdo (B); basal enzyme activity shown in B also applies to A. Each value shown in the inset to B was obtained by dividing the mean basal, PGE_1-dependent, or ClAdo-dependent adenylate cyclase specific activities found with homogenates of PGE_1-treated cells by the corresponding control values at each time; thus, the ordinate of the *inset* represents the percentage of the control values obtained with homogenates of untreated cells at each time. The abscissa (*inset*) represents hours of incubation of NG108-15 cells with PGE_1.

Exposure of NG108-15 hybrid cells to 0.1 mM ClAdo resulted in time-dependent decreases in ClAdo-stimulated adenylate cyclase specific activities (Fig. 2B); however, treatment of cells with ClAdo had little or no effect on basal or PGE_1-stimulated adenylate cyclase specific activities (Fig. 2A). The half-life of the loss of responsiveness of adenylate cyclase to ClAdo was 1.8 hr; enzyme specific activity decreased exponentially with an estimated pseudo-first order rate constant of 1.1×10^{-4} sec^{-1} (Fig. 2B, *inset*). The specific activities of basal and stimulated adenylate cyclase from control cells decreased somewhat during the course of the experiment; however, ClAdo-dependent adenylate cyclase activities of cells treated with ClAdo decreased to <2% of the value obtained with untreated control cells.

The effects of exposing NG108-15 cells to different concentrations of PGE_1 or ClAdo on adenylate cyclase activities are shown in Table 1. The lowest concentration of PGE_1 tested that resulted in desensitization of adenylate cyclase was 0.25 μM. Almost maximal desensitization was obtained with 2.5 μM PGE_1. Culture of NG108-

[4] Decreases in adenylate cyclase specific activities obtained with homogenates from untreated, control cells incubated for various periods probably resulted from replacement of the culture medium at zero time. When only 50% of the medium was replaced at zero time, adenylate cyclase activities of cells incubated for 8 hr did not change appreciably (data not shown).

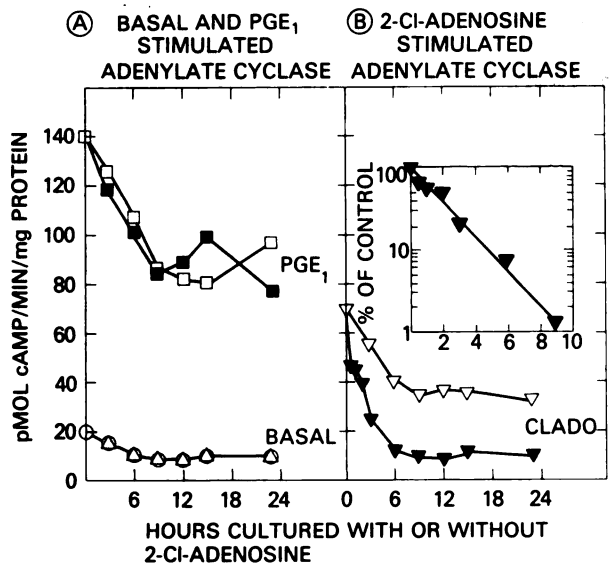

FIG. 2. *Effects of culturing NG108-15 cells in the presence of ClAdo on basal, ClAdo-stimulated, or PGE$_1$-stimulated adenylate cyclase activities*

Cells were cultured for 0–23 hr in the presence of 100 μM ClAdo (■, △, ▼) or the absence of ClAdo (□, ○, ▽) and harvested at the times shown. The data shown in A and B are from the same experiment. Homogenates were prepared and assayed for basal adenylate cyclase activity and for activity in the presence of 10 μM PGE$_1$ (A), or 50 μM ClAdo (B). Each value shown in the *inset* was obtained by dividing the mean ClAdo-dependent adenylate cyclase specific activity found with homogenates prepared from ClAdo-treated cells by the mean ClAdo-dependent adenylate cyclase specific activity found with homogenates from untreated control cells at that time. The abcissa of the *inset* represents hours of treatment of NG108-15 cells with ClAdo.

15 cells with ClAdo resulted in marked desensitization to ClAdo at each concentration tested (0.50–50 μM ClAdo), but only a small reduction in PGE$_1$-stimulated adenylate cyclase activity was observed. Basal adenylate cyclase activity decreased 21–38% after treatment of cells with ClAdo. In other experiments (not shown), treatment of cells with ClAdo had little or no effect on basal adenylate cyclase specific activity. Treatment of cells with 50 μM ClAdo in the presence of 1 or 5 mM theophylline, an adenosine receptor antagonist and an inhibitor of cyclic nucleotide phosphodiesterase, prevented the ClAdo-dependent loss of responsiveness of adenylate cyclase to ClAdo. Treatment of cells with 1 or 5 mM theophylline alone had no effect on adenylate cyclase specific activity. Theophylline had no effect on PGE$_1$-induced desensitization of adenylate cyclase (data not shown).

The effects of 10 μM PGE$_1$, 25 mM NaF, or 10 μM PGE$_1$ and 1 mM Gpp(NH)p on rates of ^{32}P-labeled cyclic AMP synthesis in homogenates prepared from NG108-15 cells cultured for 16 hr in the absence of PGE$_1$ or with 2.5 μM PGE$_1$ are shown in Fig. 3A and B, respectively. Basal, PGE$_1$-stimulated, NaF-stimulated, and PGE$_1$- and Gpp(NH)p-stimulated adenylate cyclase activities were approximately 50% lower in homogenates from cells treated with PGE$_1$ than in homogenates from untreated cells. The effects of 1, 3, 5, or 10 mM NaF were similar to those observed with 25 mM NaF, and the effect of 5 μM Gpp(NH)p was similar to that found with 1 mM

TABLE 1

Adenylate cyclase activity in homogenates prepared from NG108-15 cells treated with different concentrations of PGE$_1$ or ClAdo

NG108-15 cells were cultured in the presence of the indicated concentrations of PGE$_1$ or ClAdo for 16 hr. Homogenates were prepared and assayed for basal, PGE$_1$-stimulated, and ClAdo-stimulated adenylate cyclase activities.

Expt.	Cell treatment (16 hr)	Adenylate cyclase assay		
		No addition	10 μM PGE$_1$	50 μM ClAdo
		pmoles ^{32}P-labeled cyclic AMP/min/mg protein		
1	None	5.9	60.6	27.4
	PGE$_1$ (μM)			
	0.0025	6.4	61.4	29.7
	0.025	5.6	59.1	28.2
	0.25	4.0	42.1	21.2
	2.5	2.5	24.7	11.5
	25.0	3.9	23.4	12.7
2	None	11.9	78.9	32.2
	PGE$_1$ (μM)			
	0.25	7.8	39.0	18.3
	2.5	4.4	26.6	13.5
	25.0	3.7	22.3	15.4
	ClAdo (μM)			
	0.5	9.4	80.1	18.8
	5.0	7.4	78.0	11.2
	50.0	8.2	70.0	7.5

Gpp(NH)p (not shown). The demonstration that Gpp(NH)p inhibits PGE$_1$-stimulated adenylate cyclase activity in NG108-15 homogenates confirms previous observations (12).

NG108-15 hybrid cells were cultured with 1 mM 8-Br cyclic AMP, 1 mM Bt$_2$ cyclic AMP, or 1 mM Bt$_2$ cyclic GMP for 4 or 20 hr to determine whether elevation of cellular cyclic nucleotide levels affects adenylate cyclase activity (Table 2). Treatment of cells with 8-Br cyclic

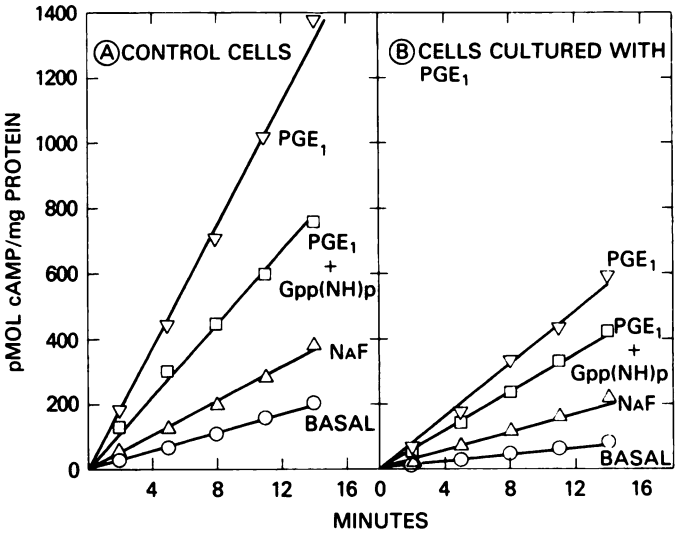

FIG. 3. *Effects of PGE$_1$, NaF, or Gpp(NH)p and PGE$_1$ on adenylate cyclase activity in homogenates prepared from control (untreated) or PGE$_1$-treated NG108-15 cells*

Cells were cultured for 16 hr in the absence of PGE$_1$ (A) or in the presence of 25 μM PGE$_1$ (B). ○, Basal adenylate cyclase activity; △, 25 mM NaF; ▽, 10 μM PGE$_1$; □, 10 μM PGE$_1$ and 100 μM Gpp(NH)p.

TABLE 2

Adenylate cyclase activity in homogenates of NG108-15 cells cultured with cyclic nucleotides or compounds that affect cyclic nucleotide levels

NG108-15 cells were incubated with the compound indicated for the times shown. Homogenates were assayed for adenylate cyclase activity.

Expt.	Cell treatment		Adenylate cyclase assay		
	Addition	Hr	No addition	10 μM PGE_1	50 μM ClAdo
			pmoles ^{32}P-labeled cyclic AMP/min/mg protein		
1	None	4	14.0	95.3	46.2
	1 mM 8-Br cyclic AMP	4	11.9	85.5	31.3
	1 mM Bt_2 cyclic AMP	4	13.5	98.5	38.6
	1 mM Bt_2 cyclic GMP	4	15.7	98.3	52.4
	None	20	14.9	101.1	47.6
	1 mM 8-Br cyclic AMP	20	7.7	74.5	17.6
	1 mM Bt_2 cyclic AMP	20	11.4	110.4	17.5
	1 mM Bt_2 cyclic GMP	20	14.4	91.0	51.0
2	None	16	8.6	68.5	27.6
	25.0 μM PGE_1	16	4.7	24.6	15.1
	25.0 μM PGA_1	16	6.3	53.7	22.8
	25.0 μM $PGF_{2\alpha}$	16	6.8	63.8	27.6
	0.5 mM Ro20-1724	16	6.9	72.4	24.0

AMP, which activates cyclic AMP-dependent protein kinase (13) and inhibits a low-K_m phosphodiesterase (14), decreased basal, PGE_1-stimulated, and ClAdo-stimulated adenylate cyclase activities. This observation suggests that PGE_1-dependent inactivation of adenylate cyclase may be mediated by 8-Br cyclic AMP-dependent protein phosphorylation. In contrast, treatment of cells with Bt_2 cyclic AMP, an inhibitor of a cyclic nucleotide phosphodiesterase (14, 15) and a relatively poor activator of cyclic AMP-dependent protein kinase (16), markedly desensitized adenylate cyclase to ClAdo but had little effect on basal or PGE_1-stimulated adenylate cyclase activities. Bt_2 cyclic AMP or a metabolite of Bt_2 cyclic AMP resembles ClAdo with respect to its ability to desensitize NG108-15 cells, which suggests that Bt_2 cyclic AMP may activate adenosine receptors. Bt_2 cyclic GMP had little or not effect on basal, PGE_1-stimulated or ClAdo-stimulated adenylate cyclase activities. Treatment of cells with 25 μM PGA_1 for 16 hr resulted in small decreases in basal, PGE_1-stimulated, and ClAdo-stimulated adenylate cyclase activities, whereas treatment of cells with 25 μM $PGE_{2\alpha}$ or 0.5 mM Ro20-1724, a cyclic AMP phosphodiesterase inhibitor, had little or no effect on basal, PGE_1-, or ClAdo-stimulated adenylate cyclase activities (Table 2, Experiment 2).

NG108-15 hybrid cells were cultured for 16 hr without PGE_1 or with 0.25, 2.5, or 25 μM PGE_1; homogenates were then prepared and assayed for adenylate cyclase activity in the presence of various concentrations of PGE_1 (Fig. 4A). The extent of desensitization of adenylate cyclase to PGE_1 was a function of concentration of PGE_1 used for cell treatment. Eadie-Scatchard plots are shown in Fig. 4B–D, and activation constants (K_{act}) for PGE_1 and maximal velocity (V_{max}) values for PGE_1-dependent adenylate cyclase activities are shown in Table 3. Treatment of NG108-15 cells with PGE_1 resulted in increases in the activation constants for PGE_1-dependent adenylate cyclase activity (i.e., decreases in apparent affinity of PGE_1 for receptor) and decreases in the V_{max} of PGE_1-dependent adenylate cyclase. Similar results have been reported recently by Homburger *et al.* (17) with C_6 glioma cells desensitized by isoproteronol.

Cells were treated with 2.5 μM PGE_1 for 16 hr; the medium was then replaced with fresh medium without PGE_1 and incubation was continued for an additional 24 hr to determine the rate of recovery of adenylate cyclase responsiveness to PGE_1. At 4, 8, 12, and 24 hr after withdrawal of PGE_1 (20, 24, 28, and 40 hr of total incubation, respectively), homogenates were prepared, and basal, PGE_1-stimulated, and ClAdo-stimulated adenylate cyclase activities were determined (Fig. 5A). On withdrawal of PGE_1, adenylate cyclase activity gradually returned toward the control value; 50% of the enzyme activity lost because of PGE_1 was recovered within 8 hr, and 80% of the activity was recovered within 24 hr. The effect of PGE_1 concentration on adenylate cyclase activity in homogenates prepared from untreated control cells cultured for 16 or 28 hr, from cells treated with 2.5 μM PGE_1 for 16 hr, or from cells treated with 2.5 μM PGE_1

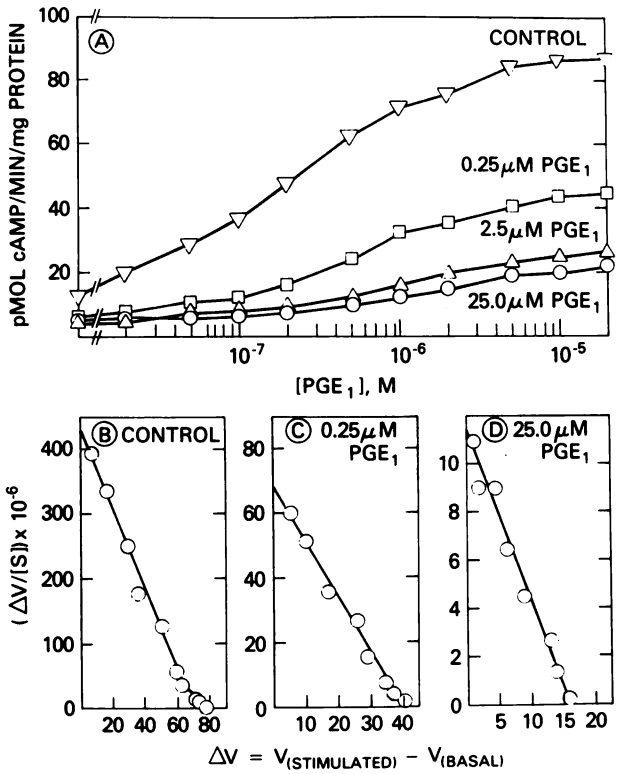

FIG. 4. *Effects of culturing NG108-15 cells with PGE_1 on the kinetics of PGE_1-stimulated adenylate cyclase activity*

A. Cells were cultured for 16 hr without PGE_1 (▽), with 0.25 μM PGE_1 (□), with 2.5 μM PGE_1 (△), or with 25 μM PGE_1 (○). Homogenates were prepared and assayed for adenylate cyclase activity in the presence of various concentrations of PGE_1 (abscissa).

B, C, and D. Eadie-Scatchard plots of the data from control cells grown without PGE_1 (B), cells treated with 0.25 μM PGE_1 (C), or cells treated with 25 μM PGE_1 (D). The velocity, V, represents picomoles of ^{32}P-labeled cyclic AMP formed per minute per milligram of protein; ΔV represents picomoles of ^{32}P-labeled cyclic AMP per minute per milligram of protein dependent on PGE_1; S represents micromolar PGE_1.

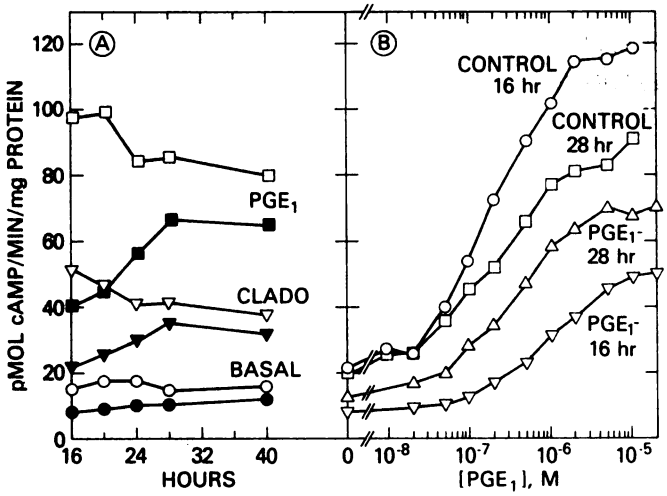

FIG. 5. *Recovery of adenylate cyclase activity following removal of PGE$_1$*

A. NG108-15 cells were cultured for 16 hr in the presence of 2.5 μM PGE$_1$ (●, ■, ▼) or absence of PGE$_1$ (○, □, ▽); at 16 hr the medium was replaced by medium without PGE$_1$. At the times indicated the cells were harvested and frozen. Homogenates were assayed for basal adenylate cyclase activity (○, ●) and for stimulated activity in the presence of 10 μM PGE$_1$ (□, ■), or 50 μM ClAdo (▽, ▼).

B. Symbols represent adenylate cyclase specific activities obtained with homogenates of NG108-15 cells from the experiment shown in A grown under the following conditions and assayed in the presence of the concentration of PGE$_1$ shown on the abscissa: ○, cells grown for 16 hr without PGE$_1$; ▽, cells grown for 16 hr with 2.5 μM PGE$_1$; □, cells grown for 28 hr without PGE$_1$; △, cells grown for 16 hr with 2.5 μM PGE$_1$ and for an additional 12 hr without PGE$_1$.

for 16 hr and for an additional 12 hr in the absence of PGE$_1$ (28 hr in Fig. 5A) is shown in Fig. 5B. Activation constants for PGE$_1$ and V_{max} values for PGE$_1$-dependent adenylate cyclase are shown in Table 3. Treatment of cells for 16 hr with 2.5 μM PGE$_1$ resulted in an increase in K_{act} and a decrease in V_{max}. The V_{max} of PGE$_1$-stimulated adenylate cyclase from control cells decreased between 16 and 28 hr; however, the activation constant did not change appreciably. Treatment of cells with PGE$_1$ for 16 hr and withdrawal of PGE$_1$ for an additional 12 hr resulted in an increase in V_{max} and a decrease in K_{act}; i.e. the values returned toward control levels.

TABLE 3

Activation constants and maximal velocity values for PGE$_1$-dependent adenylate cyclase activities shown in Fig. 4A–D and Fig. 5B

Fig.	Addition to cells		K_{act}	V_{max}
	Stage 1, 0–16 hr	Stage 2, 16–28 hr		
			μM PGE$_1$	*pmoles cyclic AMP/min/mg protein*
4	None		0.18	72.8
	0.25 μM PGE$_1$		0.59	40.0
	2.5 μM PGE$_1$		0.65	21.8
	25.0 μM PGE$_1$		1.52	16.7
5B	None		0.22	102.4
	2.5 μM PGE$_1$		0.73	41.8
	None	None	0.19	68.7
	2.5 μM PGE$_1$	None	0.32	58.6

TABLE 4

Effect of cycloheximide on PGE$_1$-dependent desensitization of adenylate cyclase and on recovery of activity after PGE$_1$ withdrawal

NG108-15 cells were incubated with the indicated compounds for the times shown. Homogenates were prepared and assayed for adenylate cyclase activity. Where indicated, the concentration of cycloheximide was 20 μg/ml of medium and the concentration of PGE$_1$ was 2.5 μM.

Expt.	Addition to cells		Adenylate cyclase assay		
	Stage 1, 0–16 hr	Stage 2, 16–24 hr	No addition	10 μM PGE$_1$	50 μM ClAdo
			pmoles ^{32}P-labeled cyclic AMP/min/mg protein		
1: Densensitization	None	None	13.2	99.7	40.5
	None	PGE$_1$	5.4	34.0	16.7
	None	Cycloheximide	11.5	98.0	38.1
	None	Cycloheximide + PGE$_1$	4.6	46.9	12.4
2: Recovery	None	Cycloheximide	10.3	76.0	28.1
	PGE$_1$	Cycloheximide	4.4	32.2	19.7
	PGE$_1$	—	5.1	23.5	18.0
	PGE$_1$	None	5.7	50.0	27.9

The effects of cycloheximide on PGE$_1$-dependent loss of adenylate cyclase activity and on recovery of responsiveness to PGE$_1$ following withdrawal of PGE$_1$ are shown in Table 4. Cycloheximide had no effect on PGE$_1$-dependent loss of adenylate cyclase activity, but inhibited the recovery of enzyme activity on withdrawal of PGE$_1$. In other experiments not shown here, 16 μM actinomycin D did not affect PGE$_1$-dependent desensitization of adenylate cyclase or recovery of enzyme activity in the absence of PGE$_1$.

DISCUSSION

Repeated exposure of cells to a receptor activator often diminishes the extent of the responses mediated by that species of receptor (18–29). In most cases the response is lost only to the ligand used for desensitization; cells retain responsiveness to ligands for other species of receptors. Yu and co-workers (28) have termed this phenomenon homologous desensitization. However, several investigators (18, 27–29) have reported that exposure of cells to a ligand for one species of receptor can result in a decrease in basal adenylate cyclase activity and/or loss of enzyme responsiveness to ligands for multiple species of receptor [termed heterologous desensitization (28)]. Heterologous desensitization thus may be due to inactivation of either adenylate cyclase, molecules that functionally couple receptors to adenylate cyclase, or to changes in membranes that affect the activities of the receptors and/or other components that are part of the adenylate cyclase complex.

Treatment of NG108-15 hybrid cells with PGE$_1$ results in decreases in basal adenylate cyclase activity and in

PGE_1-, ClAdo-, NaF-, or Gpp(NH)p- and PGE_1-stimulated activities. These results suggest that one or more components of the adenylate cyclase complex are inactivated when NG108-15 cells are treated with PGE_1. More than 95% of the specific binding sites for PGE_1 also are lost.[5] Thus, PGE_1 receptors and molecules required for activation of adenylate cyclase may be lost coordinately.

Tolkovsky and Levitzki (30) have reported that adenosine receptors of turkey erythrocytes either are coupled permanently to adenylate cyclase or form long-lived intermediates. Our results show that treatment of NG108-15 cells with ClAdo results in rapid desensitization to ClAdo with little effect on basal or PGE_1-dependent adenylate cyclase activities. The extent of activation of NG108-15 adenylate cyclase by saturating concentration of ClAdo is approximately 25–40% of that found with PGE_1 (12). The extent of desensitization of adenylate cyclase may be a function of the amount of cyclic AMP synthesized or the duration of the activated state. Assuming that the rate of cyclic AMP synthesis in intact NG108-15 cells equals that found in homogenates, exposure of cells to PGE_1 for 360 min (the time required for 50% loss of PGE_1-dependent adenylate cyclase activity) would result in the cumulative synthesis of 32 nmoles of cyclic AMP per milligram of protein above basal activity. Exposure of cells to ClAdo for 108 min (the time required for 50% loss of ClAdo-dependent adenylate cyclase activity) would result in the cumulative synthesis of 4 nmoles of cyclic AMP per milligram of protein above basal activity. Thus, ClAdo-dependent adenylate cyclase catalyzes only 12.5% as much cyclic AMP synthesis compared with PGE_1-dependent adenylate cyclase. The apparent absence of effect of ClAdo on responsiveness of adenylate cyclase to PGE_1 or on basal adenylate cyclase may be due to the lower extent of activation of the enzyme by ClAdo.

Cycloheximide had no effect on PGE_1-induced desensitization of adenylate cyclase, but markedly inhibited the recovery of adenylate cyclase activity from the desensitized state. In contrast, actinomycin D had no effect on either PGE_1-induced desensitization of adenylate cyclase or on recovery from the desensitized state. These results suggest that protein synthesis, but not mRNA synthesis, is required for recovery of PGE_1-desensitized adenylate cyclase activity in NG108-15 cells. Cycloheximide does not block the recovery of adenylate cyclase activity following beta-receptor-induced desensitization of the enzyme in frog erythrocytes (23) or in Ehrlich ascites tumor cells (26). Cycloheximide also does not affect desensitization or recovery of adenylate cyclase activity from the desensitized state in human astrocytoma cells exposed to norepinephrine or PGE_1 (28). However, cycloheximide or actinomycin D prevents the development of refractoriness to PGE_2 in cultured rat Graafian follicles (21).

In conclusion we find that prolonged activation of NG108-15 adenylate cyclase by PGE_1 results in the gradual loss of adenylate cyclase activity, much as prolonged receptor-mediated inhibition of adenylate cyclase results in gradual compensatory increase in adenylate cyclase activity. We also find that exposure of NG108-15 to 8-Br cyclic AMP, which activates cyclic AMP-dependent protein kinase, results in the loss of adenylate cyclase. Further work is needed to determine whether the loss of adenylate cyclase is dependent on cyclic AMP-mediated phosphorylation of protein (31).

[5] J. G. Kenimer, manuscript in preparation.

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