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Opposing regulatory effects of protein kinase C on the cAMP cascade in human HL-60 promyelocytic leukemia cells

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Abstract

The functional role of protein kinase C in the cAMP signaling cascade was investigated in human promyelocytic leukemia (HL-60) cells. Protein kinase C activation after short exposure to 100 nM phorbol 12-myristate 13-acetate (PMA) increased the intracellular cAMP level up to 3- to 5-fold after 30 min. Such enhancement was almost completely blocked by the selective protein kinase C inhibitor bisindolylmaleimide (GF 109203X). In addition, PMA, but not 4-α-PMA, synergistically elevated cAMP levels when adenylyl cyclase was activated directly by forskolin or indirectly by G protein activation after cholera toxin treatment or guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) treatment in digitonin-permeabilized cells. The results indicate that protein kinase C directly increases adenylyl cyclase activity and synergistically enhances it, when it is simultaneously activated otherwise. On the other hand, a 10-min treatment with PMA cut the cAMP accumulation induced by histamine, prostaglandin E_2, or isoproterenol by 50–70%. However, the binding affinity and total binding of [^{3}H]histamine to membrane receptors was not effected by PMA, suggesting that the site of protein kinase C's action is not at the receptor level. Western blot analysis of protein kinase C isozymes revealed that PMA (100 nM) caused translocation of cytosolic protein kinase C such as α, β and ε to the particulate/membrane fraction. Treatment with a lower concentration of PMA (10 nM) translocated the protein kinase C-ε within 2 min, while it had little effect on the translocation of protein kinase C-α and -β up to 20 min. However, simultaneous treatment with 10 nM PMA plus histamine for 5 min significantly inhibited the histamine-mediated cAMP generation, indicating that the protein kinase C-ε could be involved in the inhibition of receptor-mediated cAMP generation. Taken together, we conclude that PMA, through the activation of protein kinase C, has two opposite effects on the cAMP signaling cascade in HL-60 cells: a direct activation of adenylyl cyclase and an inhibition of receptor-mediated signal transduction.

Keywords: HL-60 promyelocytic leukemia cell; Adenylyl cyclase; Histamine; Protein kinase C; Phorbol 12-myristate 13-acetate

1. Introduction

The coupling of cAMP signal transduction with protein kinase C function is important for integration and modulation of various extracellular signals (Houslay, 1991; Yoshimasa et al., 1987). Many studies have shown that signal transduction through the adenylyl cyclase system can be enhanced or depressed, depending on the cell type, by the activation of protein kinase C (Fukushima et al., 1996; Morimoto and Koshland, 1994). This kinase acts at multiple sites within the adenylyl cyclase signaling system including membrane receptors (Ikeda et al., 1991), one of the GTP-binding proteins, G_s or G_i (Hernandez-Sotomayor et al., 1991; Gordeladze et al., 1989), the enzyme itself (Yoshimasa et al., 1987; Jacobowitz and Iyengar, 1994), or the cAMP phosphodiesterase that metabolizes cAMP (Bressler and Tinsely, 1990).

In human promyelocytic leukemia (HL-60) cells, histamine, prostaglandin E_1 and E_2 and isoproterenol have been shown to elevate intracellular cAMP levels by the activation of adenylyl cyclase through cholera toxin-sensitive G_s proteins (Klinker et al., 1996). The action of histamine is believed to be mediated by the histamine H_2 receptor, which is the major histamine receptor in these cells (Mitsuhashi et al., 1989). The actions of prostaglandin and isoproterenol are mediated by EP_2 (Regan et al., 1994) and β_2-adrenoceptors (Sager et al., 1988), respectively. Cyclic AMP is known to regulate several functions in the cells, such as inhibition of oxidative burst (Yu

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et al., 1995), suppression of actin polymerization and chemotaxis (Ydrenius et al., 1997), release of hepatocyte growth factor (Inada et al., 1993), protein phosphorylation (Bushkin et al., 1991), and regulation of immediate early gene expression (Kotecha et al., 1993; Slungaard et al., 1987). It is interesting that the elevation of cAMP levels can elicit spontaneous differentiation along the granulocytic pathway (Nonaka et al., 1992). Thus, it is important to understand the factors regulating the synthesis of the second messenger.

As several stimuli use signal transduction pathways involving protein kinase C activity (Deshpande et al., 1997; Huang et al., 1997), we sought to elucidate the possible regulatory role of protein kinase C on cAMP synthesis in HL-60 cells. Although the biochemical properties of the adenylyl cyclase-coupled receptors have been investigated before (Mitsuhashi et al., 1989; Regan et al., 1994; Sager et al., 1988), the multiple sites of protein kinase C activity in the cAMP signaling pathway have not yet been fully elucidated. Here, we report that protein kinase C activation led directly to the activation of adenylyl cyclase and the potentiation of cAMP responses induced by G protein activation, while it caused a decrease in cAMP accumulation in receptor-mediated responses. These dual opposite regulatory effects of protein kinase C on cAMP signaling provide an exquisite mechanism for the integration of signaling pathways and fine-tuning of cAMP synthesis.

2. Materials and methods

2.1. Materials

Histamine, prostaglandin E_2, (±)-isoproterenol, histidine, Triton X-100, potassium glutamate, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), ATP, glucose, sucrose, EDTA, EGTA, trichloroacetic acid, cholera toxin, guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS), leupeptin, pepstatin A, aprotinin, phenylmethylsulfonyl fluoride, dimethyl sulfoxide (DMSO), and bovine serum albumin were obtained from Sigma (St. Louis, MO, USA). 4-(3-butoxy-4-methoxybenzyl-2)-imidazolidinone (Ro 20-1724), phorbol 12-myristate 13-acetate (PMA), 4-α-PMA, isobutylmethylxanthine (IBMX), staurosporine, and dimaprit were purchased from Research Biochemicals (Natick, MA, USA). GF 109203X (bisindolylmaleimide) was purchased from Boehringer Mannheim (Mannheim, Germany). [^{3}H]Histamine and [^{3}H]adenine were obtained from NEN (Boston, MA, USA).

2.2. Cell culture

HL-60 cells were grown in RPMI 1640 (GIBCO, Gaithersburg, MD, USA) supplemented with 10% (v/v) heat-inactivated bovine calf serum (Hyclone, Logan, UT, USA) and 1% antibiotics (GIBCO) in a humidified atmosphere of 5% CO_2 at 37°C. Fresh medium was added to culture flasks every two days, and cells were subcultured about once a week.

2.3. Measurement of cyclic AMP accumulation

Intracellular cyclic AMP was determined by measuring the formation of cyclic [^{3}H]AMP from a [^{3}H]adenine nucleotide pool as described previously by Suh and Kim (1995). The cells were grown in 6-well dishes to confluency and loaded with [^{3}H]adenine (2 μCi/ml) in complete medium for 24 h. After loading, the cells were washed three times with Locke's solution (154 mM NaCl; 5.6 mM KCl; 1.2 mM $MgCl_2$; 2.2 mM $CaCl_2$; 5.0 mM HEPES; 10 mM glucose, pH 7.4) and preincubated with 1 mM isobutylmethylxanthine (IBMX) for 15 min in Locke's solution to inhibit phosphodiesterase. IBMX was also added to the stimulating buffer. The reaction was stopped by aspiration of the medium and addition of 1 ml of ice-cold 5% (v/v) trichloroacetic acid containing 1 μM cold cAMP. The plates were left on ice for 30 min to extract the water-soluble cAMP. Then, the extracts were transferred to Eppendorf tubes and centrifuged at $5000 \times g$ for 5 min to precipitate the cell debris. [^{3}H]cAMP and [^{3}H]ATP were separated by sequential chromatography on Dowex AG50W-X4 (200–400 mesh) cation exchanger and neutral alumina columns. The [^{3}H]ATP fraction was obtained from the Dowex column by elution with 2 ml distilled water, before the subsequent elution with 3.5 ml distilled water was loaded onto the alumina column. The alumina column was washed with 4 ml imidazole solution (0.1 M, pH 7.2) and the eluates were collected into scintillation vials containing 15 ml scintillation fluid for the counting of the cyclic [^{3}H]AMP. The increase in intracellular cAMP concentration was calculated as [^{3}H]cAMP/([^{3}H]ATP + [^{3}H]cAMP) × 10^3.

2.4. Permeabilized cell preparation

[^{3}H]adenine-loaded cells were washed with buffered Locke's solution and then incubated with vehicle (0.2% DMSO) or phorbol ester for 10 min. The cells were then permeabilized in KG (K^+-glutamate) buffer (139 mM potassium glutamate, 20 mM PIPES, 0.5 mM ATP, 1 mM $MgSO_4$, 5 mM glucose, 20 μM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724), and 50 μM IBMX, pH 7) containing 20 μM digitonin for 5 min as described (Suh et al., 1996) and then treated with GTPγS or $AlCl_3$ and NaF for 20 min at 22°C. The reaction was stopped by the addition of trichloroacetic acid to a final concentration of 5% (w/v). The amount of cAMP production was determined as described above.

2.5. Cell membrane preparation and adenylyl cyclase assay

Washed cells were separated and incubated in the presence of vehicle (0.2% DMSO), PMA (1 μM), or 4-α-PMA (1 μM) for 10 min at 25°C. The cells were then washed three times with Locke's solution and lysed by discontinuous sonication in buffer (100 mM $NaHCO_3$; 5 mM EDTA; 10% sucrose, pH 8.6; protease inhibitors). The nuclear fraction and cell debris were removed by centrifugation at $800 \times g$ for 15 min. The plasma membranes were isolated by centrifugation of the supernatant at $2000 \times g$ for 20 min. They were washed three times by resuspension and recentrifugation. Adenylyl cyclase activity tests were performed using this membrane preparation and [α-^{32}P]ATP as previously described (Salomon et al., 1974). After 10 min at 30°C, the reaction was stopped by adding 500 μl of ice-cold stopping solution (10% trichloroacetic acid) containing 10^4 cpm of [^{3}H]cAMP for the purpose of monitoring the recovery which averaged 60–70%. The product [^{32}P]cAMP was separated out by a two-step elution over Dowex and alumina columns as described above.

2.6. [^{3}H]histamine binding

The binding of [^{3}H]histamine to intact HL-60 cells was quantified by the method described previously by Mitsuhashi and Payan (1988) with some modification. Cells were collected by centrifugation at $1000 \times g$ for 1 min and preincubated for 20 min at room temperature in Locke's solution. The binding assay was carried out at 25°C in a final volume of 100 μl of Ca^{2+}-free Locke's solution supplemented with 1 mM EDTA, 0.2% bovine serum albumin, 5 mM histidine, 50 nM [^{3}H]histamine and various drugs. Assays were initiated by the addition of the cells (10^6 cells/tube) and terminated after 20 min by vacuum filtration through nitrocellulose filters (0.45 μm) using the Millipore multiscreen assay system. The filters were rinsed two times with 150 μl of ice-cold 50 mM Tris–HCl containing 1 mM EDTA (pH 7.6). The amount of bound radioactivity was measured in a liquid scintillation cocktail. Specific binding was defined as the difference in the amount of radioactivity bound in the absence and presence of 1 mM unlabeled histamine.

2.7. Cell fractionation and Western blot analysis of protein kinase C isoforms

For separation into soluble/cytosolic and particulate/membrane fraction, HL-60 cells were suspended in buffer A (20 mM Tris–HCl, pH 7.5, containing 0.25 M sucrose, 2 mM EGTA, 2 mM EDTA, 10 μg/ml pepstatin A, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml aprotinin). The cells were sonicated twice for 3 s and centrifuged at $100\,000 \times g$ for 1 h. The supernatant was saved as the cytosolic fraction. The pellet was then extracted with buffer B (20 mM Tris–HCl, pH 7.5, containing 1% sodium dodecylsulfate, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA and protease inhibitors as described above for buffer A). Following centrifugation, the supernatant was saved as the particulate/membrane fraction (Chun et al., 1996).

Proteins (30 μg) from the cytosolic and the particulate/membrane fraction were separated by electrophoresis on an 8% polyacrylamide gel containing 0.1% SDS (sodium dodecyl sulfate) and transferred to a nitrocellulose membrane. The nitrocellulose sheet was blocked

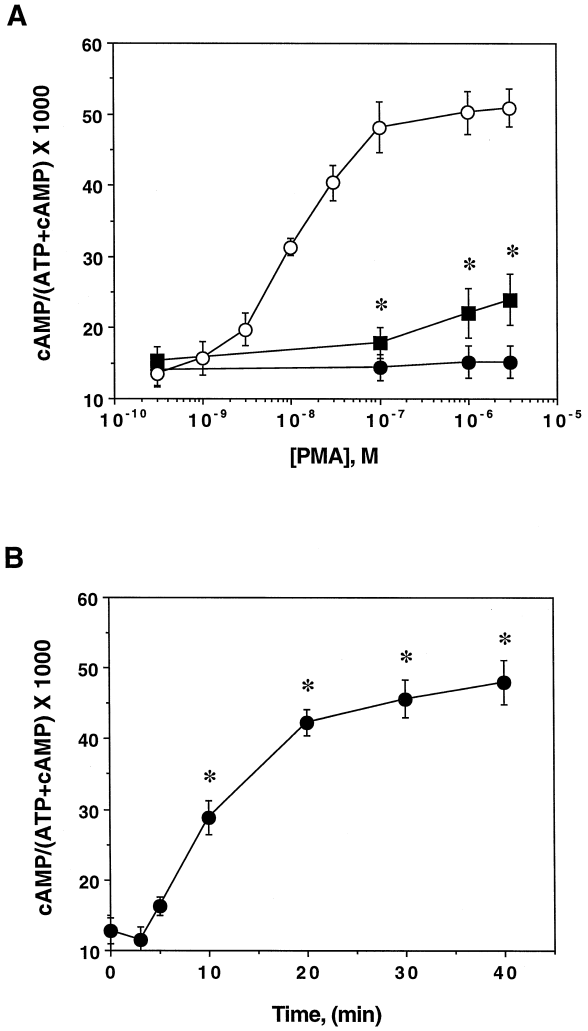

Fig. 1. Effect of PMA on cAMP generation in HL-60 cells. (A) Concentration-dependent elevation of cAMP levels during treatment with PMA. [^{3}H]adenine-loaded cells were treated with various concentrations of PMA (○) or 4-α-PMA (●) in the presence of 1 mM IBMX for 30 min. PMA-induced cAMP generation in cells pretreated with 3 μM GF 109203X for 30 min is presented (■). $*\ P < 0.05$, compared to the GF 109203X-untreated. (B) Time-course of cAMP generation induced by 1 μM PMA. Cells were stimulated with 1 μM PMA for the designated times (0, 3, 5, 10, 20, 30, 40 min) and then treated with 5% (w/v) trichloroacetic acid solution containing 1 μM unlabeled cAMP to stop the reaction. Cyclic AMP generation was assayed as described in Section 2. The experiments were done three times in triplicate and each point is the mean ± S.E.M. $*\ P < 0.05$, compared to the control.

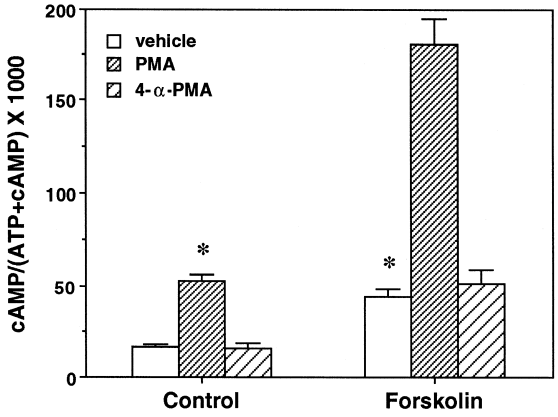

Fig. 2. Effect of PMA on adenylyl cyclase activity in intact cells. [^{3}H]adenine-loaded HL-60 cells were treated with vehicle (0.2% DMSO), 1 μM PMA, or 1 μM 4-α-PMA for 10 min and then stimulated with 10 μM forskolin for 20 min at 25°C in the presence of 1 mM IBMX. The reaction was stopped by the addition of 5% (w/v) trichloroacetic acid solution containing 1 μM unlabeled cAMP. The [^{3}H]cAMP generation was measured as described in Section 2. The experiment was done four times in triplicate and the mean ± S.E.M. values are presented. * $P < 0.05$, compared to basal.

Table 1
The effect of PMA on G protein-mediated cAMP generation

Treatment	cAMP accumulation	
	Control	Cholera toxin
Basal	13.2 ± 2.4	50.7 ± 3.5
PMA	42.2 ± 1.5[a]	131.9 ± 8.6[b]

[a] $P < 0.05$, compared to basal.
[b] $P < 0.01$, compared to basal.
[^{3}H]Adenine-loaded HL-60 cells preincubated with 1 mM IBMX for 15 min were further treated with cholera toxin (2 μg/ml) for 30 min in the presence or absence of 1 μM PMA. The reaction was stopped by the addition of 5% (w/v) trichloroacetic acid containing 1 μM cAMP. The [^{3}H]cAMP generation was measured as described in Section 2.

with 3% non-fat dry milk in Tris-buffered saline. Protein kinase C isoforms were detected with isoform-specific anti-protein kinase C monoclonal antibodies against α, β, δ, ε, θ and λ isoforms (Transduction Laboratories, Lexington, KY, USA) or with polyclonal antibodies against γ and ζ isoform (Santa Cruz Biotech, Santa Cruz, CA, USA) and η (Biomol, Plymouth Meeting, PA, USA). The blots were developed using a peroxidase-conjugated secondary

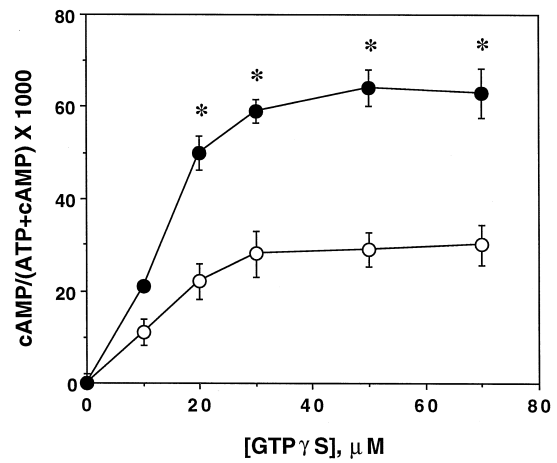

Fig. 3. Effect of PMA on GTPγS-stimulated cAMP generation in digitonin-permeabilized cells. HL-60 cells were permeabilized in KG buffer containing 20 μM digitonin and various concentrations of GTPγS in the absence (○) or presence (●) of 1 μM PMA. The amount of cAMP generation was determined as described in Section 2. The net increase in cAMP was obtained by subtracting the basal and the PMA-induced cAMP levels from the GTPγS- and the GTPγS plus PMA-stimulated cAMP levels, respectively. The data presented are the mean ± S.E.M. values of triplicate experiments. * $P < 0.05$, compared to the PMA-untreated.

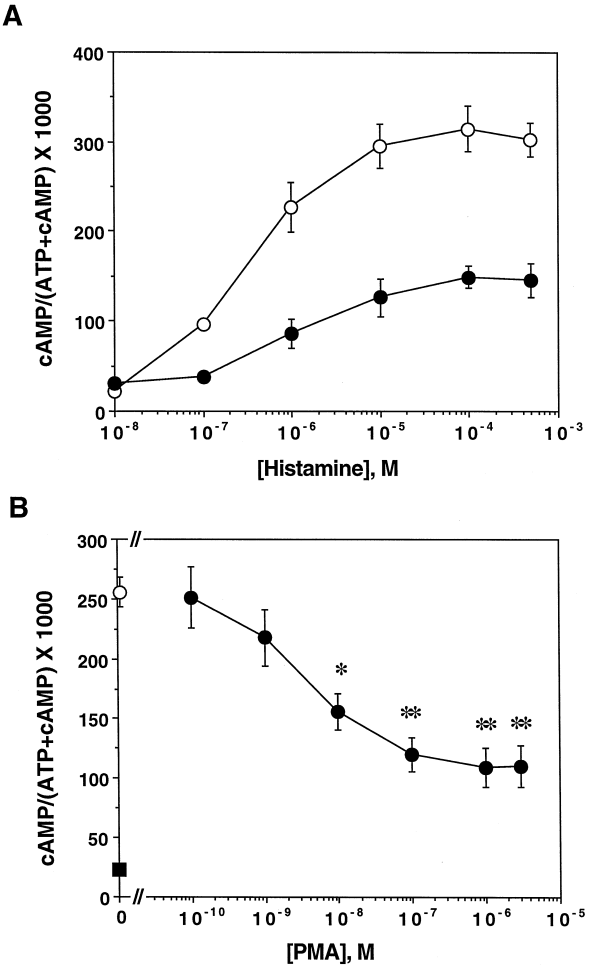

Fig. 4. Inhibitory effect of PMA on histamine-induced cAMP generation. (A) [^{3}H]adenine-loaded cells preincubated with vehicle (0.2% DMSO, ○) or 1 μM PMA (●) for 10 min were stimulated with various concentrations of histamine for 20 min. (B) Cells pretreated with various concentrations of PMA (●) were stimulated with 100 μM histamine. Cyclic AMP accumulation was measured as described in Section 2. Basal (■) and 100 μM histamine-induced cAMP generation (○) without PMA treatment are presented. The experiments were done three times in triplicate and each point is the mean ± S.E.M. * $P < 0.05$, compared to the histamine control. ** $P < 0.01$, compared to the histamine control.

antibody, either goat anti-rabbit or anti-mouse IgG, using the ECL system (Amersham, Arlington Heights, IL).

2.8. Data analyses and statistics

The results are expressed as the mean ± S.E.M. values from the number of determinations indicated. A Student's *t* test was used for comparing individual treatments with their respective control values. A probability of $P < 0.05$ was accepted as denoting a significant difference. Radioligand binding data were analyzed with the iterative-curve-fitting program LIGAND.

3. Results

To address the possible role of protein kinase C in the regulation of adenylyl cyclase activity in intact cells, we treated HL-60 cells with various concentrations of PMA in the presence of phosphodiesterase inhibitor, IBMX, for 30 min. Fig. 1A shows that PMA elevated the cAMP levels in a concentration-dependent manner with a maximal 3- to 5-fold increase of the basal cAMP level. The maximal and half maximal (EC_{50}) effective concentrations were seen at approximately 100 nM and 10 nM, respectively. However, the cAMP response to PMA could be almost completely inhibited by a 10-min pretreatment of the cells with a specific protein kinase C inhibitor bisindolylmaleimide (GF 109203X), indicating that protein kinase C is involved in the activation of adenylyl cyclase. The inactive phorbol ester, 4-α-PMA, had little effect on cAMP accumulation. The cAMP accumulation induced by PMA displayed a slow and sustained increase as shown in the time course with the 1 μM PMA treatment (Fig. 1B). A significant increase in cAMP was detectable 10 min after stimulation and the saturation of cAMP production was reached about 30 min after stimulation.

In order to further analyze the stimulatory effect of protein kinase C on adenylyl cyclase, we treated the cells with forskolin, which directly activates adenylyl cyclase, and assessed cAMP production in the presence or absence of PMA. Fig. 2 shows that the addition of forskolin caused a 2- to 3-fold increase in the cAMP production. The forskolin-stimulated cAMP generation was significantly enhanced in PMA- but not in 4-α-PMA-treated cells. This result indicates that when protein kinase C is already stimulated, it could even further enhance the activity of adenylyl cyclase. This phenomenon was also seen in the membrane fraction prepared from cells pretreated with PMA for 10 min, where the forskolin-stimulated cAMP generation was significantly enhanced (data not shown).

Using digitonin-permeabilized HL-60 cells, G proteins could be activated with increasing concentrations of the GTP analog GTPγS. In the absence of phorbol ester, increasing concentrations of GTPγS resulted in slightly higher cAMP accumulations with 30 μM of GTPγS evoking the maximal response (Fig. 3). However, the inclusion of PMA dramatically bolstered GTPγS-induced cAMP production, indicating that PMA also enhances the signaling between stimulatory G protein, G_s, and adenylyl cyclase.

We further investigated the PMA effect on adenylyl cyclase in intact cells in which G_s protein was activated by cholera toxin. The incubation of cells with 2 μg/ml cholera toxin for 30 min increased cAMP levels by ~2.5- to 4-fold, but co-treatment of the cells with PMA and cholera toxin synergistically enhanced the response (Table 1). Taken together, these results demonstrate that adenylyl cyclase is activated by protein kinase C and synergistically activated when simultaneously stimulated by forskolin or after G protein activation.

We examined the PMA effect on receptor-mediated adenylyl cyclase activation by applying histamine. Histamine is known to increase the cellular cAMP level via the activation of histamine H_2 receptors in HL-60 cells (Mitsuhashi et al., 1989). When [^{3}H]adenine-loaded HL-60 cells were stimulated with histamine, the cAMP levels increased in a concentration-dependent manner with the

Table 2
The effect of PMA on receptor-induced cAMP generation in HL-60 cells

	cAMP accumulation			
	Basal	Histamine	Prostaglandin E_2	Isoproterenol
Control	14.9 ± 1.4	248.9 ± 18.6	290.7 ± 26.5	201.1 ± 23.2
PMA	57.5 ± 2.7[a]	144.5 ± 13.1[b]	189.2 ± 22.1[b]	121.4 ± 15.6[b]
4-α-PMA	16.4 ± 2.1	243.7 ± 20.1	269.0 ± 20.2	183.2 ± 17.6
PMA + GF 109203X	16.9 ± 3.4	238.6 ± 25.2[c]	278.2 ± 19.5[c]	185.2 ± 20.6[c]

[a] $P < 0.05$, compared to control.
[b] $P < 0.01$, compared to the agonist-stimulated control.
[c] $P < 0.01$, compared to the PMA-treated cells.
[^{3}H]Adenine-loaded cells preincubated with vehicle (0.2% DMSO), or 1 μM 4-α-PMA, 1 μM PMA, 3 μM GF109203X, or 1 μM PMA plus 3 μM GF109203X for 10 min were stimulated with 100 μM histamine, 10 μM prostaglandin E_2 (PGE_2), and 1 μM isoproterenol for 20 min in the presence of 1 mM IBMX. The reaction was stopped by the addition of 5% (w/v) trichloroacetic acid containing 1 μM unlabeled cAMP. The [^{3}H]cAMP was measured as described in Section 2. GF 109302X alone did not effect the basal and the agonist-stimulated cAMP accumulations. The data presented are expressed as mean ± S.E.M. values of four independent experiments.

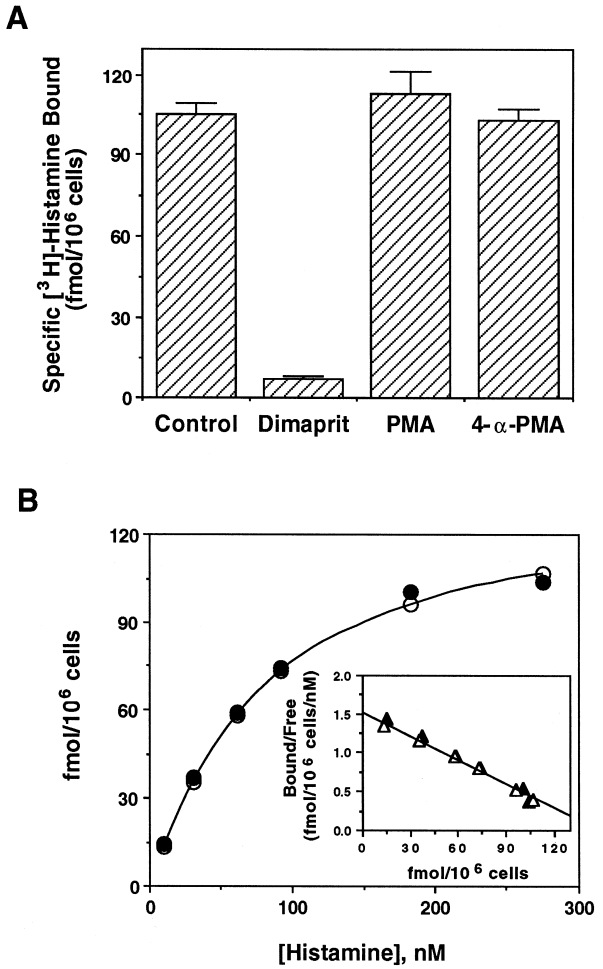

Fig. 5. Binding of [^{3}H]histamine to HL-60 cells. (A) Cells preincubated with 1 μM PMA or 1 μM 4-α-PMA for 10 min were treated with [^{3}H]histamine or [^{3}H]histamine plus 5 μM dimaprit at room temperature for 20 min. The data represent specific binding obtained by subtracting nonspecific binding from the total binding. Nonspecific binding was determined after the addition of 5 μM unlabeled histamine and was 39.0 ± 4.6 fmol/10^6 cells. (B) Saturation curve of the specific binding of [^{3}H]histamine to the control (○) and the PMA-treated (●) cells. Inset shows a Scatchard plot of the same data; the K_d and B_{max} data were: 88.3 ± 12.5 nM and 143.5 ± 18.9 fmol/10^6 cells for control cells (△), and 96.9 ± 18.2 nM and 147.3 ± 21.4 fmol/10^6 cells for PMA-treated cells (▲), respectively. Each point is the mean of triplicate determinations.

maximal effective concentration and EC_{50} seen at ~100 μM and ~0.8 μM, respectively (Fig. 4A). Interestingly, PMA treatment decreased the histamine-induced cAMP generation by about 60–70% at stimulations with various concentrations of histamine. The inhibitory effect of PMA was concentration dependent, and maximum inhibition was obtained at over 100 nM PMA (Fig. 4B). The inhibitory effect of PMA could also be seen with other receptor-mediated cAMP accumulations. Prostaglandin E_2 and isoproterenol elevated the cAMP production, but PMA treatment decreased the responses by 50–60% (Table 2). The inhibition was also mediated by the action of protein kinase C, since pretreatment of cells with GF 109203X for 10 min reversed the inhibitory effect of PMA, and 4-α-PMA did not diminish the receptor-mediated responses.

To determine whether the PMA-induced inhibition of the histamine-mediated cAMP generation is the result of a decreased number of histamine receptors or of a change in the binding affinity of the receptors, we measured the specific binding of histamine to the cells. Fig. 5A shows that dimaprit, a histamine H_2 receptor agonist, almost completely blocked [^{3}H]histamine binding, indicating that the cells mainly used the histamine H_2 receptor to bind histamine. However, pretreatment of the cells with 1 μM PMA or 1 μM 4-α-PMA did not affect histamine binding. Scatchard plots of the equilibrium saturation binding data were linear (Fig. 5B, inset). The K_d and B_{max} data were 88.3 ± 12.5 nM and 143.5 ± 18.9 fmol/10^6 cells for untreated control cells, and 96.9 ± 18.2 nM and 147.3 ± 21.4 fmol/10^6 cells for PMA-treated cells, respectively. Thus, there was no difference between the control cells and the PMA-treated cells.

Activation of protein kinase C synergistically stimulated adenylyl cyclase, when adenylyl cyclase was simultaneously activated by forskolin or after G protein activation. On the other hand, it inhibited receptor-mediated adenylyl cyclase activation. Therefore, to test whether the adenylyl cyclase, which is stimulated by forskolin, is also activated by receptor activation, we treated the cells simultaneously with receptor agonists and forskolin and measured the cAMP generation. Fig. 6 shows that forskolin slightly increased the cAMP level, but co-treatment of the cells with forskolin and receptor agonist synergistically enhanced the response. This result indicates that the same adenylyl cyclase molecule is involved in both the

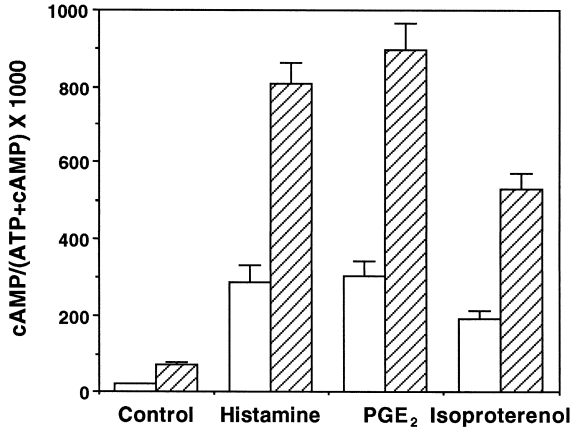

Fig. 6. Synergistic cAMP elevation by co-treatment with receptor agonists and forskolin. [^{3}H]Adenine-loaded HL-60 cells preincubated with 1 mM IBMX for 15 min were further treated with 100 μM histamine, 10 μM prostaglandin E_2 (PGE_2), or 3 μM isoproterenol in the absence (open) or presence (hatched) of 10 μM forskolin for 20 min. The reaction was stopped by the addition of 5% (w/v) trichloroacetic acid containing 1 μM unlabeled cAMP. The [^{3}H]cAMP generation was measured as described in Section 2. The experiments were done four times in triplicate and each point is the mean ± S.E.M.

forskolin-stimulated and the receptor-mediated cAMP generations.

In order to investigate the protein kinase C isoforms involved in the PMA-mediated response, we determined which isoforms of protein kinase C were expressed in these cells by Western blotting with isoform-specific antibodies. Every antibody recognized individual protein kinase C isoforms in the lysate of rat brain as the positive control (Fig. 7A). The antibodies against the protein kinase C-α, -β, -ε, -ζ, or -λ isoforms also detected immunoreactive proteins in HL-60 cell lysates, indicating that these isoforms are expressed in HL-60 cells. However, protein kinase C-δ, -η and -θ isoforms did not appear to be expressed at significant levels, since the corresponding antibodies failed to detect these proteins in the same lysates (Fig. 7A). The distribution of the expressed protein kinase C isoforms between the soluble/cytosolic and the particulate/membrane fractions was also determined. In untreated cells, all protein kinase C isoforms were detected predominantly in the cytosolic fraction (Fig. 7B). However, treatment of the cells with PMA induced translocation of cytosolic protein kinase C isoforms (i.e., α, β and ε) to the particulate/membrane fraction. The distribution of atypical protein kinase C, such as ζ, λ and ι, were not affected by the treatment with PMA (Fig. 7B). These data suggest that the specific translocation of classical protein kinase C (α and β) and/or protein kinase C-ε is involved in the regulation of the adenylyl cyclase.

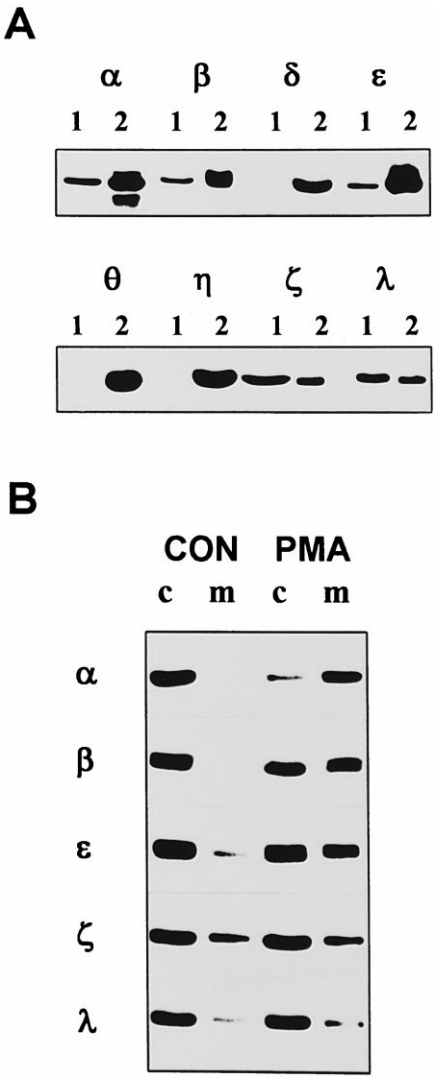

Fig. 7. Distribution of protein kinase C isoforms in HL-60 cells. (A) Total lysates (30 μg) extracted from HL-60 cells were separated by 8% SDS-PAGE, transferred to a nitrocellulose membrane, and reacted with isoform-specific antibodies (lanes 1). Rat brain extracts were used as positive controls (lanes 2). (B) HL-60 cells were treated with vehicle alone (CON, DMSO) or 200 nM of PMA for 10 min. The cells were fractionated and the lysates (30 μg) were used in immunoblotting with protein kinase C isoform-specific antibodies. Soluble/cytosolic fraction (c), particulate/membrane fraction (m).

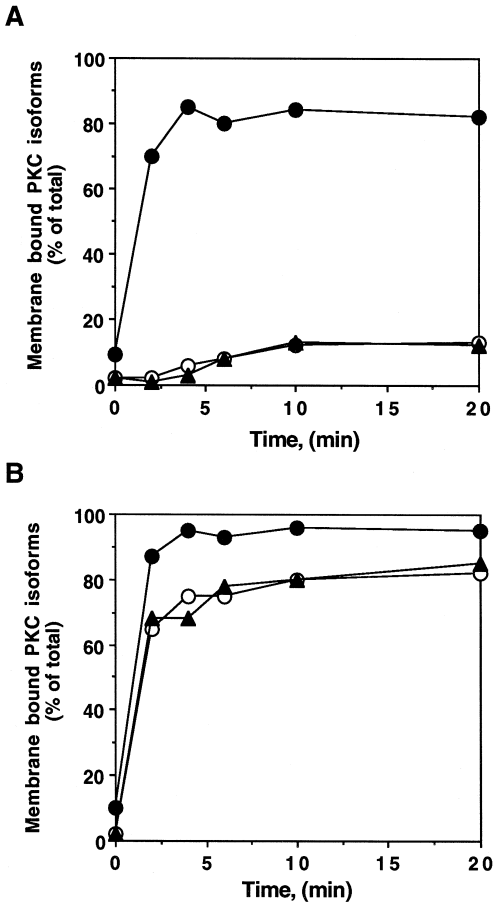

Fig. 8. Time-course of protein kinase C translocation. HL-60 cells were treated with 10 (A) or 100 nM (B) PMA for the indicated time periods. The cells were fractionated into cytosolic and particulate/membrane fractions and the distribution of protein kinase isoforms was determined by Western blotting. Relative amounts of protein kinase C isoforms were quantified by densitometry. Protein kinase C-α (○), -β (▲), and -ε (●) were presented. The data represent results of a typical experiment conducted three times with comparable results.

The time-course of the PMA-induced protein kinase translocation (Fig. 8A) shows that treatment with 10 nM PMA induced a translocation of ~70% of protein kinase C-ε within 2 min, whereas it had little effect on the

translocation of protein kinase C-α and -β up to 20 min. However, 100 nM PMA treatment rapidly translocated all the isozymes of protein kinase C within 2 min (Fig. 8B). The results indicate that the each isozyme of protein kinase C is differentially responsive to low PMA concentrations. Fig. 9A demonstrates that 10 nM PMA had little stimulatory effect on cAMP production up to 10 min after stimulation, while 100 nM PMA significantly elevated the cAMP level. In contrast, 10 nM PMA added simultaneously with histamine rapidly decreased the histamine-induced cAMP generation (Fig. 9B). These results suggest that different isozymes of protein kinase C are participating in the dual effect of PMA on cAMP production, and that the inhibition of the receptor-mediated cAMP generation induced by PMA (10 nM) results from the action of protein kinase C-ε in the cells.

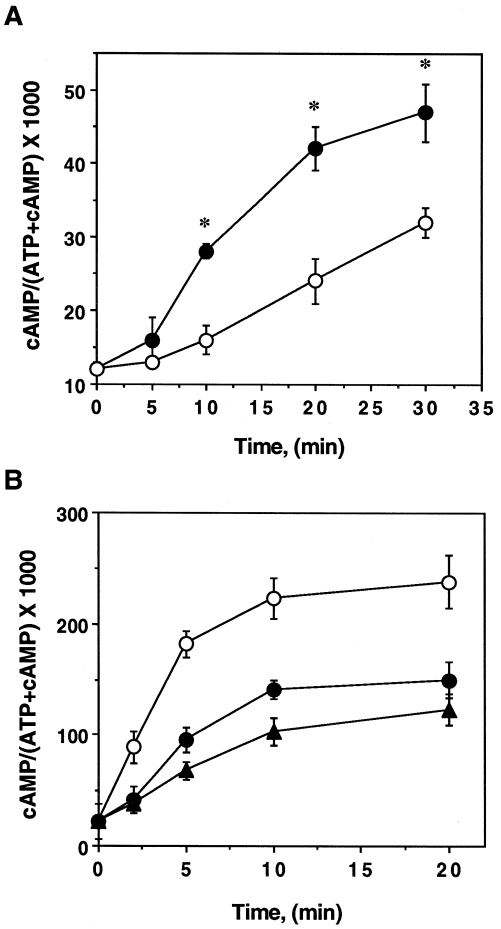

Fig. 9. Effect of PMA on cAMP levels. (A) [^{3}H]Adenine-loaded cells were treated with 10 (○) or 100 nM (●) PMA for the indicated time periods. (B) [^{3}H]Adenine-loaded cells were stimulated with 100 μM histamine alone (○), or with 10 (●) or 100 nM (▲) PMA plus histamine in the presence of 1 mM IBMX for the indicated time. The reaction was stopped by the addition of 5% (w/v) trichloroacetic acid containing 1 μM unlabeled cAMP. The [^{3}H]cAMP generation was measured as described in Section 2. The experiments were done three times in duplicate and each point is the mean ± S.E.M. $* P < 0.05$, compared to the 10 nM PMA-untreated.

4. Discussion

In the present study, we describe in detail the mechanisms by which protein kinase C accomplishes a dual, opposite regulation of the cAMP signaling cascade in HL-60 cells. The results show that protein kinase C activated, after a short exposure to the phorbol ester PMA directly activates adenylyl cyclase and synergistically enhances the forskolin- or G protein-stimulated cAMP accumulation, while it inhibits histamine-, prostaglandin E_2-, and isoproterenol-induced responses. Another protein kinase C activator, PDBu, but not 4-α-PMA which is an inactive agonist for protein kinase C stimulation, could also stimulate cAMP generation (data not shown). However, exposure of cells to GF 109203X, a protein kinase C inhibitor, almost completely reversed the dual effects of the phorbol esters, indicating that the actions of PMA are mediated through the activation of protein kinase C.

It is generally accepted that multiple protein kinase C isozymes are responsible for different specialized physiological processes and that many cell types express multiple protein kinase C isozymes (Nishizuka, 1995). Presently, 11 isozymes of protein kinase C have been identified in mammalian tissues. These isozymes can be divided into four groups based on their mechanism of activation: Ca^{2+}-dependent classical or conventional protein kinase C-α, -β and -γ; Ca^{2+}-independent novel protein kinase C-δ, -ε, -θ and -η; atypical protein kinase C-ζ and -λ; and protein kinase C-μ. In this study, we have shown that, among the expressed multiple protein kinase C isozymes, it the classical protein kinase C-α, -β, and the novel protein kinase C-ε, but not the atypical protein kinase Cs that are specifically activated by PMA treatment, as determined by the translocation of cytosolic protein kinase C to the membrane fraction. However, in experiments using the low concentration of PMA (10 nM), protein kinase C-ε, but not protein kinase C-α and -β was selectively translocated within a short time (< 5 min). The result was consistent with the inhibitory effect of PMA (10 nM), since the inhibition of histamine-induced cAMP generation was also rapidly and significantly detectable 5 min after stimulation (Fig. 9B). On the other hand, the stimulatory effect of 10 nM PMA on cAMP generation seems to match the translocation of classical protein kinase C-α and -β, but the time course of cAMP generation and protein kinase C translocation mediated by high concentration of PMA (100 nM) indicates that the cAMP generation (Fig. 1B) was much slower than the protein kinase C (i.e., α and β) translocation (Fig. 9A). The PMA-activated cAMP generation needs to be characterized in more detail. Taken together, these results suggest that the PMA-mediated actions on cAMP signal transduction might result from selective activation of the protein kinase C isozymes and that protein kinase C-ε could be specifically involved in the inhibition of receptor-mediated cAMP generation induced by PMA treatment.

Protein kinase C reportedly interacts with the cAMP signaling pathway at various levels. Our experiments with HL-60 cells show that protein kinase C activation leads to a direct increase of the basal cAMP level and the potentiation of the cAMP cascade when G protein is activated. In addition, protein kinase C enhanced the adenylyl cyclase activity in membranes obtained from PMA-treated cells (data not shown), supporting the idea that the kinase effects a direct activation of the adenylyl cyclase catalytic domain rather than an increase in the rate of the enzyme synthesis or a decrease in the hydrolysis of the cyclic nucleotide. At present, 10 distinct mammalian forms of adenylyl cyclase have been cloned, each exhibiting different regulatory properties and unequal expression in various cell types (Sunahara et al., 1996). Only some of these isoforms are substrates for protein kinase C (Jacobowitz and Iyengar, 1994; Yoshimura and Cooper, 1993). However, the mechanism by which protein kinase C regulates the adenylyl cyclase activity is controversial. It has been shown that protein kinase C induces the phosphorylation of adenylyl cyclase in an isoenzyme-specific manner (Kawabe et al., 1994) and that phosphorylation by protein kinase C is accompanied by a potentiation of its catalytic activity (Yoshimasa et al., 1987). The cAMP elevating effect of PMA in HL-60 cells is consistent with that observed in transiently transfected embryonic kidney 293 cells with type II adenylyl cyclase (Yoshimura and Cooper, 1993) or in S49 mouse lymphoma cells with type VII adenylyl cyclase (Watson et al., 1994), in which phorbol esters increased both basal activity and forskolin-stimulated adenylyl cyclase activity. In other studies, the stimulation of adenylyl cyclase may have been the result of protein kinase C-mediated inhibition of the inhibitory G protein, G_i (Gordeladze et al., 1989), enhancing the hormone-induced cAMP generation by relieving the inhibitory tonus of G_i on adenylyl cyclase. However, it is unlikely that the inhibitory G protein is involved in protein kinase C-induced activation of adenylyl cyclase in HL-60 cells, because the basal adenylyl cyclase activity could be elevated without receptor activation in the membrane preparation from PMA-treated cells. Furthermore, a 12 h-treatment of the cells with pertussis toxin (200 ng/ml), which blocked the actions of $G_{i/o}$ proteins by catalyzing the ADP-ribosylation of $\alpha_{i/o}$ subunits (Simon et al., 1991), did not affect the PMA-induced and forskolin-stimulated cAMP generation (data not shown). Therefore, the convergence of cAMP signal transduction and protein kinase C depends on the expression of specific isozymes of adenylyl cyclase. Although the pattern of expression of adenylyl cyclase isoenzymes in HL-60 is still unknown, the regulatory effect of PMA on adenylyl cyclase activity in HL-60 cells is consistent with the results obtained from the protein kinase C-activated adenylyl cyclases type II and type VII (Yoshimura and Cooper, 1993; Watson et al., 1994).

The decrease in receptor-mediated cAMP synthesis by protein kinase C activation does not appear to be due to an alteration in the activity of the receptors. Saturation binding studies indicate that the inhibitory effect of PMA on histamine-stimulated cAMP generation does not result from a decrease in the binding affinity or the binding sites of the receptors (Fig. 5). These data imply that protein kinase C acts on the coupling process between the receptor and G proteins, since the downstream signaling from G protein to adenylyl cyclase was not inhibited by protein kinase C activation. This conclusion is supported by previous observations of phorbol esters inhibiting hormone-stimulated adenylyl cyclase by acting on the level of the hormone receptor, although the exact mechanism is not clear. For example, in turkey erythrocytes, activation of protein kinase C results in the phosphorylation of the β-adrenoceptor and promotes uncoupling of the receptor from the stimulatory guanine nucleotide regulatory protein (Nambi et al., 1985). In cultured collecting tubular cells, PMA inhibited arginine vasopressin-stimulated adenylyl cyclase activity, presumably by the inhibition of the vasopressin receptor or of the coupling of the receptor to the G_s protein (Dixon et al., 1988). Other studies have also contributed evidence that homologous desensitization of hormone receptors results from receptor uncoupling rather than from a decrease in the receptor number or from receptor sequestration at least within a few minutes after the exposure of the cells to the agonist (Lefkowitz, 1993).

In previous studies, activation of the multiple subtypes of protein kinase C by PMA or diacylglycerol, the endogenous product of PLC activation, resulted in the phosphorylation of substrate proteins at the terminal consensus sequences (Kennelly and Krebs, 1991). It has been suggested that phosphorylation of these consensus sequences, found in the third intracellular domains of the β-adrenoceptor, accounts for the action that reduces receptor potency during acute PMA treatment (Yuan et al., 1994). The histamine H_2 receptors also contain in the corresponding region a consensus phosphorylation site for protein kinase C (Gantz et al., 1991). Likewise, although the site of action of protein kinase C has not been identified in prostaglandin receptors, a rapid uncoupling of these receptors from adenylyl cyclase activation may also occur after the addition of PMA. Bos et al. (1991) and Freyaldenhoven et al. (1992) showed that transient treatment with phorbol ester attenuated the prostaglandin E_2-stimulated cAMP accumulation via an effect on a different site of the adenylyl cyclase complex, while it potentiated the parathyroid hormone-, forskolin- and cholera toxin-induced cAMP generation in rat osteoblasts. In the cells, however, PMA treatment did not cause changes in receptor affinity or receptor numbers (Bos et al., 1995). Mitsuhashi and Payan (1988) also reported that the binding affinity and, up to 1 h after PMA treatment, the binding sites for histamine were not affected in cultured DDT_1MF-2 smooth muscle cells. Our experiments with HL-60 cells show that PMA exposure for 30 min does not affect the [^{3}H]histamine binding number and K_d. The results imply that the uncoupling

between the receptor and the stimulatory guanine nucleotide protein is induced by the acute activation of protein kinase C in a manner that does not effect agonist binding to the receptor.

PMA is a cell-permeable direct activator of protein kinase C which bypasses cell surface receptor signaling that leads to protein kinase activation. Therefore, the same effect obtained by treating cells with PMA can also be obtained by receptor activation. To investigate this aspect, we stimulated the cells with fMLP, a compound known to activate protein kinase C in neutrophils (Dang et al., 1995). Treatment of DMSO-differentiated HL-60 cells with fMLP decreased the histamine-stimulated cAMP generation by ~40% (data not shown). However, fMLP treatment did not affect forskolin- and GTPγS-induced cAMP generation. The discrepancy between the effects of fMLP and PMA may be due to difference in efficaciousness with regard to the translocation and the duration of the activation of protein kinase C isozymes (tsao and Wang, 1997).

In conclusion, our data show for the first time that protein kinase C plays a role of dual modulation in the receptor-mediated cAMP cascade in intact cells. The physiologically relevant cross-talk between cAMP signal transduction and protein kinase C also implies that the protein kinase C pathway can affect the process of proliferation and/or differentiation of human myeloid progenitor cells, since the cAMP in HL-60 cells is linked to the cells' differentiation to neutrophils (Nonaka et al., 1992).

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References

Bos, M.P., van Leeuwen, J.P., Herrmann-Erlee, M.P., 1991. Modulation of responsiveness of cAMP stimulating agonists by phorbol ester in fetal rat osteoblasts. J. Cell. Physiol. 147, 87–92.

Bos, M.P., Van der Meer, J.M., Feyen, J.H., Herrmann-Erlee, M.P., 1995. Down-regulation and differential restoration of cAMP responses upon transient phorbol ester treatment of primary osteoblastic cells. Cell. Signal. 7, 617–626.

Bressler, J.P., Tinsely, P., 1990. Regulation of cAMP levels by protein kinase C in C6 rat glioma cells. J. Neurosci. Res. 25, 81–86.

Bushkin, I., Roth, J., Heffetz, D., Zick, Y., 1991. pp75: a novel tyrosine-phosphorylated protein that heralds differentiation of HL-60 cells. J. Biol. Chem. 266, 11890–11895.

Chun, J.S., Ha, M.-J., Jacobson, B.S., 1996. Differential translocation of protein kinase Cε during HeLa cell adhesion to a gelatin substratum. J. Biol. Chem. 271, 13008–13012.

Dang, P.M., Rais, S., Hakim, J., Perianin, A., 1995. Redistribution of protein kinase C isoforms in human neutrophils stimulated by formyl peptides and phorbol myristate acetate. Biochem. Biophys. Res. Commun. 212, 664–672.

Deshpande, R.V., Peterson, R.H., Moore, M.A., 1997. Granulocyte colony-stimulating factor-induced activation of protein kinase-C in myeloid cells. J. Cell. Biochem. 66, 286–296.

Dixon, B.S., Breckon, R., Burke, C., Anderson, R.J., 1988. Phorbol esters inhibit adenylate cyclase activity in cultured collecting tubular cells. Am. J. Physiol. 254, C183–C191.

Freyaldenhoven, A.M., Gutierrez, G.E., Lifschitz, M.D., Katz, M.S., 1992. Protein kinase C differentially modulates PTH- and PGE_2-sensitive adenylyl cyclase in osteoblast-like cells. Am. J. Physiol. 262, E87–E95.

Fukushima, Y., Asano, T., Katagiri, H., Aihara, M., Saitoh, T., Anai, M., Funaki, M., Ogihara, T., Inukai, K., Matsuhashi, N., Oka, Y., Yazaki, Y., Sugano, K., 1996. Interaction between the two signal transduction systems of the histamine H_2 receptor: desensitizing and sensitizing effects of histamine stimulation on histamine-dependent cAMP production in Chinese hamster ovary cells. Biochem. J. 320, 27–32.

Gantz, I., Schaffer, M., DelValle, J., Logsdon, C., Campbell, V., Uhler, M., Yamada, T., 1991. Molecular cloning of a gene encoding the histamine H_2 receptor. Proc. Natl. Acad. Sci. USA 88, 429–433.

Gordeladze, J.O., Bjoro, T., Torjesen, P.A., Ostberg, B.C., Haug, E., Gautvik, K.M., 1989. Protein kinase C stimulates adenylate cyclase activity in prolactin-secreting rat adenoma (GH_4C_1) pituicytes by inactivating the inhibitory GTP-binding protein G_i. Eur. J. Biochem. 183, 397–406.

Hernandez-Sotomayor, S.M.T., Macias-Silva, M., Malbon, C.C., Garcia-Sainz, J.A., 1991. Modulation of G_s activity by phorbol myristate acetate in rat hepatocytes. Am. J. Physiol. 260, C259–C265.

Houslay, M.D., 1991. Crosstalk: a pivotal role for protein kinase C in modulating relationships between signal transduction pathways. Eur. J. Biochem. 195, 9–27.

Huang, Z.H., Hii, C.S.T., Rathjen, D.A., Poulos, A., Murray, A.W., Ferrante, A., 1997. N-6 and n-3 polyunsaturated fatty acids stimulate translocation of protein kinase Cα, -βI, -βII and -ε and enhance agonist-induced NADPH oxidase in macrophages. Biochem. J. 325, 553–557.

Ikeda, K., Sugimoto, T., Fukase, M., Fujita, T., 1991. Phorbol ester induces desensitization of PTH-stimulated cyclic AMP production by decresing the PTH receptor binding in UMR-106 cells. Biochem. Biophys. Res. Commun. 176, 764–768.

Inada, M., Koyama, H., Hino, M., Okuno, S., Terada, M., Nishizawa, Y., Nishino, T., Morii, H., 1993. Regulation of release of hepatocyte growth factor from human promyelocytic leukemia cell, HL-60, by 1,25-dihydroxyvitamin D3, 12-O-tetradecanoylphorbol 13-acetate, and dibutyryl cyclic adenosine monophosphate. Blood 82, 53–59.

Jacobowitz, O., Iyengar, R., 1994. Phorbol ester-induced stimulation and phosphorylation of adenylyl cyclase 2. Proc. Natl. Acad. Sci. USA 91, 10630–10634.

Kawabe, J., Iwami, G., Ebina, T., Ohno, S., Katada, T., Ueda, Y., Homcy, C.J., Ishikawa, Y., 1994. Differential activation of adenylyl cyclase by protein kinase C isoenzymes. J. Biol. Chem. 269, 16554–16558.

Kennelly, P.J., Krebs, E.G., 1991. Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. J. Biol. Chem. 266, 15555–15558.

Klinker, J.F., Wenzel-Seifert, K., Seifert, R., 1996. G-protein-coupled receptors in HL-60 human leukemia cells. Gen. Pharmacol. 27, 33–54.

Kotecha, S., Wilson, L., Sutcliffe, S., Wangoo, A., Shaw, R.J., 1993. Pharmacological modulation of c-fos mRNA expression in the HL60 and U937 cell lines. Pulm. Pharmacol. 6, 269–277.

Lefkowitz, R.J., 1993. G protein-coupled receptor kinases. Cell 74, 409–412.

Mitsuhashi, M., Payan, D.G., 1988. Phorbol ester-mediated desensitiza-

tion of histamine H_1 receptors on a cultured smooth muscle cell line. Life Sci. 43, 1433–1440.

Mitsuhashi, M., Mitsuhashi, T., Payan, D.G., 1989. Multiple signaling pathways of histamine H_2 receptors. J. Biol. Chem. 264, 18356–18362.

Morimoto, B.H., Koshland Jr., D.E., 1994. Conditional activation of cAMP signal transduction by protein kinase C. J. Biol. Chem. 269, 4064–4069.

Nambi, P., Peters, J.R., Sibley, D.R., Lefkowitz, R.J., 1985. Desensitization of the turkey erythrocyte β-adrenergic receptor in a cell-free system. J. Biol. Chem. 260, 2163–2171.

Nishizuka, Y., 1995. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J. 9, 484–496.

Nonaka, T., Mio, M., Doi, M., Tasaka, K., 1992. Histamine-induced differentiation of HL-60 cells. The role of cAMP and protein kinase A. Biochem. Pharmacol. 44, 1115–1121.

Regan, J.W., Bailey, T.J., Pepperl, D.J., Pierce, K.L., Bogardus, A.M., Donello, J.E., Fairbairn, C.E., Kedzie, K.M., Woodward, D.F., Gil, D.W., 1994. Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP_2 subtype. Mol. Pharmacol. 46, 213–220.

Sager, G., Bang, B.E., Pedersen, M., Aarbakke, J., 1988. The human promyelocytic leukemia cell (HL-60 cell) β-adrenergic receptor. J. Leukocyte Biol. 44, 41–45.

Salomon, Y., Londos, C., Rodbell, M., 1974. A highly sensitive adenylate cyclase assay. Anal. Biochem. 58, 541–548.

Simon, M.I., Strathmann, M.P., Gautam, N., 1991. Diversity of G proteins in signal transduction. Science 252, 802–808.

Slungaard, A., Confer, D.L., Schubach, W.H., 1987. Rapid transcriptional down-regulation of c-myc expression during cyclic adenosine monophosphate-promoted differentiation of leukemia cells. J. Clin. Invest. 79, 1542–1547.

Suh, B.C., Kim, K.T., 1995. Stimulation of adenylyl cyclase mediated by phospholipase C-linked M_3 muscarinic receptor in human neuroblastoma SK-N-BE(2)C cells. J. Neurochem. 64, 2500–2508.

Suh, B.C., Park, T.J., Kim, K.T., 1996. Synergistic activation of adenylyl cyclase is dependent upon phospholipase C-mediated processes in human neuroblastoma SK-N-BE(2)C cells. Eur. J. Pharmacol. 314, 235–242.

Sunahara, R.K., Dessauer, C.W., Gilman, A.G., 1996. Complexity and diversity of mammalian adenylyl cyclase. Annu. Rev. Pharmacol. Toxicol. 36, 461–480.

Tsao, L.T., Wang, J.P., 1997. Translocation of protein kinase C isoforms in rat neutrophils. Biochem. Biophys. Res. Commun. 234, 412–418.

Watson, P.A., Krupinski, J., Kempinski, A.M., Frankenfield, C.D., 1994. Molecular cloning and characterization of the type VII isoform of mammalian adenylyl cyclase expressed widely in mouse tissues and in S49 mouse lymphoma cells. J. Biol. Chem. 269, 28893–28898.

Ydrenius, L., Molony, L., Ng-Sikorski, J., Andersson, T., 1997. Dual action of cAMP-dependent protein kinase on granulocyte movement. Biochem. Biophys. Res. Commun. 235, 445–450.

Yoshimasa, T., Sibley, D.R., Bouvier, M., Lefkowitz, R.J., Caron, M.G., 1987. Cross-talk between cellular signalling pathways suggested by phorbol-ester-induced adenylate cyclase phosphorylation. Nature 327, 67–70.

Yoshimura, M., Cooper, D.M.F., 1993. Type-specific stimulation of adenylyl cyclase by protein kinase C. J. Biol. Chem. 268, 4604–4607.

Yu, H., Suchard, S.J., Nairn, R., Jove, R., 1995. Dissociation of mitogen-activated protein kinase activation from the oxidative burst in differentiated HL-60 cells and human neutrophils. J. Biol. Chem. 270, 15719–15724.

Yuan, N., Friedman, J., Whaley, B.S., Clark, R.B., 1994. cAMP-dependent protein kinase and protein kinase C consensus site mutations of the β_2-adrenergic receptor. J. Biol. Chem. 269, 23032–23038.