Biochemical Pharmacology, Vol. 56, pp. 561–567, 1998.



ISSN 0006-2952/98/$19.00 + 0.00
PII S006-2952(97)00660-6

# Opposing Effects of Protein Kinase A and C on Capacitative Calcium Entry into HL-60 Promyelocytes

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**ABSTRACT.** Treatment of HL-60 cells with thapsigargin, a microsomal $Ca^{2+}$/ATPase inhibitor, led to depletion of intracellular calcium stores followed by capacitative calcium entry. Stimulation of adenylyl cyclase with forskolin enhanced thapsigargin-induced $Ca^{2+}$ influx. The forskolin effect was confirmed by enhanced fluorescence quenching induced by $Mn^{2+}$ entry into fura-2 loaded cells. 1,9-Dideoxy-forskolin, an inactive analog of forskolin, did not affect capacitative calcium entry. On the other hand, phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, inhibited thapsigargin-induced $Ca^{2+}$ entry. Histamine and prostaglandin $E_2$ ($PGE_2$) elevated intracellular adenosine 3′:5′-cyclic monophosphate (cAMP) levels and enhanced the thapsigargin-induced capacitative calcium entry. Incubation with *N*-[2-(*p*-bromocynnamylamino)ethyl]-5-isoquinolinesulfonamide (H89), an inhibitor of protein kinase A (PKA), blocked the forskolin effect, and GF109203X, an inhibitor of protein kinase C (PKC), blocked the phorbol 12-myristate 13-acetate effect. The results suggest that protein kinase A regulates capacitative calcium entry positively, but that protein kinase C regulates $Ca^{2+}$ influx negatively. Furthermore, after differentiation of HL-60 promyelocytes with dimethylsulfoxide to granulocytes, the inhibitory effect of phorbol 12-myristate 13-acetate became more pronounced, whereas the stimulatory effect of prostaglandin $E_2$ did not change. This result suggests that the regulation of capacitative calcium entry by protein kinase C and protein kinase A develops differently during differentiation. BIOCHEM PHARMACOL **56**;5:561–567, 1998. © 1998 Elsevier Science Inc.

**KEY WORDS.** protein kinase A; protein kinase C; capacitative calcium entry; HL-60 cells

Elevation of cytosolic $IP_3$† levels after stimulation of receptors linked to the phosphoinositide-signaling pathway triggers $Ca^{2+}$ release from intracellular calcium stores. The depletion of these calcium stores induces calcium influx through CRACs in the plasma membrane, thereby refilling the empty calcium stores [1–3]. This mechanism has been termed "capacitative calcium entry." The relation between calcium stores and CRAC thus suggests the involvement of a diffusable messenger [4, 5].

Little is known about the regulatory mechanism of capacitative calcium entry, but the involvement of cytosolic factors has been reported. A recent study of Jurkat leukemic T lymphocytes revealed that a feedback effect of $Ca^{2+}$ inhibits CRAC [6]. It has also been suggested that tyrosine kinase and phosphatase [7, 8] as well as intracellular adenine-guanine nucleotide levels [9] regulate capacitative calcium entry. In addition, small GTP-binding protein (G-protein) has been implicated in capacitative calcium entry into rat basophil leukemia cells [10] and mouse lachrymal acinar cells [11]. Moreover, heterotrimeric G-protein is reported to be involved in the regulation of CRAC in HL-60 granulocytes [12] and *Xenopus* oocytes [13, 14] with a direct or indirect mode of action. Indirect regulation of CRAC by G-protein occurs via PKC and/or PKA, activated following stimulation of PLC and/or adenylyl cyclase. Several reports propose that PKC regulates capacitative calcium entry. PKC inhibits capacitative calcium entry into HL-60 cells and human neutrophils [15–17], whereas PKC activates calcium entry into the insulin-secreting cell line RINm5F [18]. However, the regulation of CRAC via PKA has not yet been studied extensively. Here we report that PKC and PKA regulate capacitative calcium entry into HL-60 cells in an opposite manner. Whereas PKA potentiates thapsigargin-stimulated $Ca^{2+}$ influx, PKC inhibits it.

HL-60 promyelocytes can be made to differentiate into granulocytes by culturing with DMSO for 5–7 days. Differentiated HL-60 cells show similar morphology and behavior with neutrophils [19, 20]. Montero *et al.* [16] have reported that inhibition of thapsigargin-induced $Ca^{2+}$ influx by PDBu, an activator of PKC, developed gradually along with

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† *Abbreviations:* cAMP, adenosine 3′:5′-cyclic monophosphate; $[Ca^{2+}]_i$, cytosolic calcium concentration; CRAC, calcium-release-activated calcium channel; G-protein, GTP-binding protein; H89, *N*-[2-(*p*-bromocynnamylamino)ethyl]-5-isoquinolinesulfonamide; $IP_3$, inositol 1,4,5-trisphosphate; $PGE_2$, prostaglandin $E_2$; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; and PMA, phorbol 12-myristate 13-acetate, fura-2/AM.

Received 4 March 1997; accepted 21 October 1997.

the differentiation of HL-60 cells. Here, we also studied the effect of PKA on capacitative calcium entry during differentiation of HL-60 cells, comparing it with the effect of PKC. Unlike the gradual potentiation of the PKC effect, the positive effect of PKA on thapsigargin-induced capacitative calcium entry did not significantly change during the differentiation of HL-60 cells.

## MATERIALS AND METHODS

### *Materials*

RPMI 1640 medium and penicillin-streptomycin were purchased from Life Technologies. Bovine calf serum was obtained from HyClone Laboratories (Logan, UT, U.S.A.). Thapsigargin, DMSO, forskolin, 1,9-dideoxy-forskolin, histamine, $PGE_2$, cAMP, IBMX, GF109203X, sulfinpyrazone, and EGTA were purchased from Sigma. PMA and 4-$\alpha$-PMA were purchased from Research Biochemicals Inc. H89 was purchased from Seikagaku Co. Fura-2/AM was obtained from Molecular Probes.

### *Cell Culture and Differentiation*

HL-60 cells were maintained at 37° in RPMI1640 supplemented with 10% (v/v) heat-inactivated bovine calf serum and 1% (v/v) penicillin-streptomycin in a humidified atmosphere of 95% air and 5% $CO_2$. The culture medium was changed every 2 days. HL-60 cells were induced to differentiate into granulocytes by culturing in growth medium containing 1.25% (v/v) DMSO for 2–6 days [19, 20].

### *Fluorescence Measurements*

The level of intracellular $Ca^{2+}$ was measured using fura-2 acetoxymethyl ester (fura-2/AM) as previously described [21]. Briefly, cell suspensions were incubated in fresh serum-free RPMI 1640 medium with 3 $\mu$M fura-2/AM at 37° for 40 min under continuous stirring. Afterwards, the cells were resuspended in Locke's solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, and 5 mM HEPES buffer adjusted to pH 7.4. Sulfinpyrazone (250 $\mu$M) was added to all solutions to prevent dye leakage. For fluorometric measurement of cytosolic calcium concentrations ($[Ca^{2+}]_i$), $1 \times 10^6$ cells were aliquoted, pelleted, and resuspended in Locke's solution. The aliquot of cells was placed into a quartz cuvette in a thermostatically controlled cell holder at 37°, and the cell suspension was stirred continuously. Changes in fluorescence ratios were measured at the dual excitation wavelengths of 340 nm and 380 nm and the emission wavelength of 500 nm. $[Ca^{2+}]_i$ was determined as described by Grynkiewicz *et al.* [22].

$Mn^{2+}$ influx was measured as fura-2 fluorescence quenching at the single excitation wavelength of 360 nm, which is the isosbestic wavelength, and at the emission wavelength of 500 nm.

### *Measurement of cAMP*

Intracellular cAMP was determined by measuring the formation of [$^3$H]cAMP from [$^3$H]adenine nucleotide pools as we have previously described [23]. Briefly, cells were harvested and aliquoted into half a million cells per tube. The cells were then loaded with [$^3$H]adenine (2 $\mu$Ci/mL) in complete medium for 24 hr. After loading, the cells were washed twice with Locke's solution. The stimulation reaction was stopped by adding 1 mL of ice-cold 5% (v/v) trichloroacetic acid containing 1 $\mu$M unlabeled cAMP. The tubes were left on ice for 30 min to extract the water-soluble components, including cAMP and ATP. After the extraction on ice, the tubes were centrifuged at 15,000 *g* for 10 min to precipitate cell debris. [$^3$H]cAMP and [$^3$H]ATP were separated using sequential chromatography on a Dowex AG50W-X4 (200–400 mesh) cation exchanger and a neutral alumina column. The [$^3$H]ATP fraction was recovered from the Dowex column by elution with 2 mL of distilled water. Then, a sequential elution with 3.5 mL of distilled water was loaded onto the alumina column. The alumina column was eluted with 4 mL of imidazole buffer (0.1 M, pH 7.2) into scintillation vials containing 15 mL of scintillation fluid, and the radioactivity of the [$^3$H]cAMP was measured. The increase in the intracellular cAMP concentration was calculated as $[^3H]cAMP/([^3H]ATP + [^3H]cAMP) \times 10^3$.

### *Statistical Analysis*

All quantitative data were expressed as mean ± SEM. The results were analyzed for differences using unpaired Student's *t*-test. Differences were considered to be significant only for $P < 0.05$.

## RESULTS

In our study of the regulation of capacitative calcium entry, we triggered capacitative calcium entry by thapsigargin treatment. Thapsigargin induces $Ca^{2+}$ release from intracellular stores without generating known second messengers [24]. HL-60 cells were treated with thapsigargin for 10 min in the presence of extracellular $Ca^{2+}$ to completely deplete intracellular calcium stores and to activate capacitative calcium entry. Addition of forskolin, an activator of adenylyl cyclase or of PMA to the thapsigargin-treated cells had different effects (Fig. 1A). Whereas forskolin enhanced thapsigargin-induced capacitative calcium entry, PMA inhibited it. Treatment with forskolin or PMA alone without thapsigargin incubation had little or no effect on the basal level of the $Ca^{2+}$ concentration. Addition of forskolin or PMA after thapsigargin treatment had a dramatic effect on the CRAC-induced $[Ca^{2+}]_i$ level. From the $[Ca^{2+}]_i$ level steadily elevated by thapsigargin, the net change in calcium level induced by forskolin or PMA was 54 ± 2 or 43 ± 3 nM, respectively (Fig. 1B). Addition of DMSO, which is used as vehicle for forskolin and PMA, did not affect

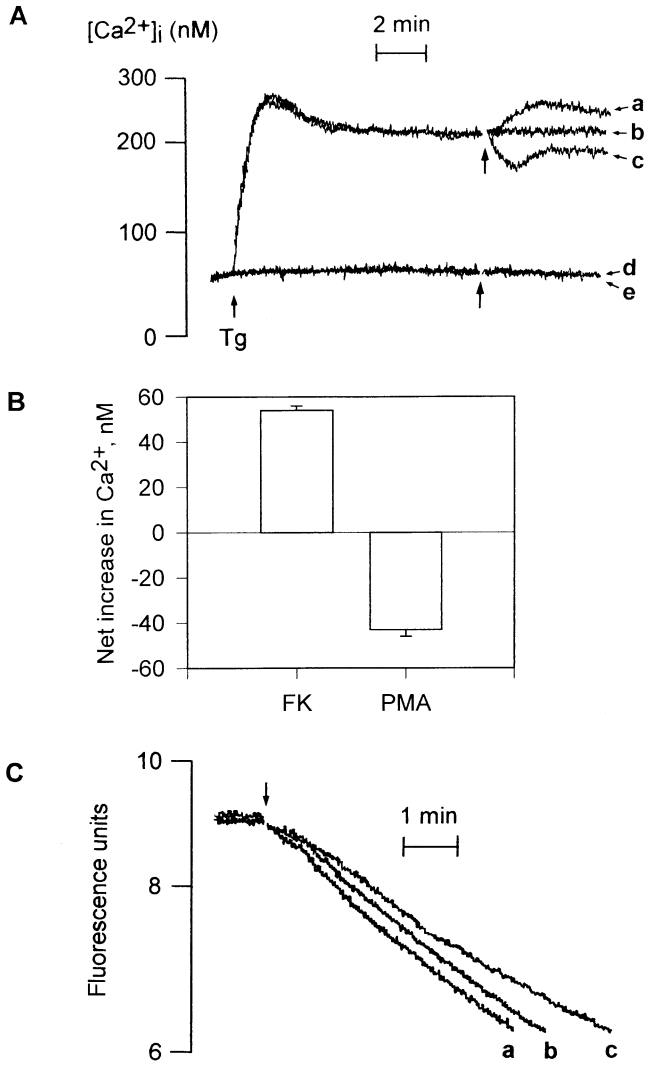


**FIG. 1. Effects of forskolin and PMA on thapsigargin-induced capacitative calcium entry and $Mn^{2+}$ influx. (A) Fura-2/AM-loaded cells were treated with 10 μM forskolin (a); DMSO as a vehicle (b); or 1 μM PMA (c) after preincubation with 1 μM thapsigargin (*Tg*) for 10 min at the time indicated by a bold arrow. In addition, cells were treated with 10 μM forskolin (d) or 1 μM PMA (e) without the preincubation of thapsigargin as a negative control. (B) Experiments were performed as in (A). Forskolin- (FK) or PMA-induced net changes in $[Ca^{2+}]_i$ were obtained from the point of maximum effect in the calcium trace. The net changes in $[Ca^{2+}]_i$ were measured as the steady-state calcium level after incubation with thapsigargin. Data are the mean ± SEM of triplicate measurements. (C) $Mn^{2+}$-induced fura-2 fluorescence quenching was recorded in cells incubated with 1 μM thapsigargin for 10 min. Stimuli were applied as follows: 10 μM forskolin (a); DMSO as vehicle (b); 1 μM PMA (c) in the presence of 1 mM $Mn^{2+}$. All experiments were carried out independently more than three times and the results were reproducible.**

thapsigargin-induced capacitative calcium entry. Enhancement of the capacitative current by the forskolin treatment developed relatively slowly as compared to the inhibitory process set in motion by PMA. 1,9-Dideoxy-forskolin or 4-α-PMA, inactive analogs of forskolin and PMA, respectively, did not affect thapsigargin-induced capacitative calcium entry (data not shown). We then examined the stimuli's effect on $Mn^{2+}$ influx. $Mn^{2+}$ is a good surrogate, since it is not pumped out of the cell, and can thereby be considered a selective tracer for entry. Both $Ca^{2+}$ and $Mn^{2+}$ can enter through CRAC, and we can measure the extent of $Ca^{2+}$ influx by recording the quenching of fura-2 fluorescence after addition of $Mn^{2+}$ to the extracellular medium. While forskolin caused fluorescence quenching by enhancing $Mn^{2+}$ influx, PMA had an inhibitory effect (Fig. 1C). The data suggest that not only PMA but also forskolin can regulate $Ca^{2+}$ influx through CRAC, although both agents do so with opposite effects.

Forskolin is known to elevate cAMP levels. We therefore tested the effects of physiological agonists that elevate cytosolic cAMP. In HL-60 cells and neutrophils, stimulation of prostanoid receptors and histamine $H_2$ receptors increases intracellular cAMP [25]. Treatment of thapsigargin-treated cells with $PGE_2$ or histamine enhanced capacitative calcium entry by 95 ± 6 and 73 ± 5 nM, respectively, as net increases of calcium level (Fig. 2A, B). $PGE_2$ and histamine also enhanced $Mn^{2+}$ fluorescence quenching (Fig. 2C). We thus concluded that a cAMP signaling pathway is positively linked to capacitative calcium entry.

We treated HL-60 cells with various agents to confirm the involvement of cAMP in enhancing capacitative calcium entry. We found, as expected, that forskolin, histamine, and $PGE_2$ increased cAMP level, while PMA increased it to a lesser extent. 1,9-Dideoxy-forskolin and 4-α-PMA did not change cAMP levels (Fig. 3). When comparing the effect of $PGE_2$ and histamine, the extent of enhancement of capacitative calcium entry seems to be related to the extent of elevated cAMP.

PMA is a well-known direct activator of PKC, and elevating cAMP induced by forskolin activates PKA. In order to test for a functional role of protein kinases in the regulation of capacitative calcium entry, thapsigargin-treated cells were incubated with inhibitors of PKC or PKA, after which we examined the effect of forskolin or PMA. We chose H89 or GF109203X as selective inhibitors of PKA or PKC, respectively. Unfortunately, one of the inhibitors, H89, somehow affects the basal $[Ca^{2+}]_i$ level in the range of their effective concentrations. To avoid the problem, we compared the effect of forskolin and of PMA in cells pretreated with each inhibitor. Forskolin could not enhance thapsigargin-induced $Ca^{2+}$ influx after pretreatment of the cells with H89, while PMA still had an inhibitory effect as effective as in untreated control cells (Fig. 4). The stimulatory effect of $PGE_2$ was also attenuated after H89 pretreated cells (data not shown). On the other hand, PMA could not inhibit thapsigargin-induced $Ca^{2+}$ influx after treatment with GF109203X, a PKC inhibitor, while the effect of forskolin was not different in the presence of GF109203X (Fig. 5). The results thus suggest that capacitative calcium entry is positively and negatively regulated by PKA and PKC.

In order to further investigate the regulation of capacitative calcium entry by PKC and PKA, we studied changes

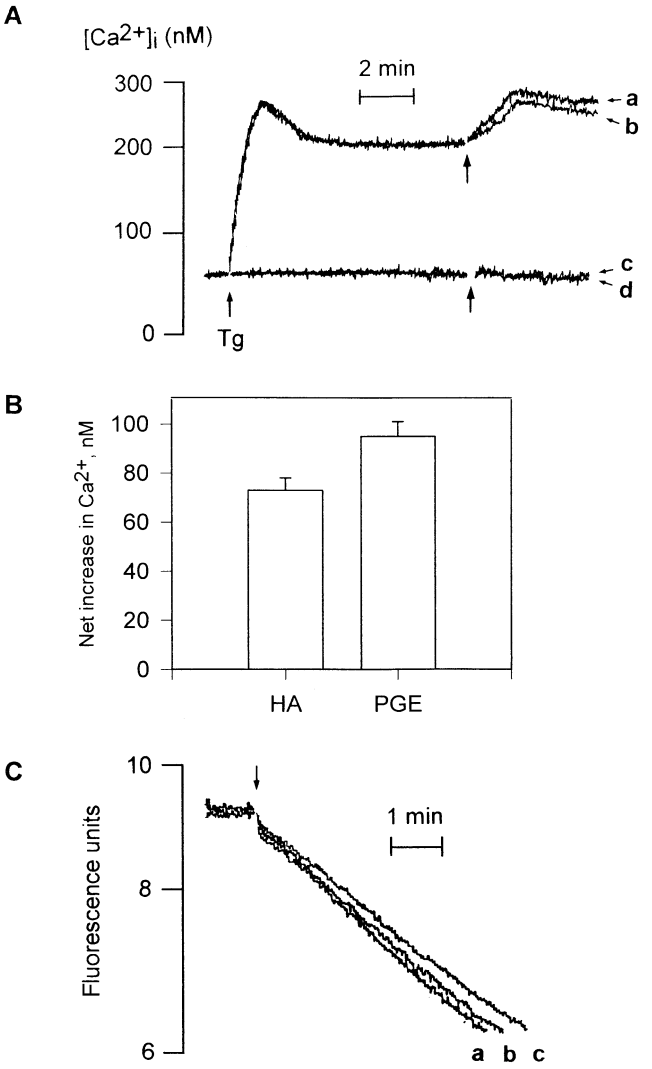


**FIG. 2. Enhancement of thapsigargin-induced capacitative calcium entry and $Mn^{2+}$ influx by histamine or $PGE_2$. (A) Fura-2/AM-loaded cells were treated with 10 μM $PGE_2$ (a) or 30 μM histamine (b) at the time indicated by a bold arrow after incubation with 1 μM thapsigargin (*Tg*) for 10 min. Cells were treated with 10 μM $PGE_2$ (d) or 30 μM histamine (e) without the preincubation of thapsigargin as a negative control. (B) $PGE_2$- or histamine-induced net increases in $[Ca^{2+}]_i$ were obtained from the point of maximum effect in the calcium trace. The net increase in $[Ca^{2+}]_i$ was measured from the steady-state calcium level after incubation with thapsigargin. Data are the mean ± SEM of triplicate measurements. (C) $Mn^{2+}$-induced fura-2 fluorescence quenching was recorded in cells preincubated with 1 μM thapsigargin for 10 min. Treatments were as follows: 10 μM $PGE_2$ (a); 30 μM histamine (b); DMSO in the presence of 1 mM $Mn^{2+}$ (c).**

in the effects during differentiation of HL-60 cells. As cells differentiated into granulocytes after incubation with DMSO, a longer time was required to reach the peak and steady-state levels of intracellular calcium after treatment with thapsigargin as seen in Fig. 6. In this experiment, $PGE_2$ was used to trigger the cAMP signaling pathway instead of forskolin, because the stimulatory effect of $PGE_2$ on capacitative calcium entry was more significant than

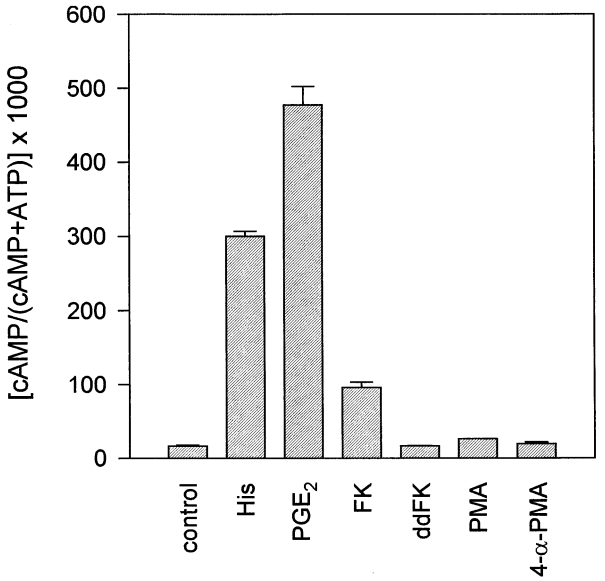


**FIG. 3. Increase in intracellular cAMP level stimulated by agonists that enhance thapsigargin-induced capacitative calcium entry. [$^3$H]Adenine-loaded cells were preincubated with 1 mM IBMX for 15 min, and then stimulated with 30 μM histamine (His), 10 μM $PGE_2$, 10 μM forskolin (FK), 10 μM 1,9-dideoxy-forskolin (ddFK), 1 μM PMA, or 1 μM 4-α-PMA. The cAMP level was measured as described in Materials and Methods. Data are the mean ± SEM of triplicate measurements.**

that of forskolin (compare the effects in Figs. 1 and 2). It was apparent that the effect of PMA developed more gradually after differentiation. The effect of $PGE_2$, however, hardly changed, and its effect on the cells at different stages of differentiation was similar (Fig. 6A–D).

## DISCUSSION

In the field of intracellular calcium homeostasis, there is growing awareness of capacitative calcium entry. The great interest in this phenomenon stems not only from its unique mechanism of activation but also from its physiological importance. For example, capacitative calcium entry plays a role in the maintenance of $Ca^{2+}$ oscillation, in the refilling of intracellular calcium stores, in phototransduction in the *Drosophila* photoreceptor, and in modulation of secretion [1]. Unlike other calcium channels, however, the mechanisms involved in regulating capacitative calcium entry are not yet well understood. Recently, G-proteins were suggested as candidates for factors regulating capacitative calcium entry.

G-proteins are known to be implicated in various $Ca^{2+}$ signaling pathways: activation of PLC, regulation of $Ca^{2+}$-dependent responses such as secretion and muscle contraction, and regulation of communication between intracellular $Ca^{2+}$ pools. Several lines of evidence for a profound effect of G-proteins on capacitative calcium entry have been presented, but the results are not compatible. It has been concluded that small G-proteins regulate capacitative calcium entry in rat basophilic leukemia cells [10] and

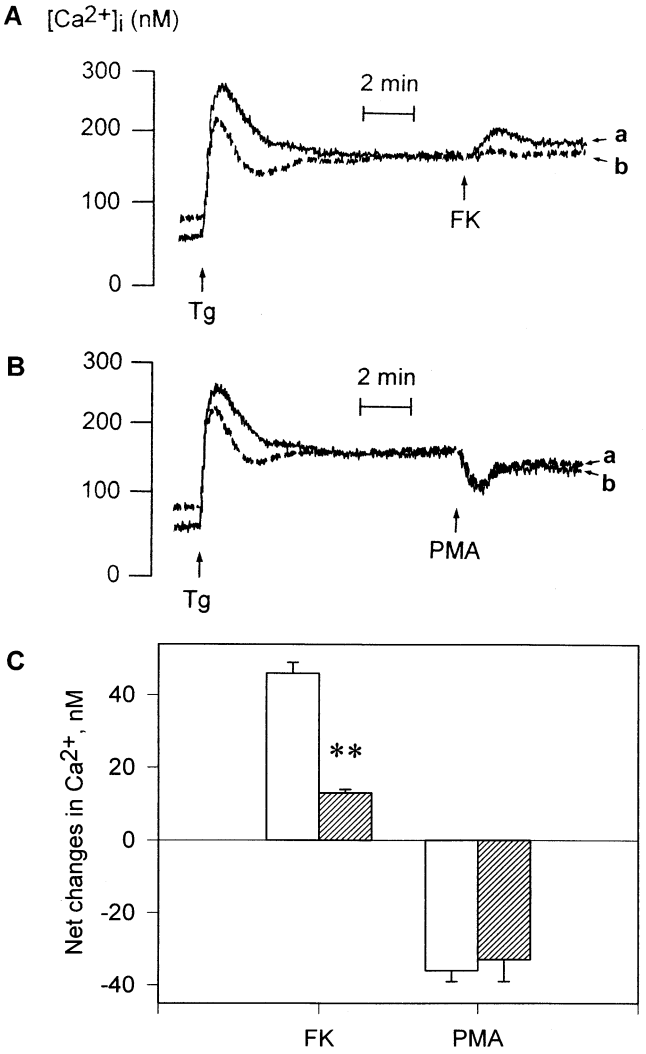


**FIG. 4. Inhibition of the forskolin effect on thapsigargin-induced capacitative calcium entry by H89. (A) After addition of 1 μM thapsigargin (Tg) for 10 min, 10 μM forskolin (FK) was added to the cells with (b) or without (a) preincubation with 10 μM H89 for 10 min. (B) After the 10-min preincubation of 1 μM thapsigargin (Tg), 1 μM PMA was added to the cells with (b) or without (a) preincubation with 10 μM H89 for 10 min. (C) Experiments were performed as in (A). Forskolin- or PMA-induced net increases in $[Ca^{2+}]_i$ were obtained from the maximum effective point in the calcium trace. The net increases in $[Ca^{2+}]_i$ were measured as the steady-state calcium level after incubation with thapsigargin. Data are the mean ± SEM of triplicate measurements. **P < 0.01.**

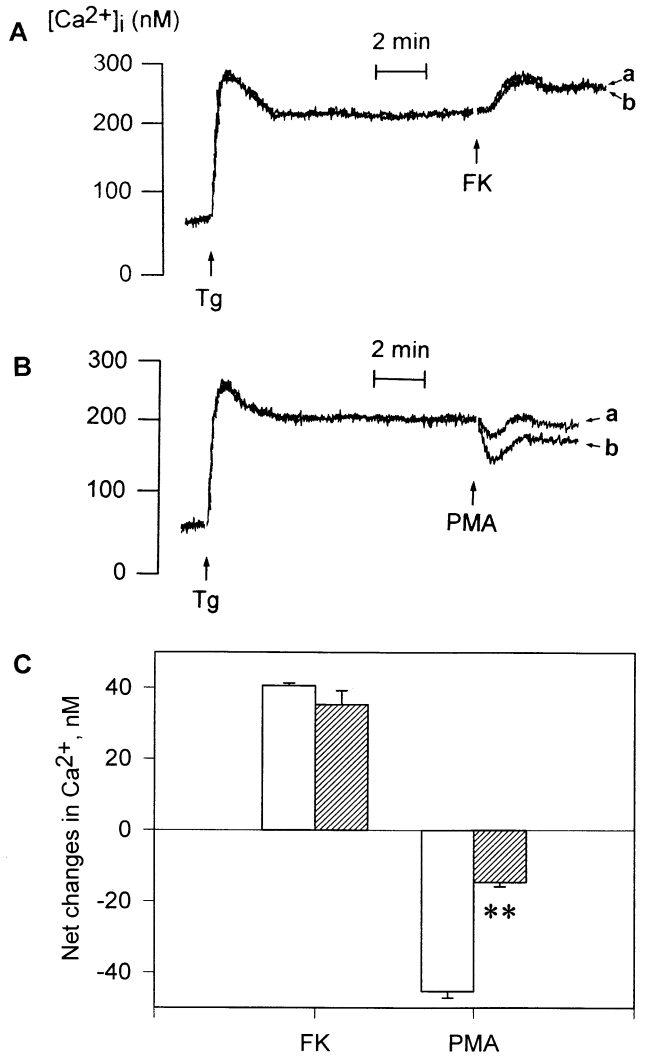


**FIG. 5. Inhibition of the PMA effect on thapsigargin-induced capacitative calcium entry by GF109203X. (A) After addition of 1 μM thapsigargin (Tg) for 10 min, 10 μM forskolin (FK) was added to the cells with (b) or without (a) preincubation with 10 μM GF109203X for 10 min. (B) After the 10-min preincubation of 1 μM thapsigargin (Tg), 1 μM PMA was added to the cells with (b) or without (a) preincubation with 10 μM GF109203X for 10 min. (C) Experiments were performed as in (A). Forskolin- or PMA-induced net increases in $[Ca^{2+}]_i$ were obtained from the maximum effective point in the calcium trace. The net increases in $[Ca^{2+}]_i$ were measured as the steady-state calcium level after incubation with thapsigargin. Data are the mean ± SEM of triplicate measurements. **P < 0.01.**

mouse lachrymal acinar cells [11], and heterotrimeric G-protein in HL-60 granulocytes [12] and *Xenopus* oocytes [13]. Regulation of capacitative calcium entry by heterotrimeric G-protein can be direct or indirect. Indirect regulation is performed by PKC or PKA through activation of PLC or adenylyl cyclase. Although reports of regulation by PKC or PKA seem to support the indirect involvement of G-proteins, we cannot exclude the possibility of a direct role of G-proteins in the regulation of capacitative calcium entry.

The results of the present study reveal that PKA and PKC have opposite regulatory effects on capacitative calcium entry into HL-60 cells. Whereas activation of PKA by treatment with forskolin, histamine, or $PGE_2$ enhances thapsigargin-induced capacitative $Ca^{2+}$ influx, activation of PKC inhibits it. Because the stimulations with histamine and $PGE_2$ clearly show positive regulation of the capacitative calcium entry, the enhancement of CRAC activity by cAMP signaling becomes physiologically relevant. When cells were treated with forskolin and PMA concomitantly, capacitative calcium entry was inhibited to a similar extent as with PMA alone (data not shown). This suggests that

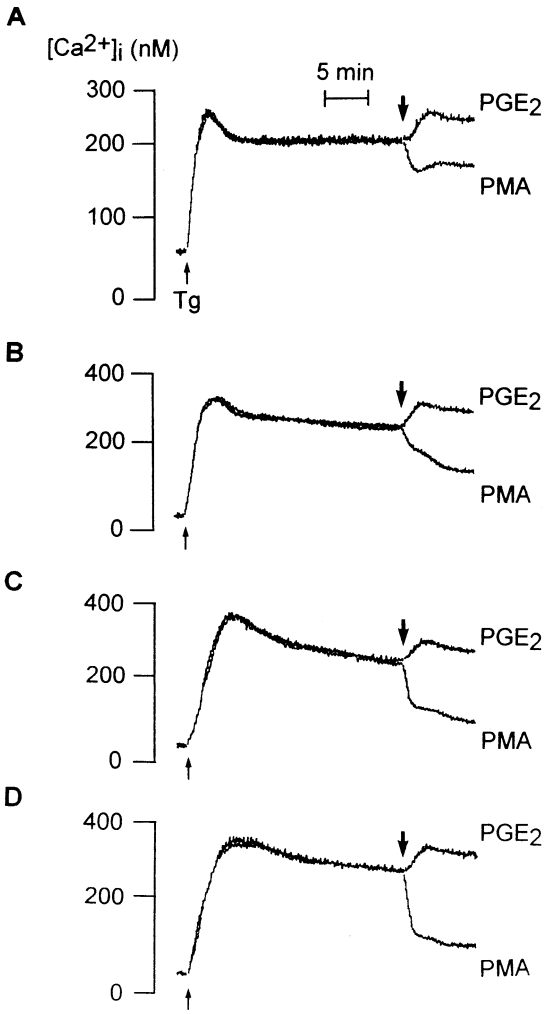


**FIG. 6. Development of the regulation of thapsigargin-induced capacitative calcium entry by PKC and PKA during differentiation of HL-60 cells to granulocytes. Either undifferentiated HL-60 cells (A) or HL-60 cells differentiated after incubation in DMSO for 2 days (B), 4 days (C), and 6 days (D) were loaded with fura-2/AM. The cells were treated with 10 μM $PGE_2$ or 1 μM PMA at the time indicated by a bold arrow after incubation with 1 μM thapsigargin (Tg) for 20 min.**

PKC may exert a more rapid and/or dominant effect on the regulation of CRAC than does PKA, thereby explaining the activation results of G-proteins on the inhibition of capacitative calcium entry in most earlier reports.

Recently, Petersen and Berridge [13,14] showed that both PKC and PKA had a biphasic regulatory effect on the capacitative calcium entry in *Xenopus* oocytes. They demonstrated that activation of PKC initially potentiated capacitative calcium current, which it then gradually inhibited. Also, whereas weak activation of PKA with 1–10 μM dibutyryl cAMP inhibited the current induced by internal $Ca^{2+}$ store depletion after thapsigargin treatment, strong activation of PKA with 1–10 mM dibutyryl cAMP potentiated calcium entry. When we used low and high concentrations of agents such as forskolin and PMA in HL-60 cells, PKA and PKC did not exhibit such biphasic effects as occurred in the *Xenopus* oocyte. This difference in the regulatory modes of PKC and PKA may be real in different cell systems. The opposing regulatory actions of PKA and PKC could provide the cells with an elaborate and highly responsive regulation of capacitative calcium entry in the achievement of calcium homeostasis. Recently, we observed that the elevation of intracellular calcium level elicited by the stimulation of ATP receptors which are coupled to adenylyl cyclase and PLC was modulated in a feedback mode with regulation of capacitative calcium entry by subsequent activation of PKA and PKC [26]. Our present study also suggests that concomitant or sequential activation of various receptors linked to adenylyl cyclase or PLC may very well affect intracellular calcium homeostasis and result in different physiological responses. The experiments performed at different stages of differentiation provide information concerning the development of regulation by PKC and PKA during differentiation. Montero *et al.* [16] reported that the inhibitory effect of PMA on capacitative calcium entry was absent in undifferentiated cells and became significant with differentiation in HL-60 cells. Our results agree with Montero's observation, except that PMA had an inhibitory effect even in undifferentiated cells. This difference in the PMA effect in our undifferentiated cells might be due to clonal differences in the HL-60 cells. An intracellular cAMP elevating reagent such as histamine triggers a rise in PKA activity and induces differentiation of HL-60 cells toward neutrophils [27]. However, our results show that the activation of PKA by $PGE_2$ did not change the extent of the stimulatory effect on capacitative calcium entry during differentiation. This suggests that there may be a limit to the stimulation of capacitative calcium entry by PKA activation inherent in each cell type.

In summary, our results suggest that PKA has a positive effect on capacitative calcium entry and stands opposed to the inhibitory effect of PKC. Our observations also suggest that the effects of PKC and PKA develop differently along the line of differentiation in HL-60 cells.

*We thank Ms. G. Hoschek for editing the manuscript. This work was supported by grants from the Hallym Academy of Sciences, Hallym University, the Korea Science and Engineering Foundation (KOSEF 95-0401-02), and the Basic Science Research Institute Program (Project BSRI-96-4435) of the Ministry of Education.*

## References

1. Putney JW Jr and Bird GSJ, The signal for capacitative calcium entry. *Cell* **75:** 199–201, 1993.
2. Fasolato C, Innocenti B and Pozzan T, Receptor-activated $Ca^{2+}$ influx: How many mechanisms for how many mechanisms for how many channels? *Trends Pharmacol Sci* **15:** 77–83, 1994.
3. Berridge MJ, Capcitative calcium entry. *Biochem J* **312:** 1–11, 1995.
4. Randriamampita C and Tsien RY, Emptying of intracellular $Ca^{2+}$ stores releases a novel small messenger that stimulates $Ca^{2+}$ influx. *Nature* **364:** 809–814, 1993.
5. Parekh AB, Terlau H and Stümer W, Depletion of $InsP_3$ stores activates a $Ca^{2+}$ and $K^+$ current by means of a

phosphatase and a diffusible messenger. *Nature* **364:** 814–818, 1993.
6. Zweifach A and Lewis RS, Slow calcium-dependent inactivation of depletion-activated calcium current: Store-dependent and -independent mechanisms. *J Biol Chem* **270:** 14445–14451, 1995.
7. Lee KM Toscas K and Villereal ML, Inhibition of bradykinin- and thapsigargin-induced $Ca^{2+}$ entry by tyrosine kinase inhibitors. *J Biol Chem* **268:** 9945–9948, 1993.
8. Meucci O, Scorziello A, Avallone A, Florio T and Schettini G, 1B, but not 1A, adrenoreceptor activates calcium influx through the stimulation of a tyrosine kinase/phosphotyrosine phosphatase pathway, following noradrenaline-induced emptying of $IP_3$-sensitive calcium stores, in PC Cl3 rat thyroid cell line. *Biochem Biophys Res Commun* **209:** 630–638, 1995.
9. Gamberucci A, Innocenti B, Fulceri R, Báhegyi G, Giunti R, Pozzan T and Benedetti A, Modulation of $Ca^{2+}$ influx dependent on store depletion by intracellular adenine-guanine nucleotide levels. *J Biol Chem* **169:** 13597–13602, 1994.
10. Fasolato C, Hoth M and Penner RA, A GTP-dependent step in the activation mechanism of capacitative calcium influx. *J Biol Chem* **268:** 20737–20740, 1993.
11. Bird GSJ and Putney JW Jr, Inhibition of thapsigargin-induced calcium entry by microinjected guanine nucleotide analogs. *J Biol Chem* **268:** 21468–21488, 1993.
12. Jaconi MEE, Lew DP, Monod A and Krause KH, The regulation of store-dependent $Ca^{2+}$ influx in HL-60 granulocytes involves GTP-sensitive elements. *J Biol Chem* **268:** 16075–16078, 1993.
13. Petersen CCH and Berridge MJ, G-protein regulation of capacitative calcium entry may be mediated by protein kinases A and C in *Xenopus* oocytes. *Biochem J* **307:** 663–668, 1995.
14. Petersen CCH and Berridge MJ, The regulation of capacitative calcium entry by calcium and PKC in *Xenopus* oocytes. *J Biol Chem* **269:** 32246–32253, 1994.
15. Montero M, Garcia-Sancho J and Alvalez J, Transient inhibition by chemotactic peptide of a store-operated $Ca^{2+}$ entry pathway in human sneutrophils. *J Biol Chem* **268:** 13055–13061, 1993.
16. Montero M, Garcia-Sancho J and Alvalez J, Inhibition of the calcium store-operated calcium entry pathway by chemotactic peptide and by phorbol ester develops gradually and independently along differentiation of HL60 cells. *J Biol Chem* **268:** 26911–26919, 1993.
17. Montero M, Garcia-Sancho J and Alvalez J, Phosphorylation down-regulates the store-operated $Ca^{2+}$ entry pathway of human neutrophils. *J Biol Chem* **269:** 3963–3967, 1994.
18. Bode HP and Göke B, PKC activates capacitative calcium entry in the insulin-secreting cell line RINm5F. *FEBS Lett* **339:** 307–311, 1994.
19. Collins SJ, Ruscetti FW, Gallagher RE and Gallo RC, Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl silfoxide and other polar compounds. *Proc Natl Acad Sci USA* **75:** 2458–2462, 1978.
20. Collins SJ, Ruscetti FW, Gallagher RE and Gallo RC, Normal functional characteristic of cultured human promyelocytic leukemia cells (HL-60) after induction of differentiation by dimethylsulfoxide. *J Exp Med* **149:** 969–974, 1979.
21. Suh BC and Kim KT, Inhibition by ethaverine of catecholamine secretion through blocking L-type $Ca^{2+}$ channels in PC12 cells. *Biochem Pharmacol* **47:** 1262–1266, 1994.
22. Grynkiewicz G, Poenie M and Tsien RY, A new generation of $Ca^{2+}$ indicators with greatly improved flourescence properties. *J Biol Chem* **260:** 3440–3450, 1985.
23. Choi SY and Kim KT, Extracellular ATP-stimulated increase of cytosolic cAMP in HL-60 cells. *Biochem Pharmacol* **53:** 429–432, 1997.
24. Takemura H, Hughes AR, Thastrup O and Putney JW Jr., Activation of calcium entry by the tumor promoter thapsigargin in parotid acinar cells. *J Biol Chem* **264:** 12666–12271, 1989.
25. Talpain E, Armstrong RA, Coleman RA and Vardey CJ, Characterization of the PGE receptor subtype mediating inhibition of superoxide production in human neutrophil. *Br J Pharmacol* **114:** 1459–1465, 1995.
26. Lee HS, Suh BC and Kim KT, Feedback regulation of ATP-induced $Ca^{2+}$ signaling on HL-60 cells is mediated by protein kinase A- and C-mediated changes in capacitative $Ca^{2+}$ entry. *J Biol Chem* **27:** 21831–21838, 1997.
27. Nonaka T, Mio M, Doi M and Tasaka K, Histamine-induced differentiation of HL-60 cells: The role of cAMP and protein kinase A. *Biochem Pharmacol* **44:** 1115–1121, 1992.