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Differential Effects of Phorbol Ester on Apoptosis in HL-60 Promyelocytic Leukemia Cells

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ABSTRACT. The role of the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA) in apoptosis of HL-60 cells was investigated. PMA inhibited DNA fragmentation induced by thapsigargin (TG) and 4-bromo-calcium ionophore (Br-A23187). The inhibitory effect of PMA was concentration-related and was abolished by a specific PKC inhibitor, bisindolylmaleimide (GF109203X). In addition, TG-induced apoptosis was decreased in cells in which PKC activity was down-regulated by long-term pretreatment with PMA. These results indicate that PKC activation by PMA inhibits HL-60 cell apoptosis induced by TG and Br -A23187, and that this inhibition is not influenced by the down-regulation of PKC. However, PMA did not inhibit DNA fragmentation induced by 1-β-D-arabinofuranosylcytosine (Ara-C) and cycloheximide. PMA suppressed TG- or Br-A23187-induced DNA fragmentation probably by interfering in the modulation of calcium homeostasis by TG and Br-A23187. Our results indicate that PKC participates in the regulation of apoptosis only by some pathways. Down-regulation of PKC is not responsible for the diverse effects of PKC activators on apoptosis. The effect of a PKC modulator on apoptosis is dependent upon interaction with individual apoptotic stimulus. BIOCHEM PHARMACOL 51;9:1229–1236, 1996.

KEY WORDS. apoptosis; leukemia; PKC; calcium; PMA

Protein phosphorylation is widely recognized as an important event in transmembrane signal transduction in diverse cellular processes ranging from cell growth and differentiation to nerve cell communication, learning, and memory. PKC† is a serine/threonine kinase and plays a central role in the phosphorylation of different proteins, consequently influencing a variety of cell functions. Apoptosis is a universal biological phenomenon with specific biochemical and morphologic characteristics such as cell shrinkage, chromatin condensation, apoptotic body formation, and DNA degradation [1]. The role of PKC in the regulation of apoptosis has been studied widely in various cell types. However, conflicting results have been reported. Activation of PKC by phorbol esters has been reported to suppress apoptosis in murine thymocytes [2], in normal and leukemia hematopoietic cells [3, 4], in chronic lymphocytic leukemia following exposure to different chemotherapeutic agents [5, 6], and in *in vitro* cultured HL-60 cells [7]. Phorbol esters also promote the induction of apoptosis in T cell hybridoma [8], in thymocytes [9], and in the serum-deprived neuronal cell line ND7 [10]. In addition, PKC activator bryostatin 1 has been reported to inhibit apoptosis in hematopoietic cells [11] and to promote Ara-C-induced apoptosis in HL-60 cells [12, 13]. Other conflicting reports about PKC inhibitors include the promotion of apoptosis in mature thymocytes [14], in HL-60 cells [15], and in hepatocytes [16], and the inhibition of apoptosis in immature [14, 17] or normal mouse thymocytes [18].

Several reasons have been suggested as responsible for the different actions of PKC modulators in the regulation of apoptosis. First, differences exist between PKC isotypes with regard to substrate phosphorylation *in vitro*, tissue distribution, subcellular localization, and cofactor dependence [19]. Second, down-regulation of PKC usually follows the activation of the enzyme by phorbol esters and other PKC activators [13, 20, 21].

In the present study, the role of PMA in the regulation of apoptosis was examined further using HL-60 cells. We tried to determine whether PMA has a homologous effect on apoptosis in the same cell type and what influence PKC down-regulation has on apoptosis. We demonstrated that in HL-60 cells PMA affected apoptosis induced by various stimuli differently. Down-regulation of PKC did not reverse PMA-supported inhibition of apoptosis induced by TG. The possible mechanism of PKC action in apoptosis was discussed.

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† *Abbreviations:* PKC, protein kinase C; Br-A23187, 4-bromo-calcium ionophore; TG, thapsigargin; PMA, phorbol 12-myristate 13-acetate; $[Ca^{2+}]_i$, cytosolic calcium concentration; Ara-C, 1-β-D-arabinofuranosylcytosine; and GF109203X, bisindolylmaleimide.

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MATERIALS AND METHODS

Materials

Br-A23187, TG, PMA, Ara-C, EGTA, Fura-2/AM, diphenylamine, perchloric acid, glacial acetic acid, and RPMI 1640 medium powder were purchased from Sigma (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS) was from Gibco (Grand Island, NY, U.S.A.) and GF109203X from Calbiochem (San Diego, CA, U.S.A.).

Cell Culture

The HL-60 cell line was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Cells were cultured in RPMI 1640 medium (containing 100 U/mL penicillin and 100 μg/mL streptomycin) supplemented with 10% FBS at 37° in humidified air containing 5% CO_2. They were sub-cultured twice a week, and only those in the exponential growth period were used in these experiments.

Evaluation of HL-60 Cell Apoptosis

Apoptosis was confirmed by checking the morphology of treated cells (the formation of apoptotic bodies in apoptotic cells) and by analyzing the specific DNA fragmentation. Cell viability was also examined after the scheduled treatments to verify the absence of necrosis. None of the treatments used in this study altered cell viability significantly.

Qualitative and Quantitative Analysis of Internucleosomal Fragmented DNA

The internucleosomal DNA fragmentation was assayed by a modified method of Bhalla *et al.* [22]. HL-60 cells were treated with the test agents for 4 hr. Then the cells were washed with isotonic PBS (pH 7.4) and disrupted by a lysis buffer [5 mM/L Tris–HCl, 0.5% (v/v) Triton X-100, and 20 mM/L EDTA]. The cellular lysates were centrifuged at 13,000 *g* for 20 min to separate the low molecular weight DNA from the intact chromatin. Fragmented DNA in the supernatant was extracted with phenol: chloroform:isoamyl alcohol (25:24:1). The total purified DNA from each sample of 5×10^6 cells was dissolved in 15 μL of Tris/EDTA loading buffer (pH 8.0) and 3 μL of tracking dye (50% glycerol, 1% xylene cyanol) and electrophoresed through a 1% agarose gel (60 V, 90 min). DNA was visualized by UV illumination.

For quantitative DNA analysis, 10^7 cells were treated with test reagents for 4 hr. At the end of the incubations, cells were disrupted, and the supernatant was collected as described above. The diphenylamine method [22] was used to measure the DNA content in the supernatant.

Preparation and Assay of Protein Kinase C

Total cytosolic protein kinase C activity was measured by a modification of the method of Yasuda *et al.* [23] with the PKC assay kit provided by Gibco-BRP. Cells were collected and washed with PBS and then homogenized on ice in Tris–EGTA buffer (2×10^{-2} M Tris, 5×10^{-4} M EGTA, and 10^{-2} M β-mercaptoethanol, pH 7.5), containing 2.5 g/mL protease inhibitors (aprotinin, leupeptin) and 0.5% Triton X-100. The homogenate was incubated on ice for 30 min, centrifuged at 1200 *g* for 2 min, and then partially purified by passage through DEAE-cellulose with elution homogenization buffer containing 2×10^{-1} M NaCl. The enzyme fractions were normalized for total protein content and added directly to an assay reaction system containing mixed micelles of phosphatidylserine and PMA in suspension. The reaction was initiated by the addition of 2.5×10^{-5} Ci/mL [γ-^{32}P]ATP, 2×10^{-5} M non-isotopic ATP, and 5×10^{-5} M synthetic peptide substrate (acetylated myelin basic protein N-terminal peptide $AcMB_{4\text{-}14}$).After a 5-min incubation at 30°, aliquots of the reaction mixture were transferred to phosphocellulose filters, and the reaction was terminated by immersion of the discs in 1% phosphoric acid. Radioactivity was determined by liquid scintillation. A parallel reaction system was set up in the presence of a specific PKC inhibitor (2×10^{-5} M PKC pseudosubstrate inhibitor peptide PKC(19-36) and 4×10^{-2} M Tris) to confirm the specificity of PKC.

Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured by a method described by Lambert and Nahorski [24]. HL-60 cells at approximately 80% confluence were collected, washed, and resuspended to a cell density of 2×10^7/mL with RPMI medium. Finally, they were loaded with 5 μM Fura-2 for 45 min at 37°. At the end of loading, the cells were washed three times with calcium-free HEPES-buffered Hanks' balanced salts (HBSS, pH 7.4, with 100 μM EGTA) and were resuspended to 10^6/mL with the same buffer. A 2-mL suspension was added to the quartz cuvette, and the fluorescence was measured at 37° using a Hitachi F-4000 fluorescence spectrometer with a thermally controlled cuvette holder and a magnetic stirrer. The excitation and emission wavelengths used were 340 and 500 nm, respectively. Reagents were added after a stable fluorescence base was obtained. F_{max} was obtained by adding 0.1% Triton X-100, and F_{min} by 10 mM EGTA after the addition of 1.25 mM calcium. $[Ca^{2+}]_i$ was calibrated with the following formula:

$$[Ca^{2+}]_i \text{ (nM)} = (F - F_{min})/(F_{max} - F) \times 224.$$

F = the recorded fluorescence value.

Statistical Analysis

Student's *t*-test was used to analyze the data, and values are expressed as means ± SD.

RESULTS

Effects of PMA on HL-60 Cell Apoptosis Induced by Different Stimuli

Apoptosis was identified by the morphologic features of extensive membrane bleb formation and chromatin condensation and was qualitatively and quantitatively evalu-

ated by the analysis of characteristic apoptotic DNA fragmentation. In HL-60 cells, apoptosis spontaneously occurs even in normal culture conditions. This process can be promoted by various agents with different mechanisms of action. Thapsigargin, the calcium ionophore Br-A23187, Ara-C, and cycloheximide all induced apoptosis in HL-60 cells. Zinc ions inhibited spontaneous apoptosis of HL-60 cells and abolished DNA fragmentation induced by all four agents (Fig. 1).

PMA suppressed spontaneous apoptosis in HL-60 cells, and decreased DNA fragmentation induced by TG and Br-A23187 ($P < 0.01$) (Fig. 2); these effects were concentration dependent. PMA at a concentration of 2 nM was effective in inhibiting apoptosis. The maximum response occurred at a concentration of 100 nM PMA (data not shown). GF109203X is a highly specific PKC inhibitor, which competitively combines with the ATP-binding site on PKC and inhibits the activity of the enzyme [25]. GF109203X slightly promoted HL-60 cell apoptosis. In the presence of GF109203X (1 μM), 100 nM PMA-induced suppression of apoptosis was abolished almost completely (Fig. 3). These results indicated that PKC activation by PMA inhibited HL-60 cell apoptosis induced by TG and Br-A23187. However, the role of PMA in apoptosis induced by Ara-C and cycloheximide differed from that observed in the cases of TG and Br-A23187. As shown in Fig. 4, instead of inhibiting apoptosis, PMA slightly increased DNA fragmentation initiated by Ara-C ($P > 0.05$) and significantly enhanced the effect of cycloheximide ($P < 0.05$).

Effects of PMA-Induced PKC Down-Regulation on HL-60 Cell Apoptosis

As shown in Fig. 5, PMA increased total cellular PKC activity. However, the increased PKC activity persisted for

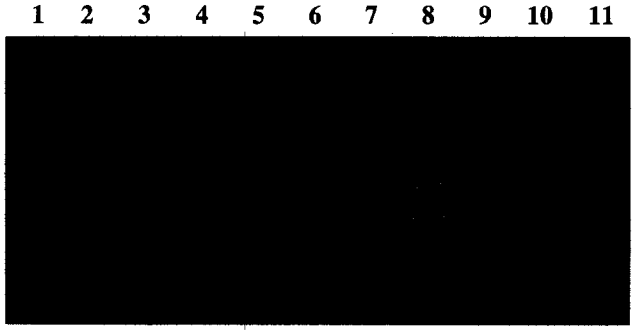

FIG. 1. Induction of apoptosis by different stimuli and the inhibitory effect of zinc sulfate. HL-60 cells (5×10^6) were cultured in serum-free medium for 4 hr with 5 nM TG, 250 nM Br-A23187, 10 μM Ara-C, or 5 μg/mL cycloheximide (CHX) in the absence or presence of 1 mM zinc sulfate. Then the cells were pelleted, and fragmented DNA was purified and checked on agarose gel. Lane 1, ØX174 *Hae* III DNA marker; Lane 2, control; Lane 3, zinc; Lane 4, TG; Lane 5, zinc + TG; Lane 6, Br-A23187; Lane 7, zinc + Br-A23187; Lane 8, Ara-C; Lane 9, zinc + Ara-C; Lane 10, CHX; and Lane 11, zinc + CHX. Four experiments yielded similar results.

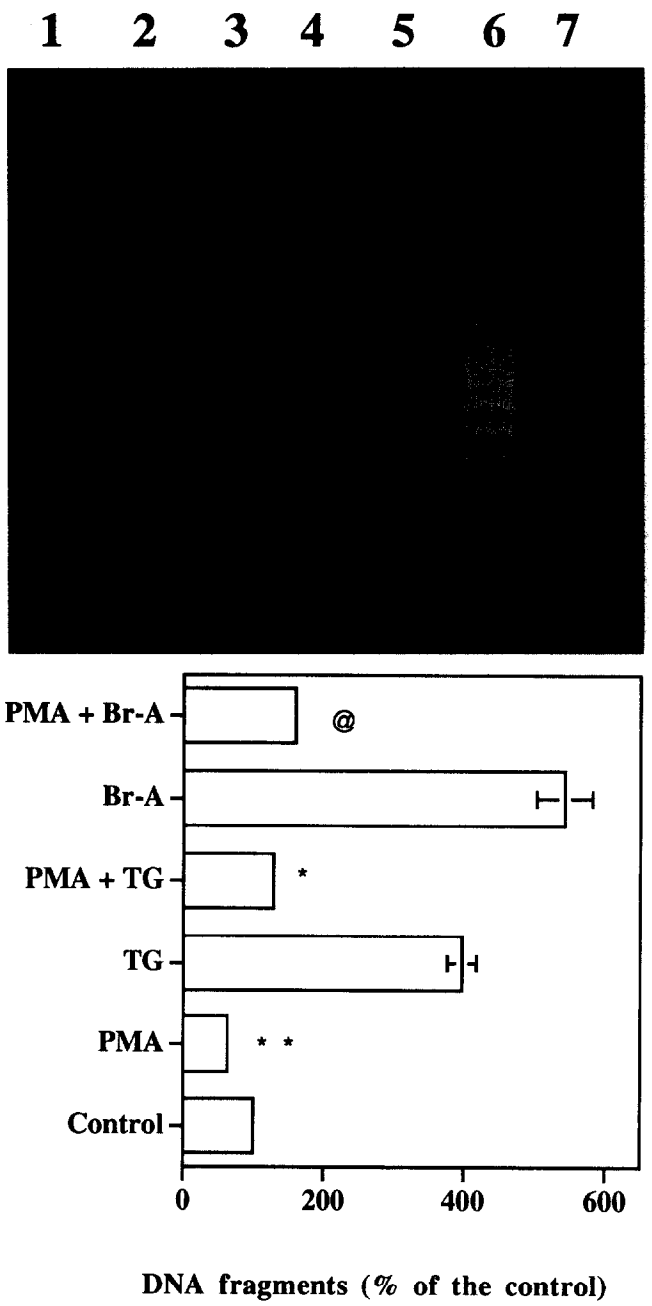

FIG. 2. Modulation of TG- or Br-A23187-induced DNA fragmentation by PMA. Top panel: Cells (5×10^6) were cultured in serum-free medium for 4 hr with 2.5 nM TG or 250 nM Br-A23187 in the absence or presence of 100 nM PMA. Fragmented DNA of treated cells was checked as described in Fig. 1. Lane, ØX174 *Hae* III DNA marker; Lane 2, control; Lane 3, PMA; Lane 4, TG; Lane 5, PMA + TG; Lane 6, Br-A23187; and Lane 7, PMA + Br-A23187. Four experiments yielded similar results. Bottom panel: HL-60 cells (1×10^7) were cultured in serum-free medium for 4 hr in the presence of different agents as defined on the figure [control, no agent; PMA, 100 nM; TG, 5 nM; Br-A23187 (Br-A), 500 nM]. The amount of fragmented DNA was then measured with the method described in Materials and Methods. In these untreated cells, the amount of DNA fragments was 2.29 ± 0.15 μg/10^7 cells. Values are means ± SD, N = 6. Key: (**) $P < 0.01$, compared with untreated cells; (*) $P < 0.01$, compared with cells treated with TG alone; and (@) $P < 0.01$, compared with cells treated with Br-A23187 alone.

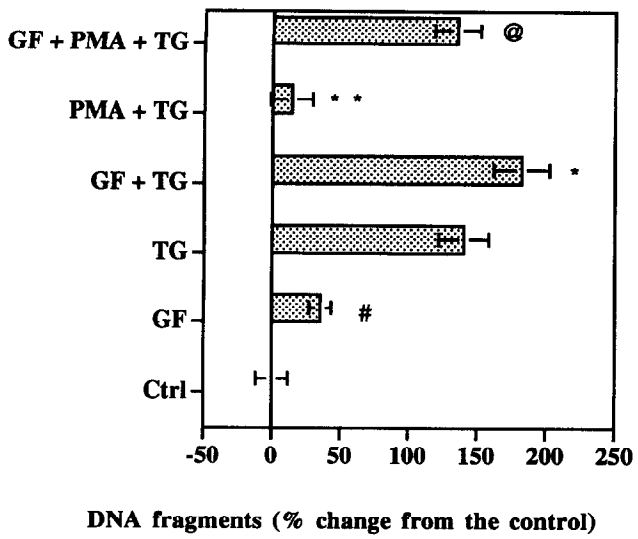

FIG. 3. Effect of the specific PKC inhibitor GF109203X on PMA-induced inhibition of HL-60 cell apoptosis. HL-60 cells were incubated in serum-free medium for 4 hr in the presence of different agents as defined on the figure [control (Ctrl), no agent; GF109203X (GF), 1 μM; TG, 2.5 nM; PMA, 100 nM]. The amount of fragmented DNA was then measured. In the untreated cells, the amount of DNA fragments was 1.75 ± 0.08 μg/10^7 cells. Values are means ± SD, N = 6. Key: ($^{\#}$) $P < 0.05$, compared with untreated cells; (*) $P < 0.05$, (**), $P < 0.01$, and ($^{@}$) $P > 0.05$, compared with cells treated with TG alone.

only a short time and returned to normal level in 30 min. Closely followed was the down-regulation of PKC. Four hours after the addition of 100 nM PMA, total cellular PKC activity decreased to 60% of control.

To understand the roles of PMA-induced down-regulation of PKC in apoptosis, we measured changes in DNA fragmentation induced by TG and Ara-C in cells pretreated with 200 nM PMA for 12 hr; this treatment has been found to induce PKC down-regulation [13, 20, 21]. After treatment with 200 nM PMA for 12 hr, total cellular PKC activity of HL-60 cells decreased to an undetectable level. In pretreated cells, TG-induced apoptosis was clearly attenuated ($P < 0.01$), whereas there was no significant ($P > 0.05$) effect with Ara-C (Fig. 6). These results were similar to those found when PMA and TG or Ara-C were administered simultaneously. The effect of PKC down-regulation on apoptosis differed from that of PKC inhibition by specific PKC inhibitors. As reported by other authors [15], we also observed that PKC inhibitors (such as calphostin C and GF109203X) induced apoptosis in HL-60 cells and enhanced the apoptotic effects of TG and Br-A23187 (the effect of GF109203X is shown in Fig. 3).

Effect of PMA on TG- and Br-A23187-Induced Modulation of Calcium Homeostasis

It is well-known that PKC and calcium are closely related in regulating cell functions. Both TG and Br-A23187 are calcium modulators. We found that neither Ara-C nor cycloheximide had an effect on $[Ca^{2+}]_i$ of HL-60 cells. The results showing that PMA inhibited apoptosis induced by TG and Br-A23187 but not that by Ara-C and cycloheximide suggest the possibility that PMA influenced calcium modulation by TG and Br-A23187 to inhibit apoptosis. We

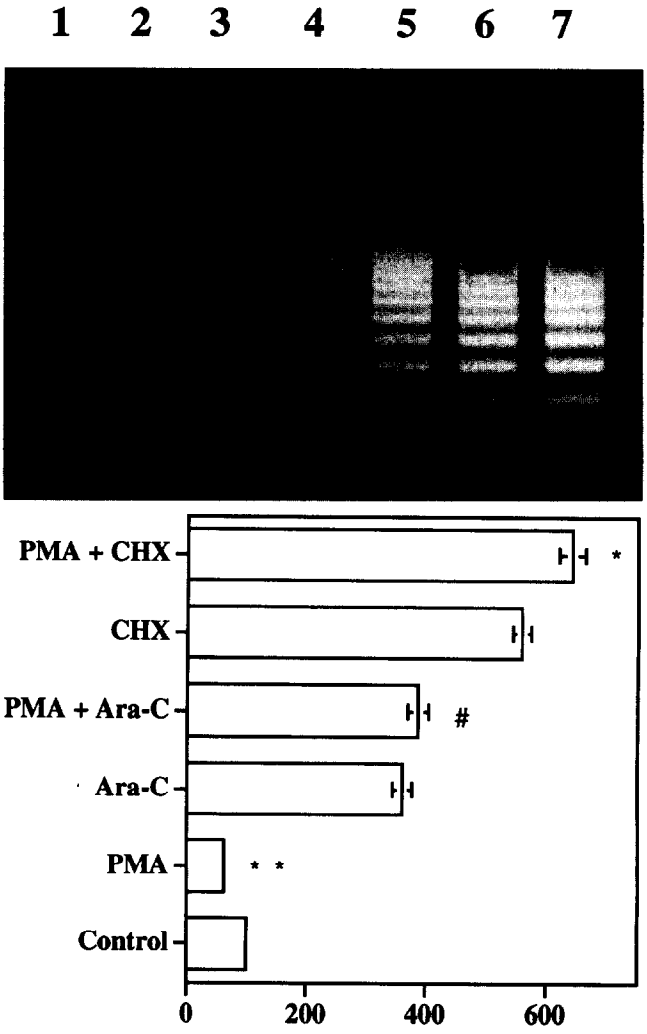

FIG. 4. Effect of PMA on Ara-C- or cycloheximide-induced DNA fragmentation of HL-60 cells. Top panel: HL-60 cells (5×10^6) were cultured in serum-free medium for 4 hr with 10 μM Ara-C or 5 μg/mL cycloheximide (CHX) in the absence or presence of 100 nM PMA. Fragmented DNA of treated cells was checked as described in Fig. 1. Lane 1, ØX174 *Hae* III DNA marker; Lane 2, control; Lane 3, PMA; Lane 4, Ara-C; Lane 5, PMA + Ara-C; Lane 6, CHX; and Lane 7, PMA + CHX. Four experiments yielded similar results. Bottom panel: HL-60 cells (1×10^7) were cultured in serum-free medium for 4 hr in the presence of different agents as defined on the figure (control, no agent; PMA, 100 nM; Ara-C, 10 μM; CHX, 10 μg/mL). The amount of fragmented DNA was then measured. In the untreated cells, the amount of DNA fragments was 2.04 ± 0.10 μg/10^7 cells. Values are means ± SD, N = 6. Key: (**) $P < 0.01$, compared with untreated cells; ($^{\#}$) $P > 0.05$, compared with cells treated with Ara-C alone; and (*) $P < 0.05$, compared with cells treated with CHX alone.

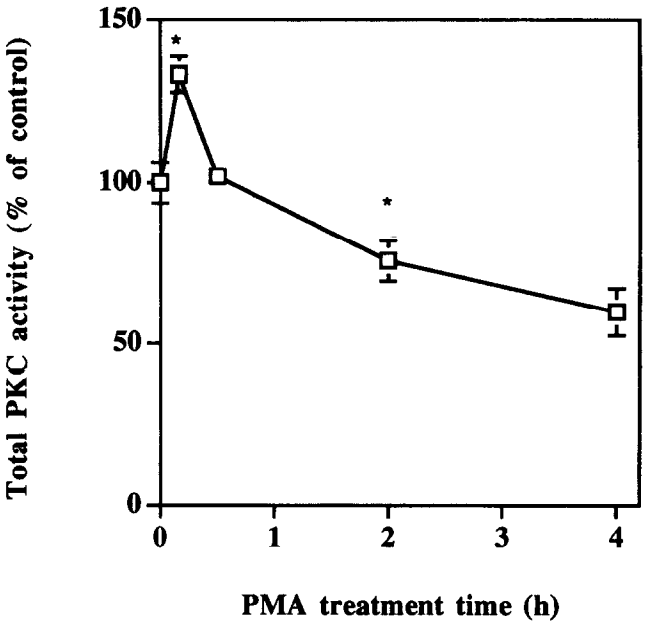

FIG. 5. Modulation of total cellular PKC activity by PMA. HL-60 cells were treated with 100 nM PMA for different periods of time in serum-free medium. Total cellular PKC activity was measured with the assay kit provided by Gibco-BRP. In untreated cells (control), the measured total cellular PKC activity was 45.7 ± 2.93 pmol/min/10^6 cells. Values are means ± SD, N = 4. Key: (*) $P < 0.01$, compared with the control.

have demonstrated previously that mobilization of intracellular calcium (or depletion of intracellular calcium stores) is closely related to the induction of HL-60 cell apoptosis by TG and Br-A23187 [26]. Therefore, the effect of PMA on TG- and Br-A23187-induced intracellular calcium mobilization was studied in detail.

PMA itself did not influence the $[Ca^{2+}]_i$ of HL-60 cells. In calcium-free medium, both TG and Br-A23187 increased $[Ca^{2+}]_i$ by mobilizing calcium from intracellular stores. PMA decreased the magnitude of the increase in $[Ca^{2+}]_i$ induced by both TG and Br-A23187 (Fig. 7). The inhibitory effects of PMA on calcium mobilization were concentration dependent, the maximum response being achieved with 100 nM PMA. PMA, however, did not block completely calcium mobilization by TG and Br-A23187.

DISCUSSION

The role of PKC modulators in the regulation of apoptosis differs with cell types, a result due perhaps to the differential modulation of PKC isotypes with their separate and unique functions in different cells [19]. However, whether a PKC modulator influences apoptosis induced by different stimuli in a certain cell type is not well defined. Forbes *et al.* [5] reported that in chronic lymphocytic leukemia cells phorbol esters inhibit cell death induced by colchicine, etoposide, and methylprednisolone and suggested that phorbol esters act on an event common to apoptosis caused by diverse stimuli.

We found that in the HL-60 cell line, PMA had different effects on apoptosis induced by different agents. PMA concentration-dependently inhibited the apoptotic effect of TG and B_r-A23187. PMA-induced suppression of apoptosis was directly related to activation of PKC as the PKC inhibitor GF109203X abolished the effects of PMA. However, the same treatment with PMA did not inhibit DNA fragmentation induced by Ara-C and cycloheximide. In contrast, zinc ions ubiquitously inhibited apoptosis induced by TG, Br-A23187, Ara-C, or cycloheximide. It is known that zinc ions inhibit apoptosis by blocking the activation of the endonuclease, the final common step associated with the induction of apoptosis [27]. These findings indicate that in HL-60 cells apoptosis can be initiated via different mechanisms. Unlike zinc ions, PKC does not act on a common pathway to influence apoptosis. PKC is only involved in the regulation of apoptosis initiated through certain pathways.

Down-regulation of PKC reportedly follows the activation of the enzyme by some external PKC activators [13, 20, 21, 28]. Jarvis *et al.* [13] compared the effects of bryostatin 1 and other PKC activators on Ara-C-induced HL-60 cell apoptosis and suggested that the ability of bryostatin 1 to potentiate Ara-C-induced apoptosis was mediated by down-regulation of one or more isoforms of PKC. In HL-60 cells, long-term treatment with PMA also down-regulated

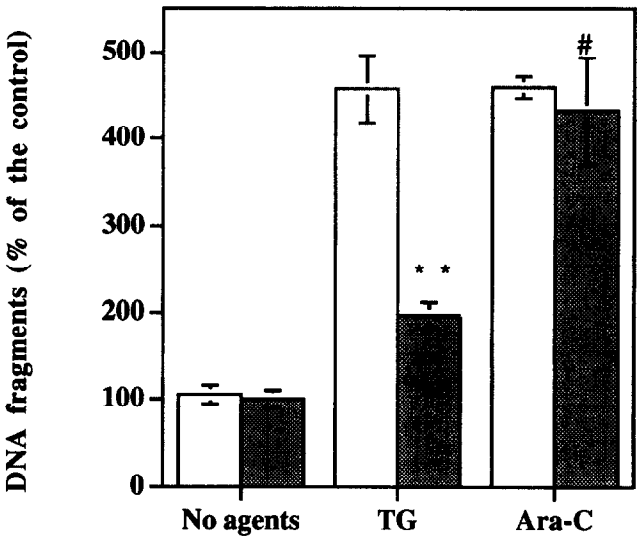

FIG. 6. Effect of PMA-induced PKC down-regulation on DNA fragmentation induced by TG and Ara-C. HL-60 cells were cultured in serum-free medium for 12 hr in the absence (open bars) or presence (stippled bars) of 200 nM PMA. The cells were then washed three times and incubated in a serum-free medium for another 4 hr with 5 nM TG, 10 μM Ara-C, or no agent. The amount of fragmented DNA was then measured. The amount of fragmented DNA from cells that did not receive PMA pretreatment and treatment of other agents (1.95 ± 0.20 μg/10^7 cells) was used as the control. Values are means ± SD, N = 6. Key: (**) $P < 0.01$, compared with result obtained from TG-treated cells, but without PMA pretreatment; and (#) $P > 0.05$, compared with result obtained from Ara-C-treated cells, but without PMA pretreatment.

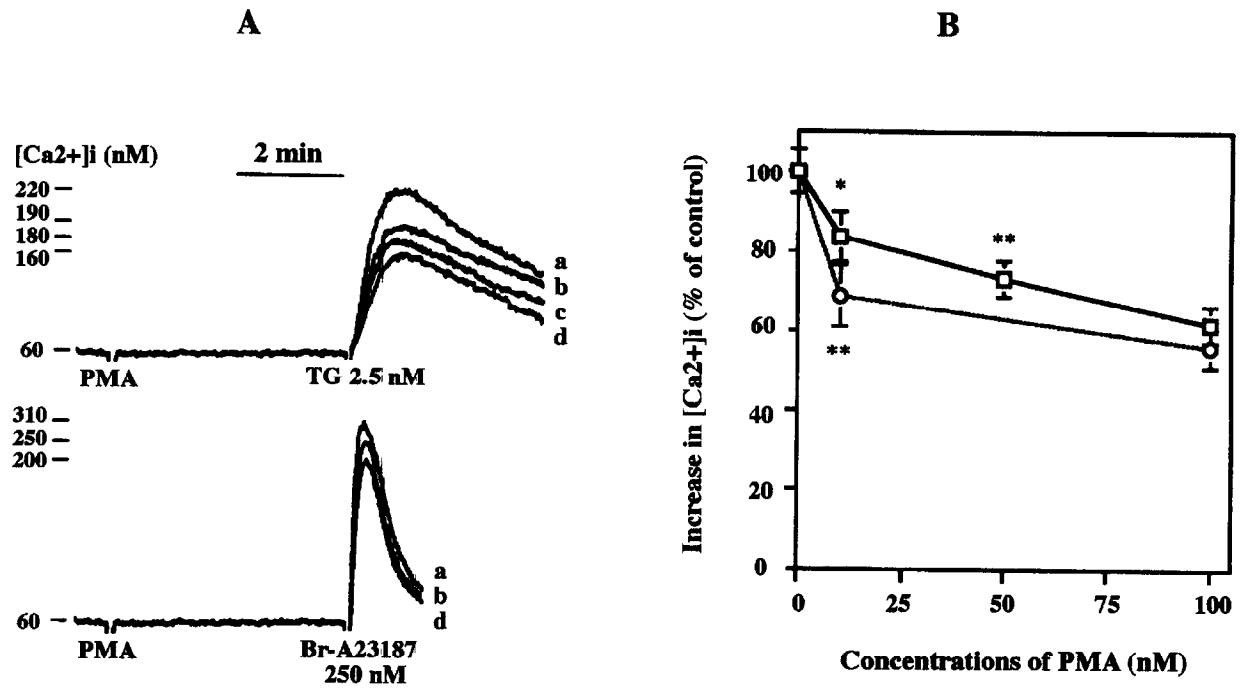

FIG. 7. Effect of PMA on TG- and Br-A23187-induced increase in $[Ca^{2+}]_i$. HL-60 cells were loaded with Fura-2/AM and suspended in calcium-free HBSS buffer (with 100 nM EGTA). After pretreatment with different concentrations of PMA for 5 min, the increase in $[Ca^{2+}]_i$ induced by 2.5 nM TG or 250 nM Br-A23187 was measured. (A). A typical recording of $[Ca^{2+}]_i$ measurement. (a) Control; (b) 10 nM PMA; (c) 50 nM PMA; and (d) 100 nM PMA. (B). Pooled results of four experiments. Key: (□)2.5 nM TG; and (○) 250 nM Br-A23187. Increase in $[Ca^{2+}]_i$ induced by TG (163.2 ± 9.5 nM) or Br-A23187 (253.7 ± 14.1 nM) in the absence of PMA was used as controls. Values are means ± SD, N = 4. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared with the corresponding control.

PKC activity. In cells in which PKC was down-regulated by pretreatment with 200 nM PMA for 12 hr, however, the ability of TG to induce DNA fragmentation was decreased significantly. Although PMA induced the differentiation of HL-60 cells to monocytes and macrophages and it has been reported that monocyte/macrophage differentiation induced by the PKC activator 12-O-tetradecanoylphorbol-13-acetate (TPA) was associated with a decreased sensitivity to various apoptosis-inducing stimuli [7, 29], it was unlikely to be responsible for the suppression of TG-induced apoptosis in cells pretreated with PMA for 12 hr because DNA fragmentation induced by Ara-C was not affected. These results indicate that down-regulation of PKC did not interfere with the apoptosis-inhibiting effects of PMA. Activation of PKC by PMA, although transient and followed by the down-regulation of the enzyme, conferred on cells the ability to resist apoptosis initiated by TG. Our results suggest that down-regulation of PKC may not be responsible for the inability of some PKC activators to inhibit apoptosis.

The effect of PMA-induced PKC down-regulation on apoptosis differed from that of PKC inhibition by specific PKC inhibitors. PKC inhibitors such as calphostin C and GF109203X promoted apoptosis in HL-60 cells and enhanced the apoptotic effects of TG and Br-A23187. The possible reason is that down-regulation of PKC is preceded by the activation of PKC and the activation of a series of intracellular events, whereas the inhibition of PKC by specific inhibitors is the direct inhibition of the enzyme without the activation of concerned cellular events. Therefore, down-regulation of PKC does not equate to the inhibition of the enzyme.

The mechanism by which PMA affects apoptosis induced by different stimuli is still not well-defined. However, an obvious difference exists among the agents in the present study. Both TG and Br-A23187 caused a profound change in intracellular calcium homeostasis. TG increased intracellular concentration by inhibiting the Ca^{2+}-ATPase on the endoplasmic reticulum membrane, thereby blocking the refilling of intracellular calcium stores with a consequent depletion of the TG-sensitive calcium store [30]. Br-A23187 acted as a calcium ionophore, which increased intracellular free calcium concentration by enhancing the influx of external calcium and mobilizing calcium from intracellular calcium stores [31]. On the other hand, Ara-C and cycloheximide had no effect on calcium homeostasis. In calcium-free medium, the increase in $[Ca^{2+}]_i$ represents the mobilization of calcium from intracellular stores. PMA decreased $[Ca^{2+}]_i$ elevation induced by both TG and Br-A23187, implying that activation of PKC inhibits the mobilization of intracellular calcium. It is well-known that increased $[Ca^{2+}]_i$ is required for the activation of several types of PKC isoforms. Increased PKC activity may, in turn, provide negative feedback signals to prevent calcium mobilization. Disturbance of intracellular calcium homeostasis has been proposed to be responsible for the initiation of

apoptosis in some cells [32]. We have also demonstrated that mobilization of intracellular calcium is closely related to the induction of HL-60 cell apoptosis by TG and Br-A23187 [26]. Therefore, PMA may inhibit DNA fragmentation induced by TG and Br-A23187 by interfering with their induced modulation of intracellular calcium homeostasis.

Based on these observations, we suggest that the role of PKC in the regulation of apoptosis is limited. Apoptosis can be induced by a variety of stimuli; PKC may only regulate apoptosis initiated via certain pathways. Down-regulation of PKC is unlikely to be responsible for the different effects of PKC activators on apoptosis. The role of PKC modulators in apoptosis is dependent on the interaction with individual apoptotic stimulus. This is probably an important reason for the different effects of PKC modulators on apoptosis.

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