

Elevation of Intracellular Calcium Ion by Prostaglandin E₁ and Its Inhibition by Protein Kinase C in a Human Megakaryocyte Leukemia Cell Line

Masashi Adachi,¹ Ryukichi Ryo, Akinori Yoshida, Keisuke Teshigawara, Nobuo Yamaguchi, Masahiko Hoshijima, Yoshimi Takai, and Takeyuki Sato

Departments of Laboratory Medicine [M. A., A. Y., K. T., N. Y.] and Biochemistry [M. H., Y. T.], Kobe University School of Medicine and Blood Transfusion Service, Kobe University Hospital [R. R.], Kobe 650; and Department of Pediatrics, Chiba University School of Medicine, Chiba 280 [T. S.], Japan

ABSTRACT

In the present study, we examined the effect of prostaglandin E₁ (PGE₁) on Ca²⁺ mobilization in a human megakaryocyte (the progenitor of platelets) leukemia cell line, designated as CMK. PGE₁ caused a rapid and dose-dependent increase in the intracellular free calcium level ([Ca²⁺]_i) associated with the elevation of cyclic AMP. The PGE₁-induced elevation of [Ca²⁺]_i was decreased by the prior addition of ethylene glycol bis(2-aminoethylether)tetraacetic acid to the medium by approximately 25% of the control. This result indicates that the PGE₁-induced elevation of [Ca²⁺]_i is due to influx of Ca²⁺ from the external medium and to mobilization of Ca²⁺ from intracellular stores. Pretreatment of CMK cells with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a stimulus for protein kinase C, further enhanced the PGE₁-induced increase in the cellular cyclic AMP level. Inversely, pretreatment of CMK cells with TPA (10 nM), prior to the addition of PGE₁, inhibited the PGE₁-induced elevation of [Ca²⁺]_i. Dibutyl cyclic AMP and forskolin did not elevate [Ca²⁺]_i or affect the PGE₁-induced Ca²⁺ mobilization. The inhibitory action of TPA in the PGE₁-induced elevation of [Ca²⁺]_i was mimicked by other protein kinase C-activating agents, such as 1-oleoyl-2-acetyl-glycerol and *N*-(6-phenylhexyl)-5-chloro-1-naphthalenesulfonamide, and was selectively restored by protein kinase C inhibitors, such as 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride and *N*-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride. Thus, the inhibitory modulation of TPA on the PGE₁-induced elevation of [Ca²⁺]_i is mediated through protein kinase C activation. PGE₁ had no induction effect of megakaryocytic phenotypic changes in CMK cells. The biological role of PGE₁, which increased [Ca²⁺]_i and cyclic AMP levels in the CMK cells, remains to be determined.

INTRODUCTION

Ca²⁺ plays essential roles in the induction of platelet aggregation and secretion by thrombin, collagen, ADP, platelet-activating factor, stable thromboxane A₂ analogues, and other agonists (1-6). PGE₁² inhibits these platelet functions by increasing the intracellular concentration of cAMP (7, 8). cAMP presumably decreases the elevation of [Ca²⁺]_i and thereby inhibits the platelet functions (9, 10). In contrast to such an inhibitory action of PGE₁, PGE₁ and prostaglandin E₂ have been shown to cause an elevation of [Ca²⁺]_i in some cell types and stimulate their cellular functions (11-13).

Recently, we have established a megakaryocytic cell line, designated as CMK (14), which releases platelet-like particles that possess α -granule and platelet peroxidase activity, as well as exhibit platelet GPIIb/IIIa antigen on their membranes (15).

Received 12/7/88; revised 3/20/89; accepted 4/12/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ To whom requests for reprints should be addressed.

² The abbreviations used are: PGE₁, prostaglandin E₁; cAMP, cyclic adenosine-3',5'-monophosphate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EGTA, ethylene glycol bis(2-aminoethyl ether)tetraacetic acid; [Ca²⁺]_i, intracellular free calcium level; GP, platelet glycoprotein; OAG, 1-oleoyl-2-acetyl-glycerol; SC9, *N*-(6-phenylhexyl)-5-chloro-1-naphthalenesulfonamide; H7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride; H8, *N*-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride; HA1004, *N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide hydrochloride; fura 2-AM, fura-2 acetoxymethyl ester.

Since PGE₁ has an inhibitory effect on the agonist-induced elevation of [Ca²⁺]_i in platelets through cAMP accumulation, it is interesting to examine whether PGE₁ shows a similar effect in CMK cells. In this paper, we describe for the first time that PGE₁ is a potent activator of [Ca²⁺]_i in CMK cells.

Another line of study has shown that a phorbol ester such as TPA, a potent protein kinase C activator, inhibits the agonist-induced Ca²⁺ mobilization in platelets (16-21). On the basis of this finding, it is hypothesized that protein kinase C activation may have a role in the termination of the agonist-induced signal transduction in platelets. To clarify the relationship between protein kinase C activation and the PGE₁-modulated Ca²⁺ mobilization in CMK cells, we have examined the effects of protein kinase C activators and inhibitors on the PGE₁-induced Ca²⁺ mobilization in the cells. This paper also describes protein kinase C inhibition of the PGE₁-induced Ca²⁺ mobilization in CMK cells.

MATERIALS AND METHODS

Chemicals. Thrombin was a product of Mochida Pharmaceutical Co. (Tokyo, Japan). PGE₁ was donated by Ono Pharmaceutical Co. (Tokyo, Japan). TPA, dibutyl cAMP, and forskolin were purchased from Sigma Chemical Co. (St. Louis, MO). OAG was from Nakarai Chemicals (Kyoto, Japan). H7, H8, HA1004, and SC9 were from Seikagaku Kogyo Co. (Tokyo, Japan). Fura 2-AM was from Dojindo Laboratories (Kumamoto, Japan). The cAMP radioimmunoassay kit was from Amersham Japan Co. (Tokyo, Japan).

Cell and Cell Cultures. The human megakaryocytic leukemia cell line (CMK) (14, 15) was maintained in polystyrene tissue culture flasks (Corning Glass Works, Corning, NY) that contained RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd, Tokyo) supplemented with 10% (v/v) fetal bovine serum (M. A. Bioproducts, Walkersville, MD). The cells were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. The medium was changed every 2-4 days to maintain a density of 2 × 10⁵ to 8 × 10⁵ cells/ml. Stock CMK cells were stored in liquid nitrogen in a solution of 10% dimethyl sulfoxide in otherwise standard growth medium. Fresh cells were thawed every month during the course of the study to maintain the biological stability in the culture.

Assay for [Ca²⁺]_i. [Ca²⁺]_i was measured by using a fluorescent Ca²⁺ indicator, fura 2-AM. Cells (5 × 10⁶ cells/ml) were suspended in RPMI 1640 medium that contained 20 mM HEPES and were loaded by incubation with 2 μM fura 2-AM, final concentration. After incubation for 20 min at 37°C, the cells were washed twice with medium that contained 10% fetal bovine serum and were kept in the medium at room temperature until use. Just before use, aliquots of the cells (3 × 10⁶ cells) were centrifuged in a microcentrifuge (Hitachi Koki Co., Tokyo), resuspended in 0.5 ml of a HEPES-Tyrode solution (10 mM HEPES at pH 7.4, 129 mM NaCl, 8.9 mM NaHCO₃, 0.8 mM KH₂PO₄, 0.8 mM MgCl₂, and 5.6 mM dextrose), and transferred to the cuvet. Before experimental manipulations, the external Ca²⁺ was adjusted by adding 1 mM Ca²⁺ or 3 mM EGTA as required. The fura 2 fluorescence of these cells, stirring at 1000 rpm at 37°C, was recorded using a dual excitation wavelength spectrofluorometer (CAF100; Nihon Bunko Co., Tokyo, Japan), with excitation at 340 and 380 nm and emission at 500 nm. Values of [Ca²⁺]_i were calculated from fluorescence signals, as described by Tsien *et al.* (22).

Assay for cAMP. Cells (3 × 10⁶ cells/ml) in suspension were washed

twice with a HEPES-Tyrode solution, at 37°C, and were incubated with PGE₁ in 500 μl of the same solution. After the incubation, the medium was rapidly removed and cAMP was extracted with 500 μl of 12% perchloric acid, neutralized with KHCO₃, and kept at -80°C until measured. The cAMP concentrations produced by agonists were measured using the radioimmunoassay kit.

Flow Cytometric Analysis. To examine quantitatively the phenotypic differentiation of CMK cells induced by PGE₁ or TPA, cell surface antigenic changes toward greater maturation were measured using a flow cytometer (Epics; Coulter Electronics Inc., Hialeah, FL), with monoclonal antibodies in indirect immunofluorescent assays, as described in the previous paper (23). The monoclonal antibodies 224B, 227A, and Thr-4 (Serotec; Blackthorn, Bicester, United Kingdom) recognize GPIIb/IIIa, GPIb, and GPIIIa, respectively (23, 24). PLT1 (Coulter Immunology, Hialeah, FL) also reacts with platelets.

RESULTS

Fig. 1 shows the effect of 1 μM PGE₁ on [Ca²⁺]_i of fura 2-loaded CMK cells in the presence or absence of extracellular Ca²⁺ in the medium. In the presence of extracellular Ca²⁺, PGE₁ provoked an acute rise in [Ca²⁺]_i (depending on the concentration used), which was followed by a rapid decrease. PGE₁ at 1 μM caused an elevation of [Ca²⁺]_i from a resting value of 143 ± 11 to 1128 ± 48 nM (means ± SD; three experiments). A second addition of PGE₁, 4 min after the first one, triggered a small elevation in [Ca²⁺]_i. The PGE₁-induced spike phases were reduced by the prior addition of 3 mM EGTA but still induced a marked rise in [Ca²⁺]_i, as shown in Fig. 1B. These results imply that the increase in [Ca²⁺]_i after the addition of PGE₁ was mainly dependent on the extracellular Ca²⁺ but

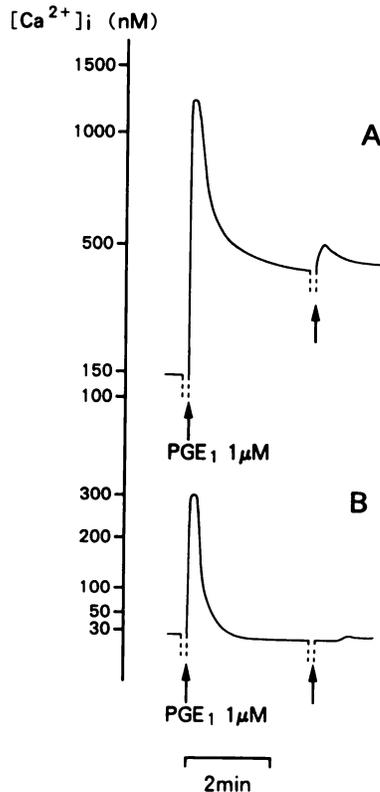


Fig. 1. PGE₁-induced elevation of [Ca²⁺]_i and its dependency on extracellular Ca²⁺ in medium. The cells loaded with fura 2-AM were washed, resuspended in either HEPES-Tyrode solution that contained 1 mM Ca²⁺ or Ca²⁺-free medium that contained 3 mM EGTA, and then stimulated with 1 μM PGE₁ twice. Other details are described under "Materials and Methods." A, with PGE₁ in HEPES-Tyrode solution containing 1 mM Ca²⁺; B, with PGE₁ in the Ca²⁺-free medium. Arrows, two points of addition of PGE₁. The panel shows a typical pattern of fluorescent monitoring from three different experiments.

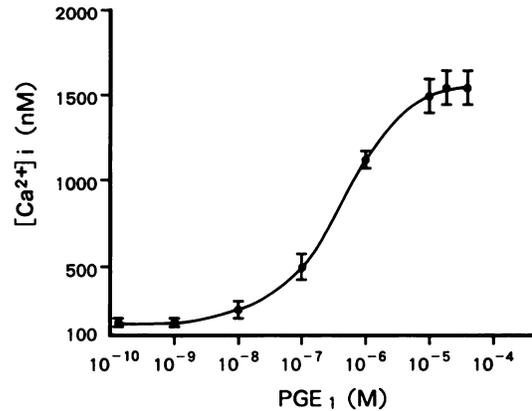


Fig. 2. Dose-dependent elevation of [Ca²⁺]_i by PGE₁. The cells loaded with fura 2-AM were washed, resuspended in HEPES-Tyrode solution that contained 1 mM Ca²⁺, and then stimulated with various doses of PGE₁. Other details are described under "Materials and Methods." Points and vertical bars, mean ± SD of three separate experiments.

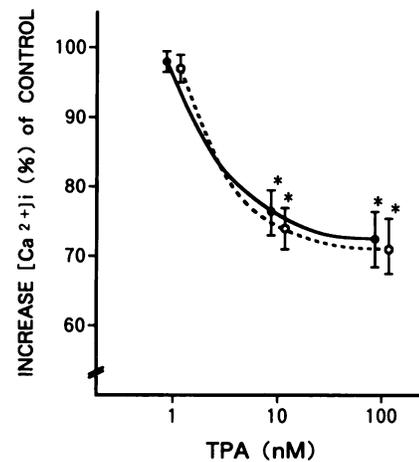


Fig. 3. Effect of TPA on the PGE₁- or thrombin-induced elevation of [Ca²⁺]_i. The cells loaded with fura 2-AM were washed, resuspended in HEPES-Tyrode solution that contained 1 mM Ca²⁺, exposed to the indicated concentration of TPA for 5 min, and then stimulated with 1 μM PGE₁ (—) or 0.5 units/ml thrombin (---). The increase in [Ca²⁺]_i over basal level without TPA addition was taken as 100% control. Other details are described under "Materials and Methods." Results are expressed as means ± SD of three independent experiments. *, values significantly different (*P* < 0.01) compared to the corresponding values without TPA, as measured by Student's *t* test.

was partly attributed to mobilization from intracellular Ca²⁺ storage sites.

Fig. 2 shows the dose-response curve for the PGE₁-induced increase in [Ca²⁺]_i. The effect of 1 μM PGE₁, which increased [Ca²⁺]_i over the resting level by 1128 ± 48 nM, was taken as 100% control. The maximal and half-maximal increases in [Ca²⁺]_i were found at about 20 and 0.5 μM PGE₁, respectively. The concentrations of PGE₁ necessary for the increase in [Ca²⁺]_i were similar to those necessary for the inhibition of the agonist-induced elevation of [Ca²⁺]_i in platelets (9, 10).

As shown in Fig. 3, incubation of CMK cells with 10 nM TPA, a stimulus for protein kinase C, prior to the addition of PGE₁ impaired the PGE₁-induced increase in [Ca²⁺]_i by about 30%. TPA did not alter the resting level of [Ca²⁺]_i (data not shown). This inhibitory effect of TPA on the PGE₁-induced rise was dose dependent (1–100 nM). Thrombin (0.5 units/ml), which is a potent Ca²⁺-mobilizing agonist in platelets, caused an elevation of [Ca²⁺]_i from a resting value to 1114 ± 82 nM (three experiments).³ TPA caused the same effect on thrombin-

³ R. Ryo, M. Adachi, A. Yoshida, W. Sugano, N. Yamaguchi, and T. Sato. Cytosolic Ca²⁺ mobilization and thromboxane synthesis in a human megakaryocytic leukemia cell, submitted for publication.

induced Ca²⁺ mobilization. The doses of TPA necessary for these inhibitions were the same as those necessary for the activation of protein kinase C (25). The inhibitory effect of TPA was mimicked by OAG or SC9, other protein kinase C activators, as shown in Table 1. Preincubation of CMK cells for 5 min with protein kinase C inhibitors, H7 and H9, abolished the inhibitory effect of TPA on the PGE₁-induced elevation in [Ca²⁺]_i in a dose-dependent manner (1–100 nM), as shown in Fig. 4. These inhibitors did not alter the resting [Ca²⁺]_i (data not shown). With H8 at a concentration of 100 nM, the PGE₁-induced increase in [Ca²⁺]_i of the TPA-treated CMK cells was restored to the level of the cells treated with H8 alone. In contrast, the same doses of HA1004, which had little inhibitory action for protein kinase C, failed to restore the TPA-induced inhibition of [Ca²⁺]_i.

As illustrated in Fig. 5, TPA alone had little measurable effect on the basal cellular cAMP level, while TPA enhanced the PGE₁-induced cAMP accumulation in a time-dependent manner. The cAMP levels in the control samples in these experiments (i.e., without TPA or PGE₁), or in the samples containing TPA alone, were less than 1.0 pmol/10⁵ cells. TPA caused the remarkable increase in the cAMP accumulation in response to PGE₁. The TPA-induced augmentation was demonstrated at 30 s after the addition of PGE₁.

Table 1 Effect of protein kinase C activators on the PGE₁-induced elevation in [Ca²⁺]_i

The cells, loaded with fura 2-AM, were washed, resuspended in HEPES-Tyrode solution containing 1 mM Ca²⁺; preincubated with TPA (10 nM), OAG (300 μM), or SC9 (10 nM) at 37°C for 5 min; and then stimulated with 1 μM PGE₁. The effect of 1 μM PGE₁, which increased [Ca²⁺]_i over the resting level, was taken as 100% control for the inhibition of PGE₁-induced elevation of [Ca²⁺]_i by protein kinase C activators. Other details are described under "Materials and Methods." Results are expressed as means ± SD of three independent experiments.

	Increase in [Ca ²⁺] _i (% of control)
TPA (10 nM)	76.6 ± 2.8 ^a
OAG (300 μM)	75.1 ± 3.9 ^a
SC9 (10 nM)	75.7 ± 3.6 ^a

^a The values are significantly different ($P < 0.01$) compared to the corresponding values without protein kinase C activators, as measured by Student's *t* test.

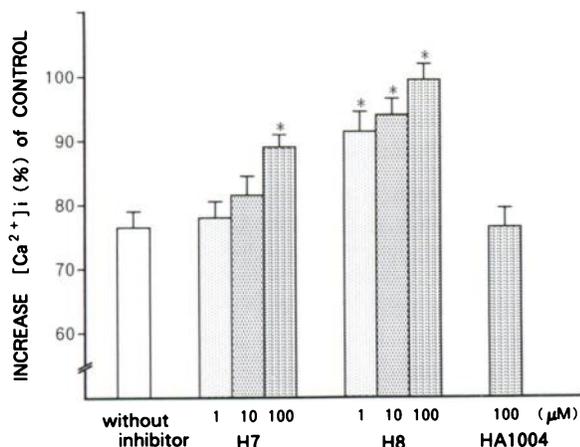


Fig. 4. Effect of protein kinase C inhibitors on the inhibitory effect of TPA in the PGE₁-induced elevation of [Ca²⁺]_i. The cells loaded with fura 2-AM were washed, resuspended in HEPES-Tyrode solution that contained 1 mM Ca²⁺, exposed to H7, H8, or HA1004 for 5 min at 37°C, treated with TPA (10 nM) for 5 min, and then stimulated with 1 μM PGE₁. The effect of 1 μM PGE₁, which increased [Ca²⁺]_i over the resting level without TPA and inhibitors, was taken as 100% control. Other details are described under "Materials and Methods." Results are expressed as means ± SD of three independent experiments. *, values are significantly different ($P < 0.01$) compared to the corresponding values without inducers, as measured by Student's *t* test.

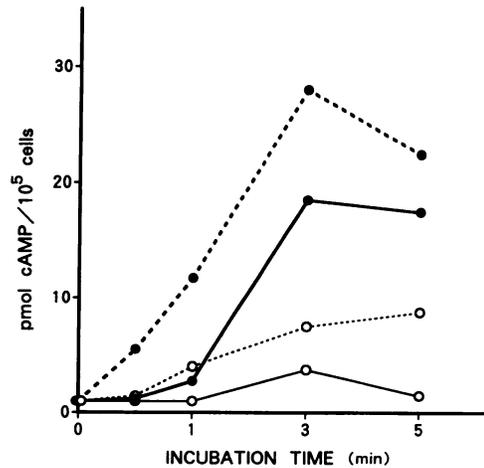


Fig. 5. Kinetics of modulation by TPA (10 nM) of the PGE₁-stimulated cAMP accumulation in CMK cells. CMK cells were treated with (---) or without (—) 10 nM TPA for 5 min and then stimulated with 1 μM (○) or 10 μM (●) PGE₁ for various periods of time. Other details are described under "Materials and Methods." No change in cAMP was observed in the absence of PGE₁, and the basal level of cAMP was less than 1.0 pmol/10⁵ cells in the unstimulated CMK cells. This figure represents the mean of duplicates for independent experiments and data points were within 10% of the mean.

Table 2 Phenotypic differentiation of CMK cells in the absence and presence of TPA and PGE₁

CMK cells were treated with TPA for 48 h. The cell surface antigenic changes toward more mature megakaryocytes were analyzed with monoclonal antibodies (224B, 227A, Thr-4, and PLT1). Other details are described under "Materials and Methods." Results are expressed as means ± SD of three independent experiments.

Inducers	% of positive cells			
	224B (GPIIb/IIIa)	227A (GPIb)	Thr-4 (GPIIIa)	PLT1
Control	76.1 ± 1.3	19.9 ± 5.2	74.2 ± 3.8	10.8 ± 2.4
TPA (10 nM)	91.0 ± 7.5	60.5 ± 4.1	90.2 ± 8.3	64.8 ± 2.7
PGE ₁ (1 μM)	78.2 ± 1.3	17.7 ± 6.1	72.3 ± 5.0	9.7 ± 1.1
PGE ₁ + TPA	97.1 ± 1.1	64.3 ± 4.1	97.0 ± 1.3	64.6 ± 4.7

As shown in Table 2, treatment of CMK cells with 10 nM TPA enhanced the reactivity with antiplatelet antibodies (anti-GPIIb/IIIa, -GPIb, and -GPIIIa and PLT1). In contrast, 1 μM PGE₁ had no inductive effect on the maturation of CMK cells. PGE₁ plus TPA also enhanced the expression of megakaryocytic markers, which was similar to augmentation with TPA alone.

DISCUSSION

We have previously observed the thrombin-induced thromboxane A₂ synthesis and calcium mobilization in CMK (26), a megakaryocytic leukemia cell line, as well as the release of platelet-like particles from these cells (15). These results indicate that CMK cells have the same cellular transduction systems for thrombin as do platelets. Our present study, however, has shown for the first time that PGE₁ has a stimulatory effect on calcium mobilization in megakaryocytic cells, although PGE₁ inhibits the agonist-induced elevation of [Ca²⁺]_i in platelets (7, 8). Prostaglandin E₂ and prostaglandin I₂ similarly cause the elevation of [Ca²⁺]_i in CMK cells, while these prostaglandins are known to inhibit platelet activation (7–9) (data not shown).

PGE₁ is known to possess many biological actions through cAMP accumulation (27). Since PGE₁ increases the cAMP level in CMK cells, as in platelets, the elevation of [Ca²⁺]_i may be postulated to be related to the cAMP accumulation. However, the increase in [Ca²⁺]_i is not mimicked by the addition of dibutyryl cAMP (1–5 μM) and forskolin (10–100 μM). More-

over, TPA alone fails to stimulate the cAMP formation in CMK cells, but TPA causes about a 2-fold increase in the PGE₁-induced cAMP synthesis. Nevertheless, TPA has an inhibitory effect on the PGE₁-induced elevation of [Ca²⁺]_i. These results suggest that cAMP is not involved in the PGE₁-induced Ca²⁺ mobilization in CMK cells. Similar findings have been shown in Swiss 3T3 fibroblasts (28). In contrast to CMK cells, cAMP plays an inhibitory role in controlling Ca²⁺ mobilization in platelets (9, 10). Thus, it seems likely that the transmembrane signaling mechanism for PGE₁ might be different in cells with platelet-megakaryocyte lineage. Since CMK cells are induced into more differentiated cells by TPA, CMK cells and platelets may be good models for studying the possible mechanisms of PGE₁ and cAMP on the Ca²⁺ mobilization in cells with platelet-megakaryocyte lineage.

Our study has also revealed that TPA produces about a 30% inhibition of the PGE₁- and thrombin-induced Ca²⁺ signal. Several works have demonstrated that the inhibitory effects of phorbol esters on the agonist-induced cellular processes are mediated through protein kinase C activation (16–21). We have examined the role of protein kinase C in the inhibition by TPA of the PGE₁-induced elevation of [Ca²⁺]_i. In our study, the TPA-induced inhibition is mimicked by OAG (29) and SC9 (30, 31), known to be other protein kinase C activators. Moreover, H7 and H8, known to be protein kinase C inhibitors (32), restore the inhibition by TPA of the PGE₁-induced elevation of [Ca²⁺]_i. These results indicate that protein kinase C is related to the regulation of the PGE₁-induced Ca²⁺ mobilization and support the notion that protein kinase C plays an important role in negative feedback regulation in various cells (33).

PGE₁ is known to be one of the growth factors present in some types of cells. The elevation of [Ca²⁺]_i and the accumulation of cAMP induced by PGE₁ have been reported to be independently involved in the expression of *c-myc* and *c-fos* genes in mouse 3T3 fibroblasts (11). We have studied the effects of PGE₁ on the phenotypic differentiation of CMK cells. However, PGE₁ shows no inductive effect on CMK cells. The biological role of PGE₁ remains to be determined in this cell line.

ACKNOWLEDGMENTS

The authors are grateful to K. Doi for her skillful assistance.

REFERENCES

- Gerrard, J. M., Peterson, D. A., and White, J. G. Calcium mobilization. *In: J. L. Gordon (ed.), Platelets in Biology and Pathology*, Vol. 2, pp. 407–436. Amsterdam: Elsevier/North-Holland Biomedical Press, Inc., 1981.
- Rink, J. T., Smith, S. W., and Tsien, R. Y. Cytoplasmic free Ca²⁺ in human platelets: Ca²⁺ thresholds and Ca²⁺-independent activation for sharp-change and secretion. *FEBS Lett.*, **148**: 21–26, 1982.
- Brass, L. F., and Shattil, S. J. Changes in surface bound and exchangeable calcium during platelet activation. *J. Biol. Chem.*, **257**: 14000–14005, 1982.
- Kawahara, Y., Yamanishi, J., Furuta, Y., Kaibuchi, K., Takai, Y., and Fukuzaki, H. Elevation of cytoplasmic free calcium concentration by stable thromboxane A₂ analogue in human platelets. *Biochem. Biophys. Res. Commun.*, **117**: 663–669, 1983.
- Hallam, T. J., and Rink, T. J. Responses to adenosine diphosphate in human platelets loaded with the fluorescent calcium indicator quin2. *J. Physiol. (Lond.)*, **368**: 131–146, 1985.
- Murer, E. H. The role of platelet calcium. *Semin. Hematol.*, **22**: 313–323, 1985.
- Haslam, R. J., Davidson, M. M. L., Davies, T., Lynham, J. A., and McClenaghan, M. D. Regulation of blood function by cyclic nucleotides. *Adv. Cyclic Nucleotide Res.*, **9**: 533–552, 1978.
- Feinstein, M. B., Rodan, G. A., and Culter, L. S. Cyclic AMP and calcium in platelet function. *In: J. L. Gordon (ed.), Platelets in Biology and Pathology*, Vol. 2, pp. 437–472. Amsterdam: Elsevier/North-Holland Biomedical Press, Inc., 1981.
- Feinstein, M. B., Egan, J. J., Sha'afi, R. I., and White, J. The cytoplasmic concentration of free calcium in platelets is controlled by stimulators of cyclic AMP production (PGD₂, PGE₁, forskolin). *Biochem. Biophys. Res. Commun.*, **133**: 598–604, 1983.
- Yamanishi, J., Kawahara, Y., and Fukuzaki, H. Effect of cyclic AMP on cytoplasmic free calcium in human platelets stimulated by thrombin: direct measurement with quin 2. *Thromb. Res.*, **32**: 183–188, 1983.
- Yamashita, T., Tsuda, T., Hamamori, Y., and Takai, Y. Possible involvement of cyclic AMP and calcium ion in prostaglandin E₁-induced elevation of *c-myc* mRNA levels in Swiss 3T3 fibroblasts. *J. Biol. Chem.*, **261**: 16878–16882, 1986.
- Lieberherr, M. Effect of vitamin D₃ metabolites on cytosolic free calcium in confluent mouse osteoblasts. *J. Biol. Chem.*, **262**: 13168–13178, 1987.
- Yokohama, H., Tanaka, T., Ito, S., Negishi, M., Hayashi, H., and Hayashi, O. Prostaglandin E receptor enhancement of catecholamine release may be mediated by phosphoinositide metabolism in bovine adrenal chromaffin cells. *J. Biol. Chem.*, **263**: 1119–1122, 1988.
- Sunami, S., Fuse, A., Simizu, B., Eguchi, M., Hayashi, Y., Sugita, K., Nakazawa, S., Okimoto, Y., Sato, T., and Nakajima, H. The *c-sis* gene expression in cells from a patient with acute megakaryoblastic leukemia and Down's syndrome. *Blood*, **70**: 368–371, 1987.
- Sato, T., Fuse, A., Eguchi, M., Hayashi, Y., Ryo, R., Adachi, M., Kishimoto, Y., Teramura, M., Mizoguchi, H., Shima, Y., Komori, I., Sunami, S., Okimoto, Y., and Nakajima, H. Establishment of a human leukemic cell line (CMK) with megakaryocytic characteristics from a Down's syndrome patient with acute megakaryoblastic leukemia. *Br. J. Haematol.*, in press, 1989.
- MacIntyre, D. E., McNicol, A., and Drummond, A. H. Tumor-promoting phorbol esters inhibit agonist-induced phosphatidate formation and Ca²⁺ flux in human platelets. *FEBS Lett.*, **180**: 160–164, 1985.
- Zavoico, G. B., Halenda, S. P., Sha'afi, R. I., and Feinstein, M. B. Phorbol myristate acetate inhibits thrombin-stimulated Ca²⁺ mobilization and phosphatidylinositol 4,5-bisphosphate hydrolysis in human platelets. *Proc. Natl. Acad. Sci. USA*, **82**: 3859–3862, 1985.
- Poll, C., and Westwick, J. Phorbol esters modulate thrombin-operated calcium mobilization and dense granule release in human platelets. *Biochim. Biophys. Acta*, **886**: 434–440, 1986.
- Krishnamurthi, S., Joseph, S., and Kakkar, V. V. Synergistic potentiation of 5-hydroxytryptamine secretion by platelet agonists and phorbol myristate acetate despite inhibition of agonist-induced arachidonate/thromboxane and β -thromboglobulin release and Ca²⁺ mobilization by phorbol myristate acetate. *Biochem. J.*, **238**: 193–199, 1986.
- Yoshida, K., Dubyak, G., and Nachmias, V. T. Rapid effects of phorbol ester on platelet shape change, cytoskeleton and calcium transient. *FEBS Lett.*, **206**: 273–278, 1986.
- Pollock, W. K., Sage, S. O., and Rink, T. J. Stimulation of Ca²⁺ efflux from fura-2-loaded platelets activated by thrombin or phorbol myristate acetate. *FEBS Lett.*, **210**: 132–136, 1987.
- Tsien, R. Y., Rink, T. J., and Poenie, M. Measurement of cytosolic free Ca²⁺ in individual small cells using fluorescence microscopy with dual excitation wavelengths. *Cell Calcium*, **6**: 145–157, 1985.
- Adachi, M., Ryo, R., Yoshida, A., Yoneda, N., Tatsumi, E., Yamaguchi, N., and Kishimoto, Y. Kinetic study of platelet glycoproteins and PPO activity in TPA-induced K562 cells. *Acta Haematol. Jpn.*, **51**: 1069–1079, 1988.
- Yoshioka, M., Ryo, R., Hirai, K., Yasunaga, K., and Yamaguchi, N. Preparation of platelet GPIIb/IIIa complex monoclonal antibody and its clinical application. *Acta Haematol. Jpn.*, **51**: 1138–1146, 1988 (in Japanese).
- Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., and Nishizuka, Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.*, **257**: 7847–7851, 1982.
- Ryo, R., Sato, T., Adachi, M., Yoshida, A., Yamaguchi, N., and Konaka, Y. Thrombin-induced thromboxane synthesis and cytosolic calcium ion mobilization in human megakaryoblastic leukemic cells. *Congr. Int. Soc. Hematol.*, **22**: 293, 1988.
- Samuelsson, B., Goldyn, M., Granström, E., Hamberg, M., Hammarström, S., and Mamsten, C. Prostaglandins and thromboxanes. *Annu. Rev. Biochem.*, **47**: 997–1029, 1978.
- Yamashita, T., and Takai, Y. Inhibition of prostaglandin E₁-induced elevation of cytoplasmic free calcium ion by protein kinase C-activating phorbol esters and diacylglycerol in Swiss 3T3 fibroblasts. *J. Biol. Chem.*, **262**: 5536–5539, 1987.
- Kaibuchi, K., Takai, Y., Sawamura, M., Hoshijima, M., Fujikura, T., and Nishizuka, Y. Synergistic functions of protein phosphorylation and calcium mobilization in platelet activation. *J. Biol. Chem.*, **258**: 6701–6704, 1983.
- Ito, M., Tanaka, T., Inagaki, M., Nakanishi, K., and Hidaka, H. *N*-(6-Phenylhexyl)-5-chloro-1-naphthalenesulfonamide, a novel activator of protein kinase C. *Biochemistry*, **25**: 4179–4184, 1986.
- Nishino, H., Kitagawa, K., Iwashima, A., Ito, M., Tanaka, T., and Hidaka, H. *N*-(6-Phenylhexyl)-5-chloro-1-naphthalenesulfonamide is one of a new class of activators for Ca²⁺-activated, phospholipid-dependent protein kinase. *Biochim. Biophys. Acta*, **889**: 236–239, 1986.
- Hidaka, H., Inagaki, M., Kawamoto, S., and Sasaki, Y. Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry*, **23**: 5036–5041, 1984.
- Nishizuka, Y. Studies and perspectives of protein kinase C. *Science (Wash. DC)*, **233**: 305–312, 1986.