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1,2-DIOCTANOYL-GLYCEROL INDUCES A DISCRETE BUT TRANSIENT TRANSLOCATION OF PROTEIN KINASE C AS WELL AS THE INHIBITION OF MCF-7 CELL PROLIFERATION

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Exposure of MCF-7 human breast cancer cells to phorbol esters such as 12-0-tetradecanoylphorbol-13-acetate (TPA) results in a dose-dependent inhibition of cell proliferation. One of the earliest biochemical events induced by TPA is the translocation of protein kinase C from the cytosolic to the particulate compartment. We have investigated the effects of permeant diacylglycerol 1,2 - dioctanoyl-glycerol (diC_8) on both protein kinase C activity and MCF-7 cell proliferation. DiC_8 induces a discrete but significant translocation of protein kinase C within the first minutes of MCF-7 cell treatment (26 ± 6 %, mean ± SD of 5 different experiments, upon 5 min incubation in the presence of 43 µg/ml diC_8). However, this effect is only transient as the enzymatic activity returns to the control value after 60 min. DiC_8 mimics the effect of TPA on MCF-7 cell proliferation. The dose-response curves for both protein kinase C translocation and cell growth inhibition show that diC_8 exerts its effects on both parameters in the same range of concentrations, despite some discrepancies at the lowest doses. We also report that long-term treatment of the cells with diC_8 does not lead to the protein kinase C disappearance observed during prolonged exposure to TPA. All together, our results reinforce the hypothesis of a negative modulatory role of protein kinase C in MCF-7 cell proliferation and suggest that the enzyme translocation but not its down-regulation could be a pre-requisite in the biological cell response.

Protein kinase C has emerged as a pivotal element of the transmembrane signaling system in a wide variety of cell responses to exogenous stimuli (1,2). Diacylglycerol (DG) generated from the receptor - coupled breakdown of inositol phospholipids is considered as the intracellular messenger for the activation of protein kinase C (3). Phorbol esters such as 12-0-tetradecanoyl phorbol-13-acetate can substitute for diacylglycerol to activate the enzyme (4), which most probably represents the high affinity phorbol ester receptor in

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ABBREVIATIONS : TPA, 12-0-tetradecanoylphorbol-13-acetate ; diC_8, 1,2-dioctanoyl-glycerol ; DG, diacylglycerol.

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target cells (5,6). A large body of evidence indicates that treatment of cultured cells with TPA results in an immediate subcellular redistribution of protein kinase C from the cytosolic to the particulate compartment (7-9). As a consequence, the enzyme translocation has been considered as the first step in the protein kinase C activation and as a pre-requisite in the ligand-receptor mediated cell response (10). However, such an enzyme movement upon physiological cell stimuli has been demonstrated only in a few cases by now (11-13). Permeant diacylglycerols appear as useful tools to demonstrate the physiological relevance of the translocation phenomenon but again few reports (14,15) are so far available about a DG-induced protein kinase C translocation. We have previously shown that treatment of MCF-7 human breast cancer cells with active phorbol esters such as TPA led to a dose-dependent inhibition of cell proliferation (16). TPA caused very rapidly the intracellular translocation of both phorbol ester binding and protein kinase C activities (17). We report now that permeant diacylglycerol diC_8 mimics the effect of TPA on MCF-7 cell proliferation. We show that diC_8 induces a discrete but transient translocation of protein kinase C in the range of concentrations which are effective in inhibiting cell growth.

MATERIALS AND METHODS

Chemicals

Histone H1, TPA, phosphatidyl-serine, 1,2-dioleoyl-glycerol and 1,2 dioctanoyl-glycerol were obtained from Sigma. (γ-^{32}P) ATP (0.5-3 Ci/mmol) was purchased from Amersham. DEAE-cellulose (DE52) was from Whatman. Leupeptin was obtained from Peptide Institute Inc., Japan. All other chemicals were from Merck.

Cell cultures

MCF-7 cells were grown at 37° C in RPMI 1640 (Gibco), pH 7.3, supplemented with 2g/l of sodium bicarbonate, 2 mM L-glutamine, 1 µM insulin (Novo Laboratories) and 5 % foetal calf serum (FCS, Seromed). Culture media were changed every two days. For cell growth measurement, MCF-7 cells were plated at an initial density of 1-1.5 x 10^4 cells per 35 mm dish. After 48 h (day 0) the medium was replaced by fresh RPMI, 5 % FCS and various concentrations of diC_8 were added. Addition of the permeant diacylglycerol was repeated three times a day. Control dishes received the same volume of the solvent acetone (final concentration of 0.1 %). Cells were counted after 5 days of treatment (day 5).

Subcellular fractionation

Subconfluent cells were scraped from the culture dishes into phosphate-buffered saline (PBS), then homogenized in 20 mM Tris-HCl, pH 7.5, containing 0.25 M

sucrose, 2 mM EDTA, 2 mM EGTA, 100 μg/ml leupeptin, and 5 mM β-mercaptoethanol (buffer A). The cell lysate was centrifuged for 1 h at 105,000g. The supernatant was used as the cytosolic fraction. The corresponding pellet was resuspended in buffer A, containing 0.5 % Triton X-100, and briefly sonicated. After incubation at 4° C for 45 min it was centrifuged for 1 h at 105,000 g. The supernatant recovered was used as the 0.5 % Triton-extract of the particulate fraction.

DEAE-cellulose chromatography

Cytosol or 0.5 % Triton-extract of particulate fraction obtained from 40 x 10^6 cells was applied to a DEAE-cellulose column (DE52, 0.8 x 3 cm), equilibrated with 20 mM Tris-HCl, pH 7.5, containing 2 mM EDTA, 1mM EGTA, 50 μg/ml PMSF and 5 mM β-mercaptoethanol (buffer B). Columns were washed with 10ml of buffer B, and protein kinase C was eluted with 5ml of buffer B, containing 0.13 M NaCl.

Protein kinase assay

Protein kinase C was assayed by measuring, in triplicate, the incorporation of ^{32}P from (γ-^{32}P) ATP into histone H1. The standard assay mixture (200 μl) contained 20 mM Tris-HCL, pH 7.4, 40 μg histone, 10 μM ATP (550 cpm/pmol), 5mM $MgCl_2$, 40 μl of sample and either 0.5 mM EGTA or 0.5 mM $CaCl_2$, 16 μg of phosphatidyl-serine and 0.6 μg of 1,2-dioleoyl-glycerol. Enzymatic activity was expressed as pmol ^{32}P incorporated into histone per minute per 10^6 cells.

RESULTS AND DISCUSSION

As shown on Fig 1, when repeatedly added to the culture medium, the permeant diacylglycerol diC_8 causes a dose-dependent inhibition of MCF-7 cell

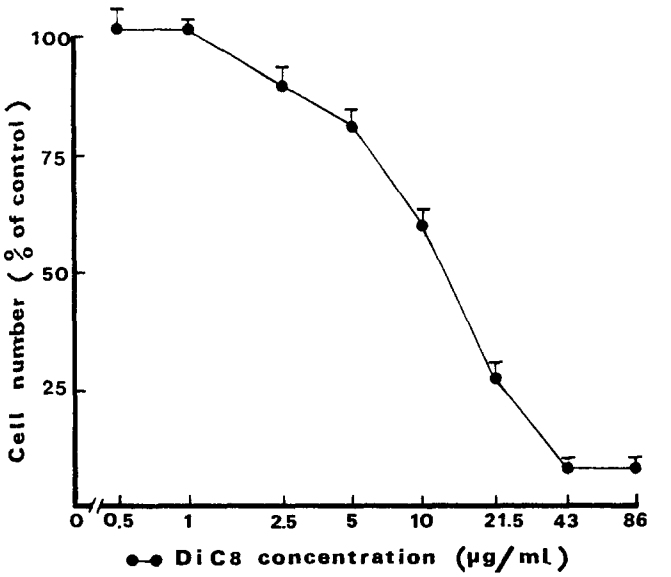

FIGURE 1 . DiC_8-induced inhibition of MCF-7 cell proliferation.
MCF-7 cells were cultured as indicated in Materials and Methods. The number of control cells increased from 4 x 10^4 cells at day 0 to 1 x 10^6 cells at day 5. Data are the mean ± SD of 5 measurements from one representative experiment. Results are expressed as percent of the control cell number.

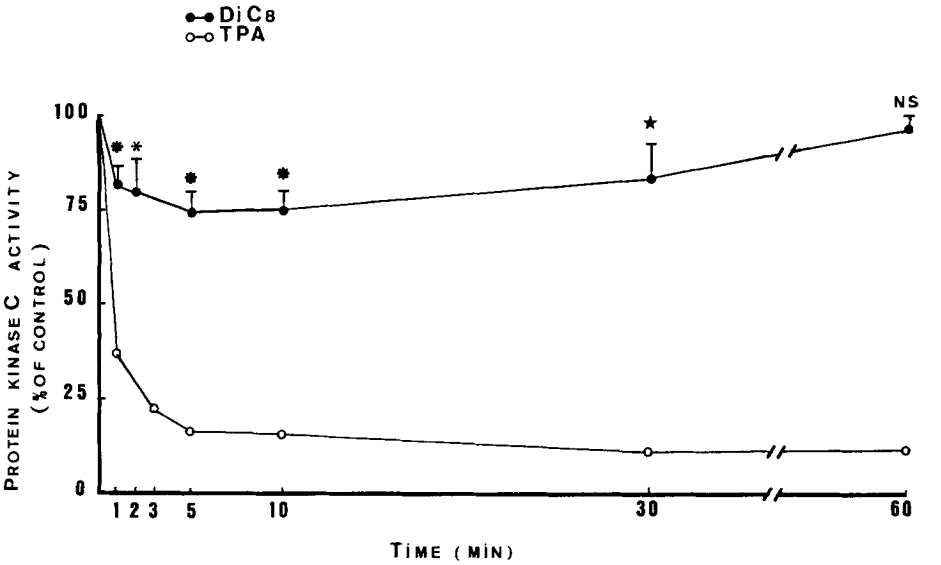

FIGURE 2 . Time course of both diC_8 and TPA effects on cytosolic protein kinase C. MCF-7 cells were incubated for various times in the absence (control) or in the presence of 43 µg/ml diC_8 (●) or 100 ng/ml TPA (○). Data are the mean ± SD of 4 to 5 different experiments. *p < 0.001, *p < 0.01, ★p < 0.05, NS : not significant (Student's t-test).

proliferation ($ED_{50} \simeq 11$ µg/ml). The fact that the intracellular messenger DG mimics the previously reported inhibitory effect of TPA on MCF-7 cell growth (16) strongly suggests the implication of protein kinase C in the phorbol ester action and reinforces the hypothesis of a negative modulatory role of the enzyme in MCF-7 cell proliferation, as precedently postulated (16-19).

Fig 2 shows that diC_8 induces very rapidly a discrete but significant translocation of protein kinase C, probed as the enzymatic activity drop in the cytosolic compartment. After 5 min of MCF-7 cell treatment with 43 µg/ml diC_8 26 ± 6 % (mean ± SD of 5 different experiments) of protein kinase C activity was lost at the cytosolic level. A concomitant increase occurred in the particulate fraction (fig 3), although the enzyme recovery at this level did not appear very reproducible from one experiment to another. The diC_8 effect on protein kinase C translocation is only transient as the cytosolic enzymatic activity was not significantly different from the control after 60 min of cell treatment (fig 2). As previously reported (17-19), TPA causes a dramatic (83 % upon 5 min of cell treatment) and irreversible protein kinase C translocation. This acute difference between the effects of TPA and diC_8 may be of interest to

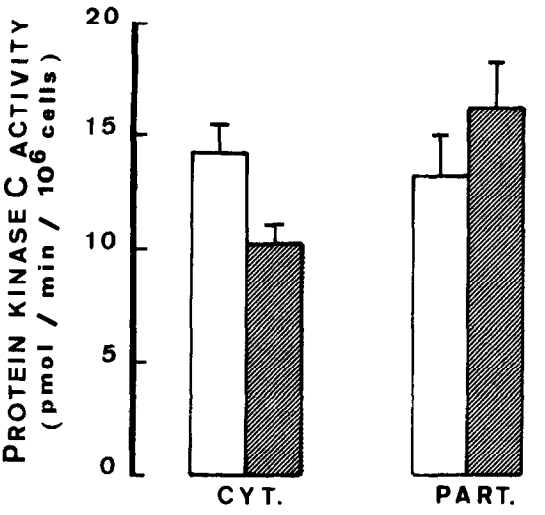

FIGURE 3 . Effect of diC_8 on both cytosolic and particulate protein kinase C. MCF-7 cells were incubated for 10 min in the absence (empty columns) or in the presence (hatched columns) of 43 µg/ml diC_8. Protein kinase C was assayed on cytosolic (Cyt) and particulate (Part) fractions. Data are the mean ± SE of 4 different experiments.

account for the tumorigenic nature of active phorbol esters. It is tempting to postulate that, following DG-induced translocation, protein kinase C is very rapidly released from the membrane compartment. On the contrary, TPA could bind the enzyme at this level in a tight and irreversible manner. Moreover, conversely to TPA, DG is known to be rapidly metabolized. It is interesting to note that the cell growth inhibition is only observed when diC_8 is added three times a day, while one addition of TPA every two days is enough to produce maximal effect (16).

Fig 4 shows that the diC_8 effect is dose-dependent. The dose-response curves for both protein kinase C translocation and cell growth inhibition (Fig 1) demonstrate that the permeant diacylglycerol exerts its effects on both parameters in the same range of concentrations albeit some discrepancies at the lowest doses, as 0.5-1 µg/ml diC_8 induced enzyme translocation but not growth inhibition.

Fig 5 shows that, contrary to TPA, diC_8 is unable to induce the down-regulation of cellular protein kinase C during long-term treatment of MCF-7 cells. Neither cytosolic nor particulate protein kinase C activity is modified upon repeated exposure of the cells to diC_8 for 48 h. These results suggest that the

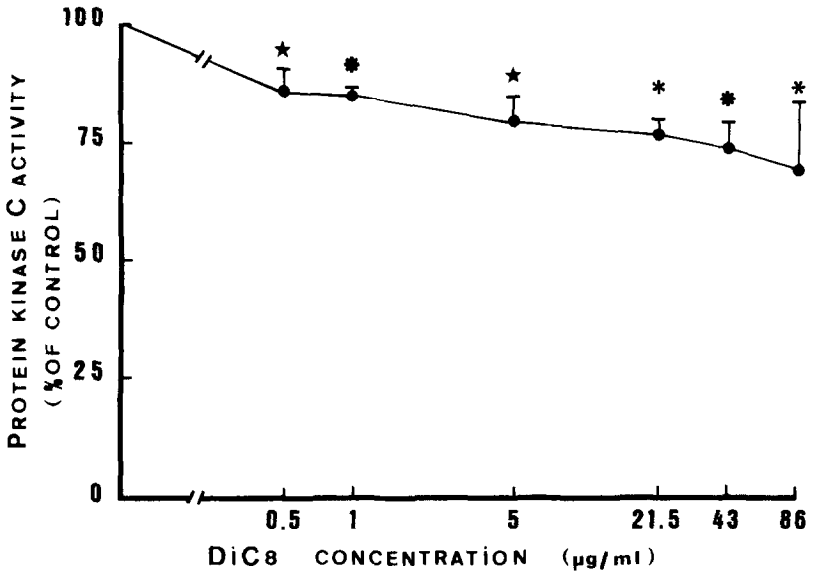

FIGURE 4 . Dose-response of the diC_8 effect on cytosolic protein kinase C. MCF-7 cells were incubated for 5 min in the absence (control) or in the presence of various concentrations of diC_8. Data are the mean ± SD of 3 to 5 different experiments. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ (student's t-test).

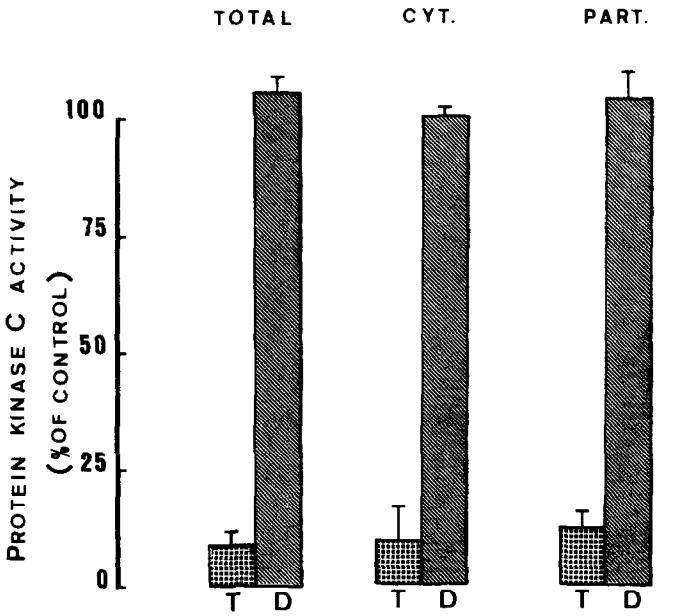

FIGURE 5 . Effects of long-term MCF-7 cell treatment with diC_8 or TPA on protein kinase C activity.
MCF-7 cells were exposed to 100 ng/ml TPA (T) or 43 µg/ml diC_8 (D) for 48 hours. Addition of the permeant diacylglycerol was repeated three times a day. Protein kinase C activities were measured on the 0.5 % Triton-extract of the cell homogenate (Total) or on cytosol (Cyt) and 0.5 % Triton-extract of the particulate fraction (Part). Data are the means ± SE of 3 to 6 different experiments and are expressed as percent of the values obtained in control cultures.

diacylglycerol cannot trigger the enzyme processing that occurs at the membrane level after TPA stimulation (17,19). We cannot rule out the possibility that a de novo synthesis of protein kinase C could balance a DG-induced degradation of the translocated enzyme. In any case, the cell growth arrest caused by TPA and diC_8 does not seem linked to a disappearance of protein kinase C as the permeant diacylglycerol is effective in inhibiting cell proliferation without affecting the cellular content of the enzyme.

Alternatively, the acute differences between the effects of TPA and DG on protein kinase C translocation and following processing could be due to the ability of the two activators to bind and activate different isozymic forms of protein kinase C (20,21). Such an hypothesis is reinforced by the fact that TPA and DG have been shown to cause differential biochemical and biological responses in several cell systems (22-24).

In conclusion, our results emphasize the role of protein kinase C in the control of MCF-7 cell proliferation, but suggest that the two activators of the enzyme, TPA and diC_8, can induce the translocation process in a distinct manner. Nevertheless, protein kinase C redistribution always appears as a possible pre-requisite for its activation and subsequent biological cell response. On the contrary, the enzyme down-regulation process does not seem involved in the cell growth inhibition.

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