Journal of Cellular Biochemistry 48:288–295 (1992)

# Protein Kinase C Involved in Zymosan-Induced Release of Arachidonic Acid and Superoxide but Not in Calcium Ionophore-Elicited Arachidonic Acid Release or Formation of Prostaglandin $E_2$ From Added Arachidonate

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**Abstract** Zymosan and phorbol ester induced in liver macrophages the release of arachidonic acid, prostaglandin $E_2$, and superoxide; the calcium ionophore A 23187 elicited a release of arachidonic acid and prostaglandin $E_2$ but not of superoxide, and exogenously added arachidonic acid led to the formation of prostaglandin $E_2$ only. The zymosan- and phorbol-ester-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide was dose-dependently inhibited by staurosporine and K252a, two inhibitors of protein kinase C, and by pretreatment of the cells with phorbol ester which desensitized protein kinase C. The release of arachidonic acid or prostaglandin $E_2$ following the addition of A 23187 or arachidonic acid was not affected by these treatments. Zymosan and phorbol ester but not A 23187 or arachidonic acid induced a translocation of protein kinase C from the cytosol to membranes in intact cells. These results demonstrate an involvement of protein kinase C in the zymosan- and phorbol-ester-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide; the release of arachidonic acid and prostaglandin $E_2$ elicited by A 23187 and the formation of prostaglandin $E_2$ from exogenously added arachidonic acid, however, is independent of an activation of protein kinase C.

**Key words:** macrophages, phospholipase $A_2$, NADPH oxidase, prostaglandins, staurosporine, phorbol ester

Resident liver macrophages (Kupffer cells) possess the ability to secrete a wide array of biologically active compounds including eicosanoids (prostaglandin $D_2$, $E_2$, $F_{2\alpha}$, thromboxane) and superoxide [1]. Eicosanoid formation has been shown to be elicited by a number of particulate (zymosan, glucan, immune complexes, latex particles coated with IgG) or soluble (calcium ionophore, fluoride, platelet activating factor, C3a) agents [2–6] while the generation of superoxide is triggered by zymosan, glucan, and platelet activating factor only [2,3]. It is widely accepted that the synthesis of eicosanoids is limited by the availability of free intracellular arachidonic acid, the precursor of all eicosanoids. The concentration of free arachidonic acid in the cell is thought to be regulated by its liberation from phospholipids, mainly by a phospholipase $A_2$ [7], and by its re-esterification into membrane lipids by an acyltransferase [5]. Superoxide generation is catalysed by a membrane-bound NADPH oxidase [8]. The release of arachidonic acid and prostanoids but not of superoxide in liver macrophages has been shown to be under the control of glucocorticoids [2], of the $Na^+/H^+$-exchanger [9], and of calcium ions [10]. Calmodulin does not seem to participate in the regulation of phospholipase $A_2$ [11] while cyclic AMP exerts no [10] or an inhibitory effect [12] on the synthesis of prostaglandins.

We [2] and others [13–16] recently showed that the formation of eicosanoids and superoxide can be elicited by phorbol esters or synthetic diacylglycerols, suggesting an involvement of protein kinase C in the formation of these mediators. In the present paper we demonstrate the effect of staurosporine and of K252a, two inhibitors of protein kinase C [17,18], and pretreatment of the cells with phorbol ester (which is known to desensitize protein kinase C [19]) on the stimulus-induced release of arachidonic acid,

Received July 22, 1991; accepted October 25, 1991.

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prostaglandin $E_2$, and superoxide in liver macrophages. Furthermore, the subcellular distribution of protein kinase C in nonstimulated and stimulated cells is presented.

## MATERIALS AND METHODS

### Materials

Cytochrome c, 4 α-phorbol 12, 13 didecanoate, arachidonic acid, 1,2-dioleoyl-rac-glycerol, L-α-phosphatidylserine, histone type III-S, and zymosan were purchased from Sigma (München, Germany). Zymosan suspensions were kept at 95°C for 30 min to destroy endogenous phospholipase $A_2$ activity. Medium RPMI 1640 and newborn calf serum were obtained from Biochrom (Berlin, Germany); newborn calf serum was heat-inactivated at 56°C for 30 min. The calcium ionophore A 23187 was obtained from Calbiochem (Gießen, Germany) and the phorbol ester, phorbol 12-myristate 13-acetate (PMA), from Pharmacia (Freiburg, Germany). Bovine serum albumin (0.99 mg lipid/g albumin) was from Behring-Werke (Marburg, Germany). Cellulose nitrate filters (pore size 0.45 μm) were purchased from Sartorius (Göttingen, Germany). Adenosine 5′-[γ-$^{32}$P] triphosphate and [5,6,8,9,11, 12,14,15-$^{3}$H] arachidonic acid (180 Ci/mmol) were obtained from Amersham Buchler (Frankfurt, Germany). The antibodies raised against prostaglandin $E_2$ and protein kinase C were generous gifts from Dr. M. Reinke (Erlangen, Germany) and Dr. U. Rapp (Frederick, USA), respectively. Samples of purified protein kinase C α, β, and γ were kindly provided by Dr. G. Kochs (Freiburg, Germany). All other chemicals were of analytical grade.

### Cell Culture

Male Wistar rats (250–350 g) were fed ad libitum with Altromin$^{R}$. The livers were removed aseptically under Nembutal$^{R}$ anesthesia and the Kupffer cells isolated by the centrifugal elutriation procedure of Brouwer et al. [20] as modified by Eyhorn et al. [21]. The cells were maintained in primary culture with RPMI medium containing 30% newborn calf serum. Changes of the medium and purity checks of the cells were performed as previously described [5]. The viability and purity of the cells was always better than 95% as judged by trypan blue exclusion, staining of β-glucuronidase, and phagocytosis of latex particles (1 μm diameter), respectively. The number of attached cells was determined microscopically. All experiments were performed with Kupffer cells kept in primary culture for 72 h. At that time the cells have attained their typical cell shape and surface structures (e.g., F-receptors).

### Determination of Released [$^{3}$H] Arachidonic Acid

Kupffer cells (48 h in primary culture) were incubated for 24 h with 2 μCi [$^{3}$H] arachidonic acid. Then the media were removed and the cells washed thoroughly and incubated without or with various stimuli in Hanks' solution containing 2% (w/v) bovine serum albumin. After 1 h the media were removed and centrifuged and the radioactivity determined in the supernatants. In the presence of albumin the radioactivity released into the cell media consists predominantly of arachidonic acid [5].

### Determination of Superoxide and Prostaglandin $E_2$

Kupffer cells (72 h in primary culture) were incubated in Hanks' solution without or with various stimuli. After 1 h the media were removed and centrifuged. Prostaglandin $E_2$ was determined in the supernatants by radioimmunoassay. In the absence of albumin almost no arachidonic acid but considerable amounts of prostanoids (prostaglandin $D_2$, $E_2$, $F_{2\alpha}$ and thromboxane $B_2$) are released into the cell media [5].

Superoxide was measured as reduction of cytochrome c inhibitable by superoxide dismutase according to Nakagawara and Minakami [22].

### Preparation of Subcellular Fractions and Determination of Protein Kinase C Activity

For the preparation of subcellular fractions the cells were scraped off the culture dishes and disrupted in isolation buffer (40 mM Hepes, 4 mM EDTA, 2 mM EGTA, 10 mM dithiothreitol, 100 μg/ml phenylmethylsulfonylfluoride) by brief sonication. The homogenate was sedimented at 100,000*g* for 60 min at 4°C and the corresponding membrane fraction resuspended by brief sonication in isolation buffer. Protein kinase C activity was determined by measuring the incorporation of radioactive phosphate from [γ-$^{32}$P] ATP into histone in the presence and absence of $Ca^{2+}$, diacylgylcerol, and phosphatidylserine. The assay (400 μl) consisted of 60 mM Hepes, 1.8 mM EGTA, 2 mM EDTA, 2 mM $CaCl_2$, 10 mM $MgCl_2$, 5 mM dithiothreitol, 50 μg/ml phenylmethylsulfonylfluoride, 63 μM

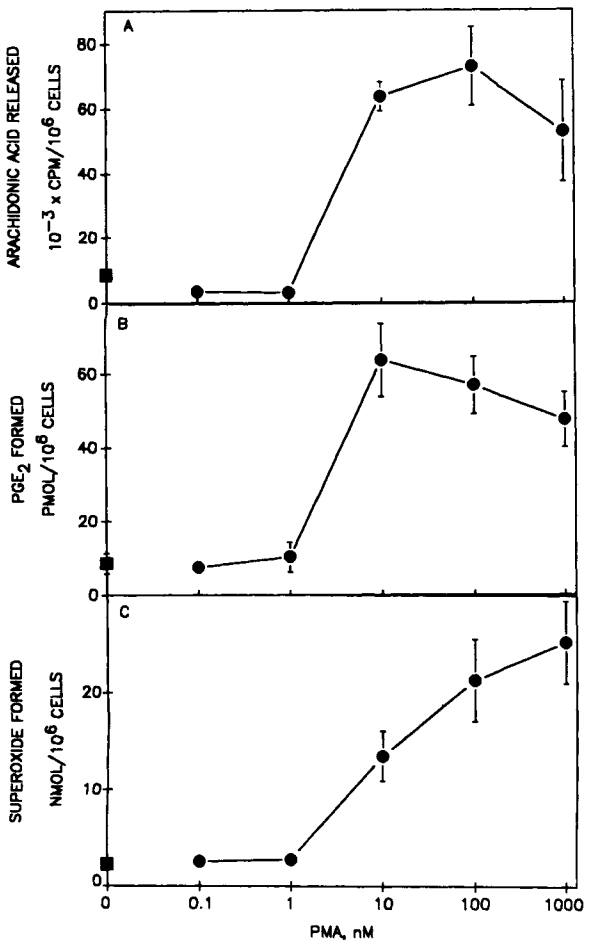


Fig. 1. Effect of PMA on the release of arachidonic acid, prostaglandin $E_2$, and superoxide in liver macrophages. Cells (48 h in primary culture) were incubated without or with 2 μCi [$^3$H] arachidonic acid. After 24 h the cells were washed thoroughly and incubated for 60 min in Hanks' solution without (squares) or with (circles) different concentrations of PMA. The release of [$^3$H] arachidonic acid (**A**), prostaglandin (PG) $E_2$ (**B**), and superoxide (**C**) was determined as described in Materials and Methods. Results are means ± SD of four independent experiments.

phosphatidylserine, 15 μM dioleoylglycerol, 200 μg/ml histone III-S, 5 μg sample protein, and 0.01 μmol ATP (300,000 cpm). The reaction was started by adding [γ-$^{32}$P] ATP to the assay, allowed to run at 37°C for 12 min and stopped by adding ice-cold 5% (v/v) $H_3PO_4$. The acid precipitable material was collected on a nitrocellulose filter (pore size 0.45 μm; Sartorius, Germany) and washed three times with 4 ml 5% (v/v) $H_3PO_4$. Incorporated radioactivity was measured by Cerenkov counting. Protein kinase C activity is expressed as the difference between the values measured in the presence and absence of $Ca^{2+}$ and lipids, respectively. Under these conditions the reaction proceded linearly with time and the amount of enzyme added (data not shown).

### Immunoblot Analysis

$10^5$ cells were lysed with 300 μl sodium dodecylsulfate (SDS) polyacrylamide sample buffer (100 mM Tris-HCl, pH 6.8, 5% SDS, 100 mM dithiothreitol), mixed vigurously, and boiled for 5 min. Samples (30 μl) were run on 7.5% SDS polyacrylamide gels [23] under reducing conditions. Proteins were transferred to Hybond ECL Nitrocellulose membranes (Amersham) and nonspecific binding blocked by incubation of the membranes in 0.02 M Tris-HCl, pH 7.5, 0.15 M NaCl, and 5% bovine serum albumin. After washing, a polyclonal antibody which recognizes the pseudosubstrate segment of protein kinase C α, β, and γ was added at a dilution of 1:2,000 in 0.02 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.1% Tween, and 1% bovine serum albumin. Specific binding was determined with the RPN 2106 Amersham ECL Western blotting detection system.

**TABLE I. Effect of Zymosan, PMA, and α-Phorbol 12,13 Didecanoate on Release of Arachidonic Acid, Prostaglandin $E_2$, and Superoxide***

| Addition | AA (cpm) | Release of $PGE_2$ (pmol) per $10^6$ cells | Superoxide (nmol) |
|---|---|---|---|
| None | 13,500 ± 838 | 5 ± 3 | 4 ± 2 |
| Zymosan | 68,502 ± 2,402 | 48 ± 7 | 38 ± 4 |
| PMA | 57,802 ± 2,002 | 43 ± 4 | 35 ± 7 |
| 4α-phorbol 12,13 didecanoate | 12,588 ± 792 | 6 ± 2 | 3 ± 4 |

*Cells (48 h in primary culture) were incubated without or with 2 μCi [$^3$H] arachidonic acid. After 24 h the cells were washed thoroughly and incubated for 60 min in Hanks' solution without or with zymosan (0.5 mg/ml), PMA (1 μM), or α-phorbol 12,13 didecanoate (1 μM). The release of [$^3$H] arachidonic acid (AA), prostaglandin (PG) $E_2$ and superoxide was determined as described in Materials and Methods. Results are mean ± SD of three to six independent experiments.

## RESULTS

The addition of PMA to cultured rat liver macrophages caused a dose-dependent stimulation of arachidonic acid, prostaglandin $E_2$, and superoxide release into the cell media (Fig. 1). The release of arachidonic acid and prostaglandin $E_2$ was elicited half-maximally at about 4 nM PMA and reached a plateau between 10 nM and 1 μM PMA (Fig. 1A,B). In contrast, superoxide generation increased almost linearly from 1 nM to 1 μM PMA (Fig. 1C). The effect of PMA on the release of arachidonic acid, prostaglandin $E_2$, and superoxide was quantitatively similar to that of zymosan (Table I). In contrast, an inactive form of the phorbol ester, 4 α-phorbol 12,13 didecanoate [24], was without any effect (Table I).

The PMA-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide was dose-dependently inhibited by staurosporine (Fig. 2) and K252a (data not shown), two inhibitors of protein kinase C [17,18]. Arachidonic acid release and formation of prostaglandin $E_2$ were almost completely inhibited at 1 μM staurosporine while lower concentrations of staurosporine were not very effective (Fig. 2A,B); in contrast, an almost complete inhibition of the PMA-induced superoxide generation occurred already at 100 nM staurosporine (Fig. 2C). The dose-inhibition curve of staurosporine for superoxide but not for arachidonic and prostaglandin $E_2$ release was dependent on the concentration of PMA (Fig. 2). The shift of the curve for superoxide release (Fig. 2C) indicates that in this case the inhibition was competitive. The basal release of the mediators was not affected by staurosporine or K252a (data not shown). The protein kinase C inhibitor H7 [25] was without any effect up to concentrations of 10 μM; at higher concentrations an inhibition of the generation of prostaglandin $E_2$ and superoxide was observed; however, this effect was accompanied by an H7-induced leakage of lactate dehydrogenase indicating cell damage at these higher concentrations (data not shown).

It has been shown that treatment of cells with phorbol ester leads to a desensitization of protein kinase C [19]. In Kupffer cells, the protein kinase C activity, measured in corresponding cell homogenates, decreased from 658 ± 57 pmol/min × mg in untreated cells to less than 30 pmol/min × mg in cells treated with 100 nM PMA for 24 h (see Fig. 5). The inactive form of

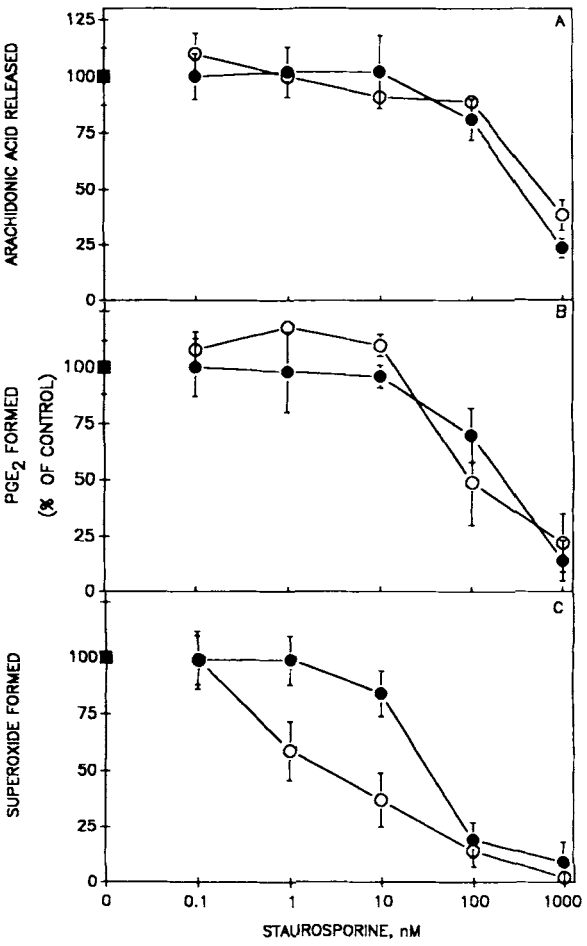


**Fig. 2.** Effect of staurosporine on the PMA-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide in liver macrophages. Cells (48 h in primary culture) were incubated without or with 2 μCi [$^3$H] arachidonic acid. After 24 h the cells were washed thoroughly and incubated for 60 min in Hanks' solution in the absence (squares) or presence (circles) of different concentrations of staurosporine. Fifteen minutes later 1 μM (closed circles) or 50 nM (open circles) PMA was added. The release of [$^3$H] arachidonic acid (**A**), prostaglandin (PG) $E_2$ (**B**), and superoxide (**C**) was determined as described in Materials and Methods. A value of 100% corresponds to a release of 121.000 ± 26.800 (118.600 ± 25.400) cpm [$^3$H] AA/$10^6$ cells, 69 ± 19 (71 ± 12) pmol $PGE_2$/$10^6$ cells, and 30 ± 6 (16 ± 8) nmol superoxide/$10^6$ cells (values in brackets: 50 nM PMA). Results are means ± SD of three to four independent experiments.

the phorbol ester, 4α-phorbol 12,13 didecanoate, did not alter the activity of protein kinase C (data not shown). Expression of protein kinase C in nontreated and phorbol-ester-pretreated cells was examined additionally by an immunoblot method using polyclonal antibodies which recognizes the pseudosubstrate segment of protein kinase C α, β, and γ. Figure 3 shows that pretreatment with phorbol ester led to a com-

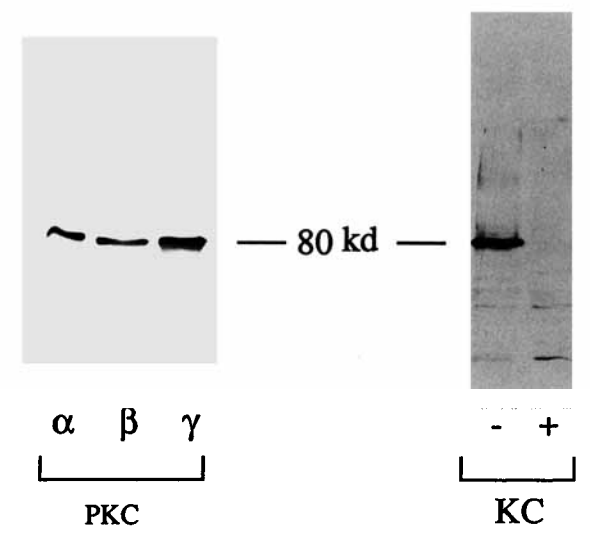


Fig. 3. Western blot analysis of protein kinase C expression in nontreated and PMA-treated liver macrophages. Cells (48 h in primary culture) were incubated without (−) or with (+) 100 nM PMA for 24 h. Cell lysates and samples of purified protein kinase (PK) C α, β, and γ were electrophoresed, transferred onto nitrocellulose, and probed with polyclonal antibodies against protein kinase C as described in Materials and Methods. KC, Kupffer cells. The figure shows a typical set of data which was reproduced at least several times.

plete disappearance of protein kinase C in liver macrophages.

Pretreatment of the cells with PMA for 24 h inhibited dose-dependently the PMA-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide (Fig. 4). Half-maximal inhibition was obtained at about 3 nM and 0.8 nM PMA for the release of arachidonic acid, prostaglandin $E_2$, and superoxide, respectively. Complete inhibition was achieved between 10 nM and 100 nM PMA (Fig. 4). The basal release of arachidonic acid, prostaglandin $E_2$, or superoxide was not affected by PMA pretreatment (data not shown).

Table II compares the effects of staurosporine, K252a, and PMA pretreatment on the release of arachidonic acid, prostaglandin $E_2$, and superoxide in cells incubated with PMA, zymosan, the calcium ionophore A 23187, and arachidonic acid. The PMA-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide was almost completely inhibited by staurosporine, K252a, or desensitization of protein kinase C (Table II). Arachidonic acid and prostaglandin $E_2$ release induced by zymosan was decreased by staurosporine, K252a, and PMA pretreatment to about 50%; zymosan-elicited superoxide generation was slightly more affected by these treatments (Table II). The PMA- and zymosan-elicited formation of other prostanoids including

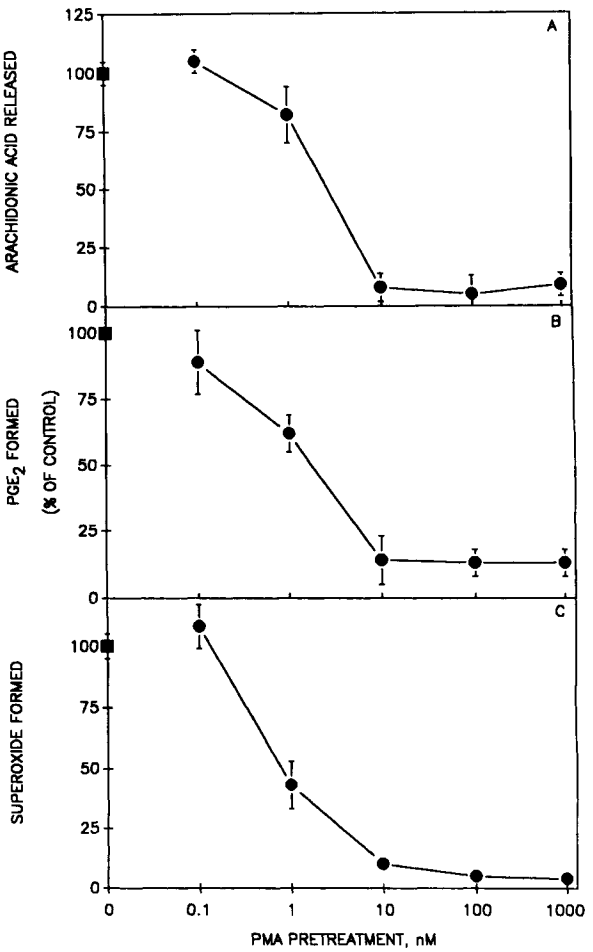


Fig. 4. Effect of PMA pretreatment on the PMA-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide in liver macrophages. Cells (48 h in primary culture) were incubated without or with 2 μCi [$^3$H] arachidonic acid in the absence (squares) or presence (circles) of different concentrations of PMA. After 24 h the cells were washed thoroughly and incubated for 60 min in Hanks' solution containing PMA (1 μM). The release of [$^3$H] arachidonic acid (A), prostaglandin (PG) $E_2$ (B), and superoxide (C) was determined as described in Materials and Methods. Results are means ± SD of four independent experiments. A value of 100% corresponds to a release of 101,000 ± 24,000 cpm [$^3$H] AA/$10^6$ cells, 69 ± 19 pmol $PGE_2/10^6$ cells, and 30 ± 6 nmol superoxide/$10^6$ cells.

prostaglandin $D_2$ and thromboxane $B_2$ was inhibited by staurosporine and by PMA pretreatment to about the same extent (data not shown). In contrast, prostaglandin $E_2$ formation from exogenously added arachidonic acid and the A 23187-elicited release of arachidonic acid and prostaglandin $E_2$ were not affected by staurosporine, K252a, or desensitization of protein kinase C (Table II).

When resting liver macrophages were homogenized about 70% of the protein kinase C activ-

**TABLE II. Effect of Staurosporine, K252a, and PMA Pretreatment on Stimulus-Induced Release of Arachidonic Acid, Prostaglandin $E_2$, and Superoxide***

| Addition | Release of AA | Release of $PGE_2$ (% of control) | Release of Superoxide |
|---|---|---|---|
| PMA | 100 | 100 | 100 |
| + staurosporine | 20 ± 5 | 10 ± 5 | 2 ± 5 |
| + K252a | 21 ± 11 | 10 ± 8 | 21 ± 3 |
| + PMA | 5 ± 2 | 3 ± 3 | 8 ± 4 |
| Zymosan | 100 | 100 | 100 |
| + staurosporine | 47 ± 9 | 40 ± 8 | 30 ± 10 |
| + K252a | 47 ± 11 | 42 ± 8 | 21 ± 1 |
| + PMA | 39 ± 14 | 50 ± 8 | 20 ± 8 |
| A 23187 | 100 | 100 | n.d. |
| + staurosporine | 95 ± 14 | 88 ± 12 | n.d. |
| + K252a | 110 ± 22 | 132 ± 29 | n.d. |
| + PMA | 120 ± 23 | 95 ± 5 | n.d. |
| AA | n.d. | 100 | n.d. |
| staurosporine | n.d. | 86 ± 18 | n.d. |
| + K252a | n.d. | 123 ± 28 | n.d. |
| + PMA | n.d. | 129 ± 19 | n.d. |

*Cells (48 h in primary culture) were incubated without or with 2 μCi [$^3$H] arachidonic acid and with or without PMA (100 nM). After 24 h the cells were washed thoroughly and incubated for 60 min in Hanks' solution without or with PMA (1 μM), zymosan (0.5 mg/ml), A 23187 (1 μM), or arachidonic acid (30 μM). Staurosporine (1 μM) or K252a (1 μM) were added 15 min prior to the stimulus. The release of [$^3$H] arachidonic acid (AA), prostaglandin (PG) $E_2$, and superoxide was determined as described in Materials and Methods. Results are means ± SD of three to five independent experiments. A value of 100% corresponds to a relase of 138.000 ± 16.800, 208.000 ± 23.000, 178.600 ± 21.200 cpm [$^3$H] AA/$10^6$ cells, 78 ± 14, 85 ± 24, 75 ± 18, 88 ± 14 pmol $PGE_2$/$10^6$ cells, and 32 ± 5, 33 ± 4 nmol superoxide/$10^6$ cells after addition of PMA, zymosan, A 23187, and arachidonic acid, respectively. n.d., not detectable.

ity was located in the cytosolic fraction, and 30% was detectable in the particulate fraction (see Table III). PMA induced a rapid and irreversible translocation of protein kinase C from the cytosol to membranes (Fig. 5A). Five minutes after exposure of macrophages to PMA almost all of the enzymatic activity was associated with the particulate fraction, whereas the activity of protein kinase C in the soluble fraction simultaneously decreased. Longer treatments with PMA (24 h) led to a complete disappearance of protein kinase C activity in both fractions (Fig. 5A). The addition of zymosan to liver macrophages resulted in an incomplete and transient translocation of protein kinase C (Fig. 5B). The enzyme activity associated to membranes increased between 10 and 15 min after exposure of macrophages to zymosan while the activity in the soluble fraction simultaneously decreased. Thirty minutes after addition of zymosan the distribution and the activity of protein kinase C was about the same as in unstimulated macrophages (Fig. 5B). Interestingly, the zymosan-induced release

**TABLE III. Effect of PMA, Zymosan, A 23187, and Arachidonic Acid on Cytosolic and Membrane-Associated Protein Kinase C Activity in Liver Macrophages***

| Addition | Protein kinase C activity: Soluble (% of total) | Protein kinase C activity: Particulate (% of total) |
|---|---|---|
| None | 71 ± 5 | 29 ± 5 |
| PMA | 9 ± 7 | 91 ± 7 |
| Zymosan | 44 ± 14 | 57 ± 14 |
| A 23187 | 66 ± 2 | 34 ± 2 |
| Arachidonic acid | 66 ± 4 | 34 ± 4 |

*Cells (72 h in primary culture) were incubated in Hanks' solution without or with PMA (1 μM, 10 min), zymosan (0.5 mg/ml, 15 min), A 23187 (1 μM, 10 min), or arachidonic acid (30 μM, 10 min). Then the cells were washed thoroughly; cytosolic and membrane fractions were prepared and protein kinase C activity determined as described in Materials and Methods. The total activity in the homogenate was set to 100% and corresponds to 658 ± 57 pmol/min × mg. Treatment of the cells with the different agents did not alter this activity (data not shown). Results are means ± SD of four to five independent experiments.

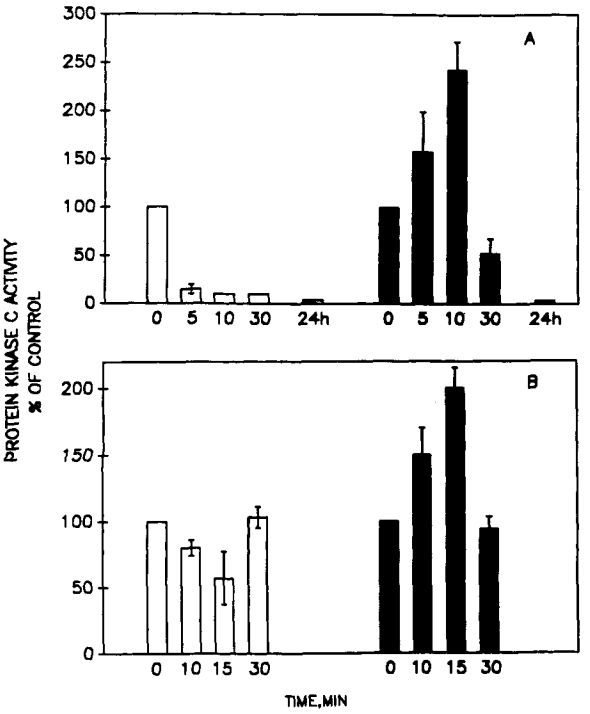


Fig. 5. Effect of PMA and zymosan on the cytosolic and membrane-associated protein kinase C activity in liver macrophages. Cells (48 h in primary culture) were incubated without or with PMA (100 nM). After 24 h the cells were washed thoroughly and incubated in Hanks' solution without or with PMA (1 μM; **A**) or zymosan (0.5 mg/ml; **B**). After the times indicated the cells were washed thoroughly; cytosolic (open bars) and membrane fractions (filled bars) were prepared and protein kinase C activity determined as described in Materials and Methods. The activities of protein kinase C in the soluble and membrane fractions from nonstimulated cells were set to 100% and correspond to 427 ± 51 pmol/min × mg and 214 ± 39 pmol/min × mg, respectively. Results are means ± SD of four to five independent experiments.

of arachidonic acid and prostaglandin $E_2$ increases almost linearly up to 60 min [2,5]. In contrast to PMA and zymosan, the calcium ionophore A 23187 and arachidonic acid did not induce a translocation of protein kinase C (Table III).

## DISCUSSION

In resident liver macrophages about one-third of the protein kinase C activity is associated with membranes while two-thirds of the enzyme activity are found in the cytosolic fraction. PMA induced a rapid and irreversible translocation of protein kinase C activity from the soluble to the particulate fraction; longer treatments with PMA led to a complete disappearance of protein kinase C. Zymosan, a compound which is recognized by cell surface receptors [26] and phagocytosed by Kupffer cells [2], led to an incomplete and reversible translocation of protein kinase C from the cytosol to membranes. No down-regulation of the enzyme activity could be detected with zymosan. The calcium ionophore A 23187 and arachidonic acid did not alter the cellular distribution or the activity of protein kinase C. These results indicate that PMA and zymosan but not A 23187 or arachidonic acid elicit an activation of protein kinase C in liver macrophages. Active phorbol esters like PMA are known to intercalate into cellular membranes and mediate a high-affinity interaction between protein kinase C and membranes [19], thus mimicking the effect of diacylglycerol, the endogenous activator of protein kinase C [27]. Recently we showed that zymosan activates a phospholipase C in liver macrophages leading to the release of inositol phosphates [28] and thereby presumably to the formation of diacylglycerol. Therefore, it is most likely that the zymosan-induced translocation of protein kinase C is mediated by diacylglycerol.

Zymosan and PMA are known to elicit in liver macrophages the release of arachidonic acid, prostanoids, and superoxide [2,5]. Recent findings demonstrate that cyclic AMP and calmodulin are not involved in these processes [10–12] indicating that cyclic AMP- and $Ca^{2+}$-calmodulin-dependent protein kinases play no major role in these pathways. In order to elucidate a possible role of protein kinase C the effects of staurosporine, K252a, and H7, three inhibitors of protein kinase C, and of down-regulation of protein kinase C by PMA pretreatment were investigated. Arachidonic acid and prostaglandin $E_2$ release elicited by A 23187 or prostaglandin $E_2$ formation from exogenous arachidonic acid was not affected by these treatments. In contrast, the PMA- and zymosan-induced release of arachidonic acid, prostaglandin $E_2$, and superoxide was dose-dependently inhibited by staurosporine, K252a, or PMA pretreatment. However, while the PMA-induced responses were almost totally suppressed by inhibition or down-regulation of protein kinase C, the zymosan-induced release of these mediators was only partially inhibited. This indicates that the action of zymosan is only partially mediated by protein kinase C and/or that activation of protein kinase C enhances the effects of zymosan. It is not yet clear at which sites of the signal transduction

pathways protein kinase C exerts its action. In the case of superoxide formation it has been postulated that protein kinase C activates directly NADPH oxidase or some endogenous NADPH oxidase regulatory proteins [8,15,16]. In the case of eicosanoid formation our results indicate that the conversion of arachidonic acid into prostaglandins is independent of protein kinase C. This favours a role for protein kinase C in the regulation of the free level of intracellular arachidonic acid. Both liberation of arachidonic acid from phospholipids by a phospholipase $A_2$ [7,29] and reacylation of the fatty acid by a lysophosphatide acyltransferase [5,29] are reported to be influenced by phorbol esters or synthetic diacylglycerols [30,31]. Therefore, lysophosphatide acyltransferase and phospholipase $A_2$ may represent linking mechanisms between activation of protein kinase C and formation of eicosanoids. It may, additionally, also be that different isozymes of protein kinase C exert different actions in the formation of superoxide and arachidonate metabolites. The dependency of the various subtypes of protein kinase C on the activation by phorbol ester has been shown to be different [32]. The fact that the concentration dependencies of arachidonic acid, prostaglandin $E_2$, and superoxide release for activation with PMA (Fig. 1), inhibition by staurosporine (Fig. 2), or PMA pretreatment (Fig. 4) are not identical supports this hypothesis.

## ACKNOWLEDGMENTS

We wish to thank Prof. K. Decker for many helpful comments and suggestions. This work was supported by grants to K. Decker from the Deutsche Forschungsgemeinschaft (through SFB 154) and from the Bundesministerium für Forschung und Technologie (Förderkennzeichen 01 GA 8816/0), Bonn/Bad Godesberg.

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