Dexamethasone down-regulates the 85 kDa phospholipase A₂ in mouse macrophages and suppresses its activation

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We have studied the effects of dexamethasone (dex) (i) on the level of the arachidonate-mobilizing phospholipase A₂ (PLA₂-85) in macrophages, (ii) on the stimulus-induced activation of this enzyme, and (iii) on the stimulus-induced release of arachidonate. Treatment of macrophages with 10 nM dex led to progressive reduction of PLA₂-85 down to approx. 35% of control levels in 20 h in the absence of stimuli. This was accompanied by a partial inhibition of calcium-ionophore-induced arachidonate release. In contrast, the ability of zymosan or phorbol ester to cause both persistent activation of PLA₂-85 and arachidonate release was greatly reduced or abolished. However, the protein phosphatase inhibitor okadaic acid, pre-

viously shown to cause enhanced phosphorylation and persistent activation of PLA₂-85, was still able to exert this effect on the dex-suppressed PLA₂-85. This suggests that the effect of okadaic acid was exerted at, or downstream of, the dex-sensitive step(s). Treatment with dex also led to inhibition of the characteristic changes in phosphoprotein labelling induced by phorbol ester or zymosan. However, phorbol-dibutyrate-binding isoforms of protein kinase C were not severely down-regulated. Thus dex was found to down-regulate PLA₂-85 and, in addition, to affect one or more component(s) in the signal chain that normally leads to its activation. However, okadaic acid retained the ability to cause activation of PLA₂-85.

INTRODUCTION

Synthetic glucocorticoids, such as dexamethasone (dex), are potent anti-inflammatory agents that also inhibit the formation of eicosanoids. The latter is long since known to be due to inhibition of the mobilization of arachidonate [1], but the mechanism for this effect has remained unclear. It was previously associated with the induction of proteins called lipocortins, the classic view being that lipocortins inhibited the enzymic activity of phospholipase A₂ and that this inhibition could be alleviated by stimulus-induced phosphorylation of lipocortin [1,2]. However, lipocortins/calpactins have been shown to be members of a family of proteins [2,3] which can bind to anionic phospholipids and actin filaments in a calcium-dependent manner and which inhibit phospholipase A₂ only under certain conditions and then by sequestration of its substrate rather than by interaction with the enzyme [4,5]. Furthermore, lipocortin I was found to be present in considerable amounts in resident mouse macrophages, but did not undergo phosphorylation upon stimulation with 4 β -phorbol 12-myristate 13-acetate (PMA) [6]. This indicated that the protein kinase C (PKC)-mediated activation of arachidonate release was not due to phosphorylation and inactivation of lipocortin I.

More recently, an intracellular 85 kDa phospholipase A₂ (PLA₂-85) has been isolated and characterized (reviewed in [7,8]). This enzyme has been implicated in the mobilization of the eicosanoid precursor arachidonate in mouse macrophages [9,10]. The mechanisms that have hitherto shown potential to regulate PLA₂-85 include changes in the cytosolic Ca²⁺ level [11–13] and phosphorylation cascades, both dependent [14] and independent [15,16] of PKC. In the present paper we investigate the effects of

the glucocorticoid analogue dex on the basal level of PLA₂-85 and on the signalling for its activation, as well as the effects on the stimulus-induced release of arachidonate in mouse macrophages.

EXPERIMENTAL

Materials

Cell culture reagents were from Flow Laboratories. All other chemicals were from Sigma Chemical (St. Louis, MI, U.S.A.), except for general laboratory reagents which were of the highest quality available. Polyclonal rabbit antibodies raised against recombinant PLA₂-85 were obtained from Genetics Institute Inc., Cambridge, MA, U.S.A.

Arachidonate release

Peritoneal macrophages were isolated and cultured by a modification of the method described previously [17]. Peritoneal cells were collected from female mice (NMRI, Bommice, Denmark) in Medium 199 containing serum (1%, v/v) and heparin (20 units/ml). The cells were allowed to adhere to plastic 12-well tissue-culture dishes. The number of adherent cells per well was approximately 1.0×10^6 . After 2 h incubation non-adherent cells were removed by washing with Dulbecco's phosphate-buffered saline (PBS) containing Ca²⁺ and Mg²⁺. The adherent macrophages were kept in culture for 20 h in Medium 199 containing 10% (v/v) serum at 37 °C in a humidified atmosphere of 5% CO₂.

Abbreviations used: dex, dexamethasone; DMSO, dimethyl sulfoxide; PLA₂-85, intracellular 85 kDa phospholipase A₂; PKC, protein kinase C; PMA, 4 β -phorbol 12-myristate 13-acetate; PDBu, 4 β -phorbol 12,13-dibutyrate; TNF- α , tumour necrosis factor- α ; IL-1, interleukin-1; TGF β , transforming growth factor β ; M-CSF, macrophage colony-stimulating factor.

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in air. During this culture period the cells were labelled with 1 μ Ci of [3 H]arachidonate (Amersham International; 212 Ci/mmol) per well, and, when indicated, dex was included in the medium. Dex was kept as a stock solution in dimethyl sulphoxide (DMSO) and for each experiment was freshly diluted in medium. The final concentration of DMSO never exceeded 0.1 %. The presence of dex did not affect labelling with [3 H]arachidonate as seen after 20 h. On the second day the cells were washed three times with PBS and Medium 199 was added for 30 min. Medium was removed and replaced with fresh Medium 199 to which appropriate stimulus, zymosan (200 μ g/ml), A23187 (1 μ M), PMA (100 nM) or okadaic acid (0.5 μ M; Bio-Zac, New Boston St., Woburn, MA, U.S.A.) was added for the time indicated. The dishes were put on ice, the medium was taken off and the cells were scraped in 0.1 % Triton X-100 (w/v). Both the tubes containing medium and the tubes containing cell extract were centrifuged for 5 min at 2500 g. The release of incorporated [3 H]arachidonate was determined from the radioactivity in the culture medium and the total radioactivity in cell extract and medium.

Dex by itself showed no inhibitory effect on the basal release of 3 H-labelled arachidonate from unstimulated peritoneal macrophages, neither did it affect uptake and esterification during the labelling period. However, a dose-dependent alteration in cellular morphology was observed after treatment with dex, in that the proportion of well-spread cells with spike-like extensions was reduced. Since no increase in lactate dehydrogenase in the medium was detected, hypothetical membrane alterations would be limited.

PLA₂ activity

PLA₂-85 was assayed according to the method described previously [9]. 1-Stearoyl-2-[1- 14 C]arachidonoyl phosphatidylcholine (Amersham International; 80 pmol/incubation) in the form of sonicated vesicles was used as substrate. Aliquots of the cytosol fraction containing PLA₂-85 were incubated in the presence of substrate, 1.4 mM CaCl₂ (approx. 400 μ M free Ca²⁺) and BSA (0.19 mg/ml) in a buffer containing 80 mM KCl, 10 mM Hepes and 1 mM EDTA, pH 7.4. The final volume was adjusted to 525 μ l. After incubation for 30 min at 37 °C the reaction was stopped by the addition of 1.5 ml of chloroform/methanol/HCl (20:10:1, by vol.), to which 0.05 % 2,6-di-*t*-butyl-*p*-cresol and carrier lipids had been added. After centrifugation the lipid phase was applied to a silicic acid column equilibrated in chloroform. Free fatty acids were eluted with 1.0 ml of chloroform and phospholipids were eluted with 2.5 ml of methanol.

Western-blot analysis of PLA₂-85

Macrophages (1.5 $\times$ 10⁷ cells/25 cm² flask) were cultured as described above. Half the volume of whole-cell extracts prepared in Laemmli sample buffer supplemented with 100 mM dithioerythritol were boiled for 5 min and electrophoresed on SDS/PAGE (8 % acrylamide). The gel was equilibrated in transfer buffer [18] and the proteins transferred to a 0.2 μ m pore size nitrocellulose membrane. Gelatin (1 %, w/v; for 2 h) was used to block the membrane. A 1/500 dilution of rabbit polyclonal antibodies against PLA₂-85 was incubated for 14 h with the membrane. After extensive washing, bound antibodies were detected with ¹²⁵I-labelled goat anti-(rabbit IgG) antibodies (0.5 $\times$ 10⁶ c.p.m./ml). The blot was dried and thereafter analysed and quantified by digital imaging (Fujix Bas 2000).

Incorporation of [35 S]methionine

Isolation and culture of macrophages was performed as described above with the exception that serum was excluded from the medium. After 10 h incubation with appropriate concentrations of dex, [35 S]methionine (Amersham International; 31 μ Ci/well) was included in the medium. After another 10 h incubation in the presence of both dex and [35 S]methionine the dishes were put on ice, the medium was taken off and the cells were washed three times in PBS and then either scraped in 1.0 % (w/v) Triton X-100 or precipitated with 12 % trichloroacetic acid.

[3 H]4 β -Phorbol 12,13-dibutyrate (PDBu) binding

Macrophage cultures were incubated in the presence or absence of dex as described above. After washing on the second day 5 pmol (approx. 2 $\times$ 10⁵ d.p.m.) [3 H]PDBu (NEN, Bedford, MA, U.S.A.) was added to each well. A concentration of 3 μ M unlabelled PDBu was used for correction for non-specific binding of [3 H]PDBu. [3 H]PDBu was allowed to bind for 2 h at 4 °C. The cells were washed three times with PBS containing 0.1 % BSA. The cells were then scraped off in 1 % Triton X-100 and the radioactivity was determined in the medium and in the cell extract.

[32 PO₄]Phosphoprotein labelling, electrophoresis and autoradiography

Each culture, in phosphate-free medium, was exposed to ³²PO₄ (50 μ Ci) for 90 min or 4 h as indicated. Phosphorylation patterns in whole-cell extracts were studied after autoradiography of SDS/PAGE gels (12 % acrylamide), as previously described [6]. After electrophoresis at constant current (16 mA) for about 18 h, the gels were fixed, stained with Coomassie Blue R250, destained and dried under vacuum. The dried gels were exposed to X-ray film (Amersham, International) for time periods ranging from 4 h to 48 h. The intensity of bands on autoradiograms, and Coomassie Blue-stained and dried gels, were quantified using a video densitometer (MAKAB, Gothenburg).

Level and ³²PO₄-labelling of ATP

Macrophage cultures were isolated, cultured and exposed to ³²PO₄ for 4 h as described above. The cells were scraped off the dishes in 40 mM KH₂PO₄, pH 2.8. After sonication for 30 s the homogenate was centrifuged for 45 min at 100 000 g to obtain a supernatant and a pellet. The supernatant was then analysed by h.p.l.c. [19] using a linear gradient from 40 mM KH₂PO₄, pH 2.8, to 0.5 M KH₂PO₄/0.8 M KCl, pH 2.7. The flow was adjusted to 1.0 ml/min. A radioisotope detector (Model 171 Radioisotope Detector, Beckman Instruments, U.S.A.) was used to determine the ³²PO₄-labelling of ATP while the amount of ATP was determined from the monitored absorbance at 254 nm.

Other methods

Gel chromatography was run on a Sephadex G-200 superfine column as described previously [9]. Protein determination was performed according to Bradford [20], on cell lysates scraped in 0.1 % (w/v) Triton X-100, with the same amount of the detergent also in standard samples.

RESULTS

Dex down-regulated PLA₂-85

Treatment of mouse macrophages with 10 nM dex for 20 h was found to reduce the activity of PLA₂-85 to 36 $\pm$ 2 %

Figure 1 Reduction of the level of PLA₂-85 by dex

Macrophages were cultured for 20 h in the absence or in the presence of dex as indicated. Then whole-cell extracts were used for Western-blot analysis as described in the Experimental section. The right-hand lane is a positive control containing 125 ng of partially purified PLA₂-85. The density of the control culture was set to 100%. After treatment with 1.0, 10, 100 or 1000 nM dex the density of the bands, as revealed by image quantification, corresponded to 75, 37, 23 and 15% of control respectively.

Table 1 Effect of dex on cellular protein and the incorporation of [³⁵S]methionine

Macrophages were treated with dex for 20 h. The protein level in control cells was approx. 4 µg/culture. During the 20 h incubation [³⁵S]methionine was included in the medium for the last 10 h. The radioactivity in the culture medium and the cell extract was determined. In control cells approx. 0.5% of the [³⁵S]methionine added was incorporated. Data are means ± S.E.M. (*n* = 3–7). N.S.: not significantly different from control (i.e. *P* > 0.05).

Dex (nM)	Cellular protein (% of control)	[³⁵ S]Methionine incorporation (% of control)
0.1	97 ± 3 (n.s.)	93 ± 4 (n.s.)
1.0	93 ± 3 (n.s.)	79 ± 4 (<i>P</i> < 0.05)
10	87 ± 7 (n.s.)	59 ± 3 (<i>P</i> < 0.01)
100	78 ± 4 (<i>P</i> < 0.01)	53 ± 4 (<i>P</i> < 0.01)
1000	83 ± 5 (<i>P</i> < 0.02)	41 ± 8 (<i>P</i> < 0.02)

(mean ± S.E.M., *n* = 27) of control level. A similar reduction of PLA₂-85 was observed by Western blotting (Figure 1), without the appearance of any other immunoreactive protein band. These effects were selective since the total protein content in macrophages preincubated in the presence of 10 nM dex was not significantly reduced (Table 1). A statistically significant, although limited, reduction in total protein level was seen only at ≥ 0.1 µM dex. The effect of dex on the rate of protein synthesis during the latter half of the 20 h period, determined as [³⁵S]methionine incorporation, is also shown in Table 1.

When cytosolic fractions from untreated cultures and from cultures treated with dex (10 nM for 20 h) were subjected to gel chromatography, the activity of PLA₂-85 was found to elute in precisely the same position whether the cells had been treated with dex or not (results not shown). Furthermore, the peak from dex-treated cells showed the same reduction in activity as that determined in samples of unfractionated cytosol. Thus, we found no evidence for any inhibitor(s) that could be separated away by this type of chromatography, nor for higher-molecular-mass complexes dissociating in the enzyme assay. Neither could we find evidence for proteolytic modification of PLA₂-85 into lower-molecular-mass forms with detectable enzyme activity or immunoreactivity.

Dex differentially inhibited the persistent activation of PLA₂-85

Treatment of macrophages with zymosan or PMA in the absence of dex up-regulated the activity of PLA₂-85 to 209 and 212 % of

Figure 2 Effects of dex on PMA-, zymosan- or okadaic acid-induced activation of PLA₂-85

Macrophages were first cultured for 20 h in the absence or presence of dex (10 nM). The cells were then treated with zymosan (200 µg/ml for 30 min), PMA (100 nM for 15 min) or okadaic acid (0.5 µM for 90 min) as indicated. A cytosolic fraction was prepared and PLA₂-85 was assayed as described in the Experimental section. Please note that the activity of PLA₂-85 in non-stimulated cells, both in control cultures and those down-regulated with dex, has been set to 100%. Data are means ± S.E.M. (*n* = 3 or 4).

control respectively (Figure 2), essentially in agreement with previous findings [9,14]. However, after treatment with dex (10 nM for 20 h), neither zymosan nor PMA was able to up-regulate the activity of PLA₂-85 to any comparable extent. This would indicate either that the down-regulated enzyme was unable to undergo persistent activation, or that the signalling to such activation was also affected by dex.

The protein phosphatase inhibitor okadaic acid has previously been shown to cause phosphorylation and activation of PLA₂-85 in macrophages, as well as release of arachidonate [15]. As shown in Figure 2, okadaic acid was able to cause such activation of PLA₂-85 equally well in dex-treated and untreated macrophages, with an increase to approx. 180% of control. Thus, the relative inability of zymosan and PMA to cause persistent activation of PLA₂-85 in dex-treated cells does not appear to be due to any defect in the down-regulated enzyme, but is more likely to be due to attenuation of the signal for its activation. We therefore decided to investigate whether dex caused any changes in the phosphoprotein pattern in resting macrophages and whether it affected the characteristic changes in this pattern induced by zymosan or PMA [6].

Dex caused a dose-dependent decrease in protein phosphorylation

A rather general reduction of protein phosphorylation after treatment with dex has previously been observed in RBL-2H3 cells [21], but not in HeLa cells [22]. Our autoradiograms clearly showed a general decrease in the ³²PO₄-labelling of phospho-

Figure 3 Effect of dex on basal phosphoprotein labelling with ³²PO₄

Dex, as indicated, was included in the culture medium for 20 h. ³²PO₄ (100 μCi) was added to each well for the last 90 min. Cell lysates were then prepared and processed for SDS/PAGE as described in the Experimental section. The experiment shown is representative of three separate experiments.

Table 2 Effect of dex on total ATP content and on [³²P]_iATP

Cells were labelled with ³²PO₄ for 4 h (see the Experimental section). H.p.l.c. was performed on cell extracts from control cells and cells treated with dex for 20 h. Total ATP in the cytosolic fraction was determined as absorbance at 254 nm. Data are means ± S.E.M. (*n* = 3). Abbreviation: n.s., not significantly different from control (i.e. *P* > 0.05).

Dex (nM)	Total ATP (% of control)	[³² P]ATP (% of control)
0.1	98 ± 3 (n.s.)	86 ± 1 (<i>P</i> < 0.01)
1.0	100 ± 9 (n.s.)	73 ± 4 (<i>P</i> < 0.05)
10	101 ± 8 (n.s.)	67 ± 2 (<i>P</i> < 0.01)
100	92 ± 10 (n.s.)	53 ± 2 (<i>P</i> < 0.01)
1000	88 ± 3 (n.s.)	49 ± 5 (<i>P</i> < 0.01)

proteins in resting macrophages as the concentration of dex was increased (Figure 3). However, analysis of the level and ³²PO₄-labelling of ATP showed that the latter was reduced to a similar extent by dex (Table 2), while the level was hardly affected at all. This may explain the general reduction of phosphoprotein labelling in dex-treated cells. Furthermore, both the difference in labelling of ATP and the difference in basal phosphoprotein labelling could be abrogated by extending the labelling time with ³²PO₄ for dex-treated cells from 90 min to 4 h (see Figure 4). In agreement with previous results [6], increased phosphorylation of

Figure 4 Effect of dex on the changes in phosphoprotein labelling induced by PMA and okadaic acid

Macrophages were cultured for 20 h in the absence (left three lanes) or presence (right three lanes) of dex (10 nM) and were labelled with ³²PO₄ (50 μCi) for the last 90 min (left three lanes) or 4 h (right three lanes) respectively. The cells were treated with PMA (100 nM for 15 min) or okadaic acid (0.5 μM for 90 min) as indicated, during the latter part of the labelling period. Cell lysates were then prepared and processed for SDS/PAGE as described in the Experimental section. Densitometric analysis of a number of bands on autoradiograms showed that PMA increased their labelling 2.5- to 5.0-fold (minus dex, 90 min labelling), and 1.1- to 1.6-fold (plus dex, 4 h labelling) respectively. Okadaic acid enhanced the labelling of the corresponding bands 3.2- to 6.0-fold (minus dex) and 4.5- to 7.2-fold (plus dex) respectively. The experiment shown is representative of three separate experiments, all of which were subjected to densitometric analysis.

many protein bands, including a prominent 45 kDa band, was seen in cells stimulated with zymosan (results not shown) or PMA (Figure 4), while in dex-treated cells (10 nM dex for 20 h) stimulation with PMA resulted in a much smaller effect (Figure 4). In accordance with results obtained by Haystead et al. [23] an increase in the phosphorylation of many bands was observed after treatment with 0.5 μM okadaic acid for 90 min. Furthermore, the phosphorylation of a band at approx. 60 kDa appeared to be selectively enhanced after treatment with okadaic acid. Addition of okadaic acid after preincubation with dex (10 nM, 20 h) resulted in a similar increase in phosphorylation as that obtained in untreated cells (Figure 4).

The dex-induced reduction in phosphorylation of the 45 kDa band and other bands in response to PMA raised the question of whether dex caused down-regulation of phorbol-ester-sensitive isoforms of PKC. However, the specific binding of [³H]PDBu decreased only to about the same degree as the total protein level

Figure 5 Effect of dex on stimulus-induced release of [³H]arachidonate

Dex, as indicated, was included in the culture medium for 4 (○), 12 (●) or 20 (▽) h. The cells were then stimulated with (a) A23187 (1 μ M) for 30 min, or (b) zymosan (200 μ g/ml) for 30 min. The results are means ($\pm$ S.E.M. where indicated) from 3–9 independent experiments. The net release in cells not treated with dex was set to 100%. It amounted to 19.5 ± 0.7 ($n = 13$) percent of total cell-associated [³H]arachidonate in the case of A23187, and 16.7 ± 1.2 ($n = 7$) in the case of zymosan.

upon treatment with dex (78 % of control at 10 nM dex for 20 h and 74 % of control at 1.0 μ M dex for 20 h; $n = 2$). This modest decrease in specific binding of [³H]PDBu argues against the possibility that down-regulation of PKC was the cause of the reduction in protein phosphorylation.

Dex differentially inhibited stimulus-induced release of arachidonate

Finally we studied the effect of dex on the release of [³H]arachidonate in prelabelled macrophages stimulated with the calcium ionophore A23187, zymosan (Figure 5) or okadaic acid. After prolonged incubation with dex (≥ 10 nM), a partial

inhibition of the ionophore-induced arachidonate release became evident (Figure 5a). However, the inhibition did not exceed 50 % even after 20 h of treatment with dex. In contrast, dex proved to be much more efficient at inhibiting zymosan-induced arachidonate release (Figure 5b). Treatment with dex (10 nM–1.0 μ M) for 20 h resulted in 90–98 % inhibition of the zymosan-induced release of arachidonate. This inhibition developed progressively with time, being limited at 4 h and approx. half-maximal at 12 h. Macrophages were also treated with okadaic acid (0.5 μ M) for 90 min after 20 h incubation with or without increasing concentrations of dex. A partial inhibition of the okadaic acid-induced release of arachidonate was observed, which amounted to 50 % at 10 nM dex and approx. 65 % at 0.1–1.0 μ M dex.

DISCUSSION

One of the findings presented here is that the synthetic glucocorticoid dex down-regulates PLA₂-85 in resting mouse macrophages. By a combination of Western blotting analysis and activity measurements we could virtually exclude that this was due to either proteolytic modification of the enzyme, or to induction of an inhibitory protein. Neither was it paralleled by any similar reduction in total cellular protein. It was also, from the outset, unlikely that it could be caused by a reduction in the activity of PLA₂-85 due to dephosphorylation of the enzyme, as *in vitro* dephosphorylation of PLA₂-85 from unstimulated macrophages causes a much smaller reduction in activity [14]. Instead, it may be due to inhibition of the formation of PLA₂-85 at the transcriptional or post-transcriptional level, or an enhanced degradation. These possibilities need to be further investigated.

In favour of regulation at the level of biosynthesis are the recent findings that PLA₂-85 can be induced by cytokines such as tumour necrosis factor- α (TNF- α) [22,24], interleukin-1 α (IL-1 α) [25], IL-1 β [26–28], transforming growth factor β 2 (TGF β 2) [26] and macrophage colony-stimulating factor (M-CSF) [29], and that, at least in the cases of TNF- α , IL-1 α and IL-1 β , this induction can be abolished by dex [22,24,25,28]. However, in contrast to our findings in primary macrophage cultures, the basal level of PLA₂-85 was only slightly, or not at all, reduced by dex in the cell lines, or the mesangial cells, employed in these studies.

The release of arachidonate induced by ionophore A23187, zymosan or okadaic acid was inhibited to different degrees by the 20 h pretreatment with dex. Besides the effect on the level of PLA₂-85 and effects on its persistent activation (see below), dex could also cause apparent inhibition of arachidonate release by exerting inhibitory effects on the cyclo-oxygenase and/or 5-lipoxygenase pathways of arachidonate metabolism, or by enhancing reacylating mechanisms. However, it is well known that ionophore A23187 and zymosan both yield prostaglandin E₂ and leukotriene C₄ as major eicosanoids in mouse peritoneal macrophages, while PMA causes selective formation of cyclo-oxygenase metabolites, primarily prostaglandin E₂ [30,31]. We found also that okadaic acid caused selective formation of prostaglandin E₂ and that dex treatment (10 nM for 20 h) inhibited arachidonate release and prostaglandin E₂ formation to a similar extent (by 50 and 66 % respectively). Thus, we find no simple relationship between the differential inhibition of arachidonate release caused by dex and possible inhibitory effects on either the cyclo-oxygenase or the 5-lipoxygenase pathways. Nor would we expect that a dex-dependent enhancement of arachidonate reacylation could lead to such differential effects.

The partial inhibition of the calcium-ionophore-induced release of arachidonate after treatment of macrophages with dex

might instead primarily reflect the reduced level of PLA₂-85. Ca²⁺ can bind *in vitro* to PLA₂-85 and cause its translocation to membranes [11–13] and the ionophore A23187 can, in macrophages, cause arachidonate release without a significant persistent activation of PLA₂-85 (J. Wijkander and R. Sundler, unpublished work). On the other hand, the release of arachidonate induced by zymosan is virtually inhibited by prior treatment with dex. By *in vitro* assay of PLA₂-85 we provide evidence that the rapid persistent activation of PLA₂-85 that normally occurs in response to zymosan [14] is impaired in dex-treated macrophages. The up-regulation induced by PMA is similarly impaired. This is consistent with the earlier finding that zymosan-induced arachidonate release is largely, although not exclusively, dependent on phorbol-ester-sensitive isoforms of PKC [6].

It has recently been demonstrated that the rapid up-regulation of PLA₂-85 in response not only to zymosan or PMA [14] and bacteria [15] in macrophages, but also to other agonists in other cells [32,33], is due to regulatory phosphorylation of the enzyme. It was also recently demonstrated that the protein Ser/Thr phosphatase inhibitor okadaic acid, with selectivity for phosphatases of type 1 and 2a, itself caused enhanced phosphorylation and up-regulation of the activity of PLA₂-85, as well as arachidonate release, in macrophages [15]. It is therefore of interest that this effect on PLA₂-85, as shown here, was not impaired by prior treatment with dex. This suggests either that the dex-induced attenuation of signalling to persistent activation of PLA₂-85 is overcome by the phosphatase inhibitor, or that its site of action is downstream of the dex-sensitive step(s).

Finally we should emphasize that both effects of dex demonstrated here, i.e. on the level of PLA₂-85 and on the signalling for its activation, are slow in onset and take several hours to become discernible. This could be one reason why no immediate effect of dex on TNF α -induced phosphorylation and activation of PLA₂-85 was observed in HeLa cells [22].

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