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Effects of Dexamethasone on Mitogen-Activated Protein Kinases in Mouse Macrophages

IMPLICATIONS FOR THE REGULATION OF 85 kDa CYTOSOLIC PHOSPHOLIPASE A_2

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ABSTRACT. In mouse macrophages, arachidonate mobilisation in response to several stimuli is severely inhibited by prolonged (16–20 hr) treatment with nanomolar dexamethasone (dex). It was shown earlier that this inhibition was accompanied by a dual effect on $cPLA_2$; down-regulation of the enzyme protein and inhibition of its activation. We now report that cycloheximide, a protein synthesis inhibitor, caused an almost complete reversion of the inhibitory effects of dex on $cPLA_2$ activation. These results indicate that the effects depend on new protein synthesis. This is consistent with other data, obtained with a glucocorticoid receptor antagonist, indicating that the effects are mediated via the glucocorticoid receptor. Northern blot results showed pronounced down-regulation of $cPLA_2$ at the level of its mRNA. The possibility that dex also targeted the level or activation of one or more of the three mitogen-activated protein kinases (MAP kinases), extracellular signal-regulated kinase (ERK), p38, or c-Jun N-terminal kinase (JNK) was also addressed. While the level of these MAP kinases and their phorbol myristate acetate (PMA)-induced activation were unaffected by dex, there was a partial inhibition of their zymosan-induced activation. However, this inhibition was not as pronounced as the dex-mediated inhibition of $cPLA_2$ activation. These data were confirmed by Western blot using antibodies against the phosphorylated forms of ERK, p38, and JNK. The results suggest that dex-mediated inhibition of PMA-induced $cPLA_2$ activation is exerted downstream of the MAP kinases, while the partial inhibition of the zymosan-induced activation may be explained by effects exerted more upstream. Thus, the MAP kinases investigated here do not appear to be main targets for the inhibitory effects of dex on $cPLA_2$ activation. BIOCHEM PHARMACOL **60**;4:545–551, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. $cPLA_2$ mRNA; cycloheximide; glucocorticoid; signal transduction; ERK; p38

$cPLA_2$† is constitutively expressed in most cell types studied and has been found to be important for stimulus-induced release of arachidonate in macrophages [1, 2]. Liberation of arachidonate from membrane phospholipids has long been regarded as a rate-limiting step in the formation of proinflammatory eicosanoids. The importance of $cPLA_2$ in macrophages was confirmed when the production of prostaglandin E_2 and leukotrienes in stimulated peritoneal macrophages from $cPLA_2$ knockout mice was found to be abolished [3, 4].

Dex, a synthetic glucocorticoid with anti-inflammatory properties, has been shown to down-regulate, or counteract the induction of, $cPLA_2$ [5–8]. Normally, glucocorticoids act by binding to their receptors in the cytoplasm and entering the nucleus, where they bind to DNA at so-called glucocorticoid response elements (GREs), resulting in the induction or repression of gene transcription. Binding to GREs may also interfere with the binding of other important transcription factors such as activator protein-1 (AP-1) or nuclear factor-κB (NF-κB) to the DNA [9, 10]. Glucocorticoids have also been proposed to exert their effects by direct protein–protein interaction [11, 12] or by destabilisation of mRNA [13, 14]. Glucocorticoids can induce the expression of proteins called lipocortins/annexins [15]. These proteins were first suggested to inhibit PLA_2, but have later been shown to interact not with PLA_2 itself but with its substrate [16]. It was shown earlier that dex reduces the level of $cPLA_2$ protein and severely inhibits its activation [8]. We now report that dex inhibits $cPLA_2$ mRNA expression in mouse peritoneal macrophages, and this may underlie the down-regulation of the protein.

The MAP kinases ERK [17, 18] and p38 [19, 20] have both been implicated in $cPLA_2$ activation. JNK [21] has also been discussed in this context. In macrophages, bacteria- and zymosan-induced activation of ERK and p38 has been shown to play a crucial role in the activation of $cPLA_2$ [22]. We therefore investigated whether these kinases

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† *Abbreviations:* ATF-2, activating transcription factor-2; $cPLA_2$, 85 kDa cytosolic phospholipase A_2; chx, cycloheximide; dex, dexamethasone; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAP kinase, mitogen-activated protein kinase; PCR, polymerase chain reaction; and PMA, phorbol myristate acetate.

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might be targets for the inhibitory effect of dex on $cPLA_2$ activation.

MATERIALS AND METHODS

Reagents

$cPLA_2$ primers for PCR were kindly provided by the UNIGEN Center for Molecular Biology, Trondheim, Norway. Rabbit polyclonal antibodies against $cPLA_2$ were a generous gift from the Genetics Institute, Cambridge, U.S.A. Antibodies against ERK-2, p38, JNK-1, and the phosphorylated form of ERK-2 were bought from SDS. The phosphorylated forms of p38 and JNK-2 were detected with antibodies from New England Biolabs and Promega, respectively.

Cell Culture

Resident macrophages were isolated from peritoneal cells obtained from female outbred NMRI mice (Bom-Mice) by adherence to either 25-cm^2 culture flasks (Nunclon, Nunc) or plastic 10-cm^2 culture dishes (Costar). The cells were incubated in an atmosphere of 5% CO_2 in air and non-adherent cells were removed 2 hr after plating, as described previously [23]. To each flask or dish was then added Medium 199 containing 10% heat-inactivated foetal bovine serum, and the cells were cultured for 16–20 hr with or without dex and chx. The cells were then washed with Dulbecco's PBS and equilibrated for 30 min in serum-free Medium 199 before the start of the experiment. At the end of the experiment, the culture medium was removed.

Preparation of RNA

Total cellular RNA was isolated with the RNeasy total RNA kit as described by the manufacturer (Qiagen).

Northern Analysis

Equal aliquots of RNA were separated on a formaldehyde–1.2% agarose gel. To visualise the RNA load in each lane, ethidium bromide was added to the gel. The gel was subsequently blotted onto Hybond-N filter (Amersham) with 20× SSC (SSC: 1 × SSC = 150 mM NaCl and 50 mM sodium citrate, pH 7.0) by capillary transfer. The blots were linked in a UV cross-linker and thereafter prehybridised and hybridised in ULTRAhyb solution (Ambion) according to the manufacturer's description. The probe used was the product from PCR run with primers with the following sequences: 5′GAG TTT TGG GAG TTT CTG GC (bp 1316–1335) and 5′ATG GCA GGT TAA ATG TGA GC (bp 1777–1758). The probe was labelled with [^{32}P]dCTP by random priming to a specific activity of approx. 10^9 cpm/μg DNA. After hybridisation, blots were washed twice at 68° for 10 min in 2× SSC, 0.1% SDS followed by two washes in 0.2 × SSC, 0.1% SDS for 15 min at 68°, and additional washing overnight at room temperature in 0.2 × SSC, 0.1% SDS. The blots were then dried and analysed by digital imaging (Fujix Bas 2000, Fuji). In order to be able to normalise each sample, the blots were rehybridised with β-actin probe.

Release of [^{3}H]Arachidonate

During the overnight incubation, the cells were labelled with 0.5–1.0 μCi [^{3}H]arachidonic acid (Amersham International; 212 Ci/mmol) per well and, when indicated, dex was included in the medium. At the end of the experiment, the culture medium was collected and the cells were scraped off the dish in 0.1% (w/v) Triton X-100. The collected medium and the cell extract were centrifuged for 5 min at 2500 × *g*. The release of arachidonate from cellular phospholipids was determined by liquid scintillation counting and expressed as a percentage of total recovered radioactivity.

Assay of $cPLA_2$

$cPLA_2$ was assayed according to the method described earlier [1]. 1-Stearoyl-2[1-^{14}C]arachidonoyl phosphatidylcholine (Amersham International; 80 pmol/incubation) was used as substrate. Aliquots of the cytosol fraction containing $cPLA_2$ were incubated in the presence of substrate, 1.4 mM $CaCl_2$ (approx. 400 μM free Ca^{2+}), 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 μL. After incubation for 30 min at 37°, the reaction was stopped by the addition of 1.5 mL of chloroform/methanol/HCl (2:1:0.1, by vol.), to which 0.05% 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 chloroform, and phospholipids were eluted with 2.5 mL methanol.

ERK, JNK, and p38 Activity

After stimulation, cells (9×10^6 cells/sample) were washed with cold PBS and lysed in buffer containing HEPES 50 mM, EDTA 1 mM, dithioerythritol (DTE) 50 mM, Triton X-100 1% (w/v), β-glycerophosphate 50 mM, Na_3VO_4 0.1 mM, and okadaic acid 0.25 μM, pH 7.4. A cytosolic fraction was obtained by centrifugation ($10^5 \times g$, 30 min). The supernatant was immunoprecipitated by shaking at 4° for 2 hr with the corresponding antibody (2.5 μg) conjugated with protein A-Sepharose. After several washes in a buffer containing 80 mM KCl, 10 mM HEPES, 1 mM EDTA, 2.5 mM DTE, 25 mM β-glycerophosphate, 10 mM NaF, and 0.1 mM ammonium vanadate, the activity of the immunoprecipitated MAP kinase was determined using myelin basic protein (10 μg/incubation) as substrate for

ERK, c-jun (2.0 μg/incubation) for JNK, or ATF-2 (2.0 μg/incubation) for p38. The immunoprecipitates were incubated with 40 mM $MgCl_2$, 3 mM EDTA, 4 μM ATP, and 1 μCi [γ-^{32}P]ATP for 30 min at 37°. The reaction was stopped by the addition of Laemmli sample buffer [24] and the samples were run on SDS–PAGE (ERK: 12% polyacrylamide, JNK: 10%, or p38: 8%). The antibody used for immunoprecipitation of JNK-1 cross-reacts with JNK-2 and the antibody used for ERK-2 cross-reacts with ERK-1. The radioactivity incorporated into the substrate was visualised by autoradiography and quantified using video densitometry.

Incorporation of [^{35}S]Met

Isolation and culture of macrophages was performed as described above, except that serum was excluded from the medium. After 10-hr incubation with appropriate concentrations of chx, [^{35}S]Met (Amersham International; 31 μCi/well) was included in the medium. After another 10-hr incubation in the presence of both dex and [^{35}S]Met, 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.

Level and $^{32}PO_4$ Labelling of ATP

Macrophage cultures were isolated, cultured, and exposed to $^{32}PO_4$ for 4 hr as described above. The cells were scraped off the dishes in 40 mM KH_2PO_4, pH 2.8. After sonication for 30 sec, the homogenate was centrifuged for 45 min at $10^5 \times g$ to obtain a supernatant and a pellet. The supernatant was then analysed by HPLC [25] using a linear gradient from 40 mM KH_2PO_4, pH 2.8 to 0.5 M KH_2PO_4/0.8 M KCl, pH 2.7. The flow was adjusted to 1.0 mL/min. A radioisotope detector (Model 171 Radioisotope Detector, Beckman Instruments) was used to determine the $^{32}PO_4$ labelling of ATP, while the amount of ATP was determined from the monitored absorbance at 254 nm.

Western Analysis

Equal aliquots of whole cell extracts prepared in Laemmli sample buffer were boiled for 5 min and electrophoresed on SDS–PAGE. Gels were then equilibrated in transfer buffer [26] and the samples transferred to a nitrocellulose (Amersham) or a polyvinylidene difluoride (PVDF; Millipore) membrane. The membrane was first blocked with 3% gelatin and was then incubated for 12–14 hr with a 1/2000 dilution of primary antibody. Bound antibodies were probed with secondary horseradish peroxidase-conjugated antibodies and detected with the supersignal chemiluminescent method (Pierce).

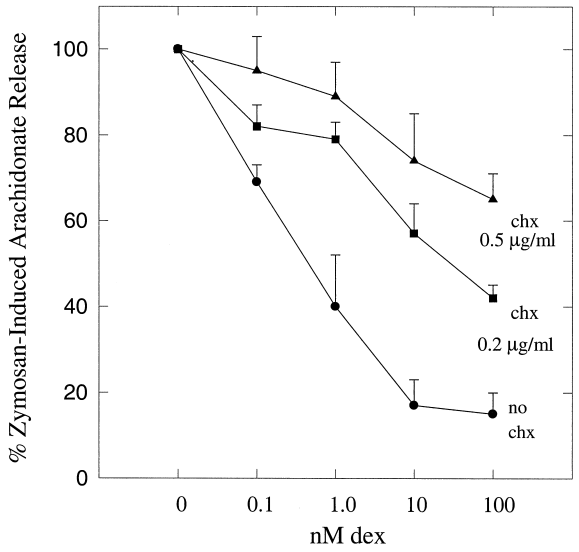

FIG. 1. Reversion of dex-inhibited arachidonate release by chx. Macrophages (3 × 10^6 cells/well) were incubated with dex, concentrations as indicated, for 20 hr. Chx was included in the medium for 20 hr. The cells were stimulated with zymosan (0.2 mg/mL, 1 hr). The release of radiolabel, corrected for the release in control cultures not exposed to zymosan, is expressed as percentage of the release in the absence of dex. The latter amounted to 15 ± 5% of total cellular [^{3}H]arachidonic acid. Data are means ± SEM, N = 5.

RESULTS

Reversal of Inhibitory Dex Effects by Cycloheximide

As previously reported, pretreatment of macrophages with dex led to a dose- and time-dependent inhibition of arachidonate release in response to PMA and zymosan [8]. This effect of dex was mediated by the glucocorticoid receptor, as it was abolished by the simultaneous addition of a molar excess of the receptor antagonist RU 38486 (not shown). We now report evidence that the effect also depends on an intact protein synthesis, as it was counteracted by the protein synthesis inhibitor cycloheximide (chx; Fig. 1). Doses of chx could be titrated that caused significant reversal of the inhibitory effect of dex on arachidonate release and rather extensive inhibition of total protein synthesis, determined by [^{35}S]methionine incorporation (Table 1). The doses used caused no apparent cytotoxicity, as judged from the lack of lactate dehydrogenase release or, more stringently, by maintenance of the cellular level of ATP (Table 1). Similar doses of chx were also found to counteract the inhibitory effect of dex on zymosan-induced cPLA$_2$ activation (Fig. 2), without any effects on the level of cPLA$_2$ protein compared to treatment with dex alone (not shown). The total protein level in control cells was approx. 4 μg/culture as determined by the method of Bradford [27]. In cultures treated with chx (0.5 μg/mL for 16–20 hr), the cellular protein was reduced to 70 ± 22% (mean ± SEM, N = 3) of that in untreated cells.

TABLE 1. Effect of chx on total ATP content, [$^{32}PO_4$]ATP, and [^{35}S]Met incorporation

Dex (nM)	Chx (μg/mL)	Total ATP (% of control)	[^{32}P]ATP (% of control)	[^{35}S]Met incorp. (% of control)
10	—	96 ± 5	69 ± 3	59 ± 3
—	0.5	124 ± 4	65 ± 4	18 ± 6
10	0.5	100 ± 2	56 ± 4	ND

Total ATP content and [$^{32}PO_4$]ATP. Cells were labelled with $^{32}PO_4$ for 4 hr (see Materials and Methods). HPLC was performed on cell extracts from control cells and cells treated with chx for 20 hr. Total ATP in the cytosolic fraction was determined as absorbance at 254 nm. Data are means ± SEM (N = 3). [^{35}S]Met incorporation. During the 20-hr incubation, [^{35}S]Met was included in the medium for the last 10 hr. The radioactivity in the culture medium and the cell extract was determined. In control cells, approx. 0.5% of the [^{35}S]Met added was incorporated. Data are means ± SEM (N = 4). ND, not determined.

Effect of Dex on the Expression of cPLA$_2$ mRNA and on Stimulus-Induced cPLA$_2$ Gel Shift

The previously reported down-regulation of macrophage cPLA$_2$ by dex [8] could in principle be due to interference with a number of different processes, including (i) cPLA$_2$ gene transcription, (ii) cPLA$_2$ mRNA turnover or (iii) translation, or (iv) an increased turnover/degradation of the cPLA$_2$ protein. As shown in Fig. 3 and determined by Northern blot analysis, dex inhibited the basal as well as the LPS-induced expression of cPLA$_2$ mRNA. Quantitative analysis showed inhibition to 14 ± 7 and 14 ± 8% of control, respectively (mean ± SEM, N = 3). This pronounced down-regulation, whether due to inhibited transcription or increased mRNA turnover, may well account for the reduced level of cPLA$_2$ protein alluded to above. The effect of chx on the expression of cPLA$_2$ mRNA has been studied by Dolan-O'Keefe and Nick [28], who found an increase in the basal expression that was not reduced by dex (1 μM for 4–12 hr). This may well explain our finding of an unaltered expression of cPLA$_2$ protein upon treatment with chx in combination with dex (see above).

Phosphorylation of cPLA$_2$ is accompanied by an electrophoretic mobility shift. In unstimulated macrophages, both the unphosphorylated and the phosphorylated band are detectable (Fig. 4). After stimulation with zymosan or PMA, a complete gel shift was obtained. Surprisingly, after pretreatment with dex the remaining cPLA$_2$ still underwent mobility shift in response to both PMA and zymosan (Fig. 4). These results indicate that the electrophoretic mobility shift, which presumably reflects phosphorylation of Ser-505 by ERK-2, is unaffected by dex, which is consistent with its limited effect on ERK activation. However, the lack of up-regulation of cPLA$_2$ catalytic activity, shown earlier [8], indicates that such up-regulation requires additional phosphorylation(s) and that these are targeted by dex.

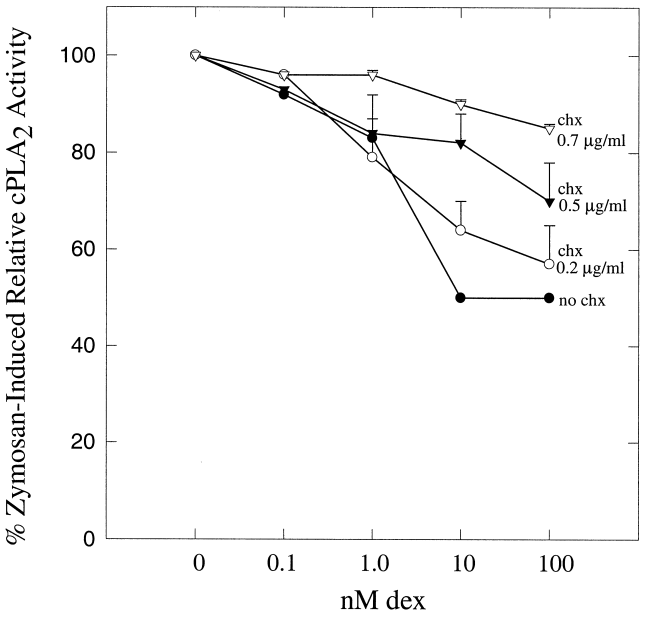

FIG. 2. Reversion of dex-inhibited cPLA$_2$ activity by chx. Macrophages (3 × 10^6 cells/well) were incubated with dex, concentrations as indicated, for 16–20 hr. Chx was included in the medium for 16–20 hr. Net hydrolysis in cytosol from unstimulated cells was 7.3 ± 0.8 pmol/30 min. Zymosan stimulation resulted in a 1.6-fold increase in cPLA$_2$ activity. The cPLA$_2$ activity in cells stimulated with zymosan was set to 100%. Data are means ± SEM, N = 5.

Effects of Dex on MAP Kinase Activation and Phosphorylation

Activation of ERK was measured as the ability to phosphorylate myelin basic protein (MBP) after immunoprecipita-

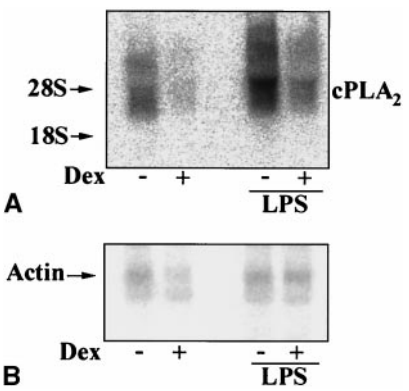

FIG. 3. Effect of dex on expression of cPLA$_2$ mRNA. Macrophages (1.5 × 10^7 cells/flask) were cultured for 16–20 hr in the absence or presence of dex (10 nM) and LPS (1 μg/mL) as indicated. Northern blot analysis as described in Materials and Methods. Representative Northern blots showing mRNA for cPLA$_2$ (A) and corresponding β-actin (B) from three independent experiments.

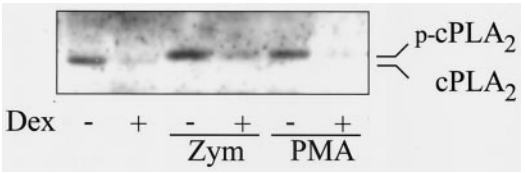

FIG. 4. Effect of dex on $cPLA_2$ protein and mobility shift. Macrophages were cultured for 16–20 hr in the absence or presence of dex (10 nM). After stimulation with zymosan (Zym; 0.2 mg/mL, 1 hr) or PMA (100 nM, 1 hr), the culture medium was removed and a cell lysate was prepared. Aliquots thereof were then subjected to 7% SDS–PAGE followed by Western blot analysis as described in Materials and Methods. This experiment is representative of twenty similar experiments. p-$cPLA_2$ denotes the phosphorylated form of $cPLA_2$, which exhibits mobility shift.

tion with antibodies against ERK-2. Since cross-reactions between different isoforms of ERK cannot be excluded, we will from now on only use the designation ERK. Dex did not inhibit the PMA-induced activation of ERK. Preincubation with dex before addition of PMA resulted in 108 ± 4% (mean ± SEM; N = 4) of the activity obtained with PMA alone. No difference in effect of dex on ERK activation was observed in the interval of 2 to 15 min of stimulation with PMA (data not shown). However, zymosan-induced ERK activation was somewhat inhibited by pretreatment with dex. Activation after dex treatment was found to be 54 ± 8% (mean ± SEM; N = 5) of that in cells treated with zymosan only (Fig. 5A).

In order to confirm data from kinase activity measurements, complementary experiments using antibodies

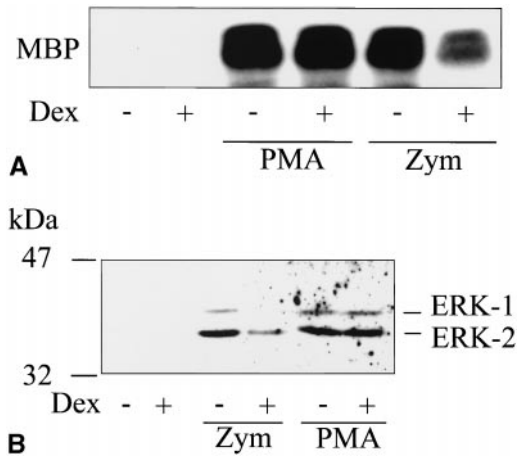

FIG. 5. Effect of dex on ERK activation. Macrophages were cultured for 16–20 hr in the absence or presence of dex (10 nM). The cells were stimulated with PMA (100 nM, 15 min) or zymosan (Zym; 0.2 mg/mL, 1 hr) as indicated below. (A) The activity assay after immunoprecipitation with ERK-2 antibodies was performed as described in Materials and Methods, using myelin basic protein (MBP) as substrate. ERK activation was not detectable in unstimulated cells. (B) Western blot analysis was performed with antibodies against the phosphorylated form of ERK-2 as described in Materials and Methods. All experiments were subjected to digital imaging analysis. This experiment is representative of three similar experiments.

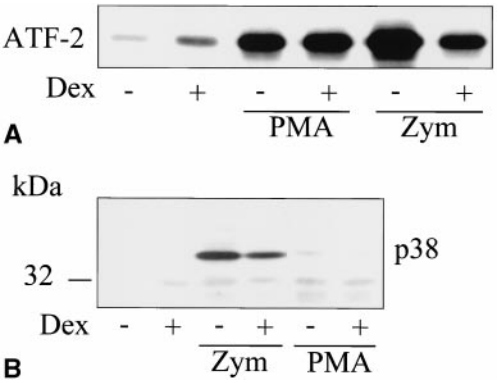

FIG. 6. Effect of dex on p38 activation. Macrophages were cultured for 16–20 hr in the absence or presence of dex (10 nM). The cells were stimulated with PMA (100 nM, 15 min) or zymosan (Zym; 0.2 mg/mL, 1 hr) as indicated below. (A) The activity assay was performed as described in Materials and Methods, using ATF-2 as substrate. An 8- to 20-fold increase in activity was found after stimulation. (B) Western blot analysis was performed using antibodies against the phosphorylated form of p38 as described in Materials and Methods. This experiment is representative of three similar experiments.

against the phosphorylated forms of MAP kinases were performed. Using such antibodies directed at phosphorylated ERK-2, Western blot revealed both PMA and zymosan to induce phosphorylation of ERK. The zymosan-induced phosphorylation of ERK was partially inhibited by dex, while that induced by PMA was not (Fig. 5B).

Dex did not inhibit the PMA-induced increase in kinase activity of p38 (Fig. 6A). Preincubation with dex before addition of PMA resulted in 117 ± 22% of the activation obtained with PMA only (mean ± SEM; N = 3). A moderate inhibition (to 69 ± 7%; mean ± SEM; N = 5) was observed when zymosan was used as stimulus. Similarly, the zymosan-induced phosphorylation of p38 was partially inhibited by dex (Fig. 6B). In the case of the PMA-induced phosphorylation of p38, only a smaller increase was observed. However, we do not know whether a linear relationship between immunoreactivity of the dually phosphorylated p38 and the kinase activity measured in our *in vitro* assay would be expected.

Antibodies against JNK-1 were used in the immunoprecipitation preceding kinase activity measurements using c-jun as substrate. This antibody probably cross-reacts with other isoforms of JNK. PMA did not induce activation of JNK. Preincubation with dex before addition of zymosan resulted in 54 ± 9% (mean ± SEM; N = 3) of the activity obtained with zymosan alone (Fig. 7A). Dex also inhibited zymosan-induced phosphorylation of JNK, while PMA treatment did not result in increased phosphorylation of this kinase (Fig. 7B). Inhibitory effects of dex on JNK have been observed by others as well [29, 30].

DISCUSSION

It was reported earlier that dex causes down-regulation of $cPLA_2$ in mouse macrophages [8]. The present study shows

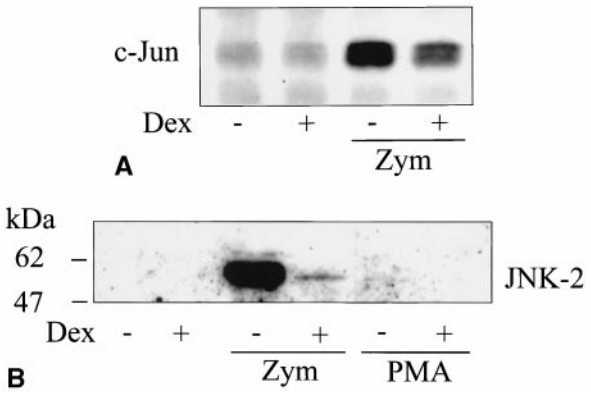

FIG. 7. Effect of dex on JNK activation. Macrophages were cultured for 16–20 hr in the absence or presence of dex (10 nM). The cells were stimulated with zymosan (Zym; 0.2 mg/mL, 1 hr) as indicated below. (A) The activity assay was performed using antibodies against JNK-1 in the immunoprecipitation as described in Materials and Methods. (B) Western blot analysis with antibodies against the phosphorylated form of JNK-2 was performed as described in Materials and Methods. All experiments were subjected to digital imaging analysis. This experiment is representative of three similar experiments.

that this can be accounted for by either inhibition of $cPLA_2$ gene transcription or increased turnover of its mRNA, as the mRNA was found to be even more down-regulated than the $cPLA_2$ protein. The almost complete reversion of zymosan-stimulated $cPLA_2$ activity shown here to be exerted by chx indicates that the suppressed activation of $cPLA_2$, the second effect of dex on this enzyme, was restored. The reversal of the effects of dex by chx indicates that it depended on synthesis of new protein(s).

Increased phosphorylation of multiple serine residues in $cPLA_2$ occurs upon activation [31, 32], but only phosphorylation on Ser-505 has been found to be responsible for the shift in $cPLA_2$ electrophoretic mobility [17, 33]. This phosphorylation has also been assumed to cause up-regulation of its catalytic activity. The present and previous [8] data, showing dissociation of mobility shift and activation, indicate that additional phosphorylation(s) is/are necessary for activation and that these, rather than that causing mobility shift, are targeted by dex. However, the identity of such phosphorylation site(s) is presently unclear. Unfortunately, the expression in insect cells of serine-mutated forms of $cPLA_2$ or forms with C-terminal truncations did not provide clear answers as to the regulatory role of the phosphorylation of individual serine residues [33].

Although both ERK and p38 have been found to be essential for activation of $cPLA_2$ [22], neither appeared to be a main target for the inhibitory effect of dex in this study, in accordance with other data [29]. The inhibitory effect of dex on $cPLA_2$ activation must instead be exerted downstream of these MAP kinases, although we found that dex partially inhibited zymosan-induced activation. However, it was reported earlier that dex leads to partial inhibition of zymosan-induced phospholipase C activation [34], and this may well account for the partial inhibition of MAP kinases. Furthermore, recent data demonstrate that phosphatidylinositol 3-kinase is positioned upstream of phospholipase C [35] and therefore could constitute an alternative target for the partial inhibition. Indeed, phosphatidylinositol 3-kinase has been found to be a target for dex [36]. In conclusion, the present study demonstrates that the transcriptional regulation of $cPLA_2$ and the regulatory phosphorylation of this enzyme are main targets for dex, rather than the expression or activation of MAP kinases.

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