CALCITRIOL-MEDIATED MODULATION OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR-2

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Abstract—Calcitriol-induced differentiation of U937 mononuclear phagocytes is known to have divergent effects on the synthesis of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-2 (PAI-2). In this study, we sought to determine whether calcitriol affects the expression of these proteins by modulating intermediate signal trasduction involving intracellular calcium and protein kinase C (PKC). U937 cells were stimulated with calcitriol (50 nM) for 6–72 hr, inducing a transient increase in specific binding of [^{3}H]phorbol dibutyrate ([^{3}H]PDBu), seen only after 24 hr. Staurosporine (2 nM), a PKC inhibitor, had no effect on calcitriol-induced secretion of plasminogen activator (PA) activity. However, staurosporine significantly ($P < 0.05$) inhibited the ability of calcitriol to enhance phorbol myristate acetate (PMA)-induced secretion of PA inhibitor activity, indicating that this priming effect of calcitriol requires expression of PKC. The calcium ionophore A23187 (0.1 µM) induced a modest increase in secreted PA inhibitor activity, in contrast to the secretion of PA activity which is consistently seen in response to calcitriol. Northern blot analysis demonstrated that A23187 induced an increase in PAI-2 mRNA and a marked reduction in uPA mRNA, while calcitriol induced opposite changes in both mRNA species. We conclude that calcitriol modulates uPA and PAI-2 expression by multiple mechanisms that are both PKC dependent and PKC independent. Our studies also demonstrated that increased intracellular calcium alters the synthesis of both uPA and PAI-2 in a manner which favors expression of PA inhibitor activity.

There is considerable evidence to suggest that mononuclear phagocytes utilize plasminogen activation in the pericellular environment to facilitate cellular migration and remodeling of extracellular matrix proteins [1, 2]. The mechanisms by which macrophages regulate plasminogen activation are complex, partly because these cells concurrently synthesize a urokinase-type plasminogen activator (uPA) and a specific plasminogen activator inhibitor, PAI-2 [3, 4]. We have demonstrated recently that uPA and PAI-2 can be modulated in an agonist-specific manner during in vitro differentiation of the human U937 monoblast-like cell line [3]. The effects of 1α,25-dihydroxyvitamin D$_3$ (calcitriol) are of particular interest because this agent concomitantly induces an increase in uPA synthesis and a marked reduction in PAI-2, resulting in expression of plasminogen activator (PA) rather than PA inhibitor activity in both conditioned media and cell lysates.

Several lines of evidence suggest that calcitriol may induce macrophage differentiation at least partly by affecting signal transduction via protein kinase C (PKC) and altered concentrations of intracellular calcium. Both uPA and PAI-2 genes are induced by phorbol esters, which act principally by activating phospholipid–Ca^{2+}-dependent PKC enzymes [3, 5]. Evidence derived largely from the phenotypically immature HL-60 line indicates that calcitriol increases PKC expression during in vitro differentiation and that the differentiation process requires PKC activity [6, 7]. Calcitriol, like phorbol ester, can induce maturation of both HL-60 and U937 cells to a monocyte–macrophage phenotype [7, 8]. Calcitriol has also been shown to increase intracellular calcium in HL-60 cells and a variety of other cell types, and in some instances the effects of calcitriol on cellular differentiation and proliferation have been shown to be calcium dependent [9–11]. Finally, calcitriol primes macrophages to respond to other exogenous stimuli in a manner similar to the effects of interferon-γ [12–15]. Studies have clearly demonstrated that a cooperative interaction between increased intracellular calcium and PKC activation underlies the ability of interferon-γ to prime macrophages for tumor cytotoxicity [14, 15]. In an attempt to elucidate more fully the factors regulating the expression of uPA and PAI-2 activities by mononuclear phagocytes, we performed the following studies to determine the role of the Ca^{2+} and PKC signal transduction pathways in mediating the effects of calcitriol on uPA and PAI-2 synthesis.

METHODS

Reagents. Calcitriol (DuPhor Co., Amsterdam, Netherlands) was maintained as a 0.1 mM stock solution in ethanol at −20°. Calcitriol purity, concentration, and structural integrity were confirmed by UV spectroscopy. Phorbol myristate

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acetate (PMA; LC Services, Woburn, MA) was prepared from stock solutions of 1 mg/mL in dimethyl sulfoxide stored at −70°. Plasminogen was prepared from outdated human plasma by lysine-Sepharose affinity chromatography (Pharmacia Chemicals, Piscataway, NJ) [16]. Plasmin contaminating the plasminogen preparations was inactivated by treatment with phenylmethylsulfonyl fluoride (PMSF; 1 mM) for 16 hr at 25°, followed by dialysis in 0.05 M phosphate buffer, pH 7.5. The human PAI-2 cDNA used was the subcloned internal PstI-DraI fragment of the pcD-1214 clone (provided by Andrew C. Webb, Ph.D., Wellesley College) [17] in pGEM-2 inserted between the PstI and SmaI sites in the polylinker. Human urokinase cDNA was obtained from the Japanese Center Resources Bank-Gene, National Institute of Health, Tokyo [18]. This is a full-length clone inserted in the PstI-Pvu II site of a pBR322 vector.

Cell culture. U937 cells (American Tissue Culture Collection, Rockville, MD) were cultured in medium consisting of RPMI-1640 (Hazleton, Denver, PA) supplemented with L-glutamine (2 mM), penicillin (100 units/mL), streptomycin (100 μg/mL), gentamicin (100 μg/mL), and 5% fetal bovine serum (Hyclone, Logan, UT). Cells were cultured $(2 \times 10^5 \text{ mL})$ for 24 hr at 37°, 5% CO_2 in 75 cm^2 flasks (Falcon Plastics, Oxnard, CA) prior to addition of calcitriol (50 nM), A23187 (0.1 μM), or diluent control (ethyl alcohol 0.05%). For most experiments, cells were then maintained in flasks for the indicated time periods. For measurement of PA and PA inhibitor activities, cells were washed and dispensed at 10^6 cells in 1 mL of serum-free medium supplemented with 0.1% human serum albumin (Transfusion Services, University of Michigan Medical Center) and cultured for 48 hr in sterile 16 mm polystyrene culture wells (Falcon). Cell counts were performed with a hemocytometer and viability was confirmed by trypan blue exclusion. Preliminary experiments confirmed that after 48 hr in serum-free medium, the cell number did not increase significantly. Further, there were no significant differences between experimental conditions in either the number or viability of cells after the 48-hr culture period (data not shown).

Phorbol ester binding. Cells were collected by centrifugation, washed twice in phosphate-buffered saline, pH 7.4, and sonicated in 500 μL of Buffer A (Tris, 25 mM; sucrose, 250 mM; dithiothreitol, 0.5 mM; EDTA, 2 mM; ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA), 10 mM; magnesium acetate, 1 mM; PMSF, 2 mM; leupeptin, 50 μg/mL, pH 7.4). The sonicates were centrifuged at 12,000 g for 20 min at 4°. The supernatant (cytosol) fraction was then collected. The pellet (membrane) fraction was washed with 200 μL Buffer A, and the wash was pooled with the supernatant fraction. Protein concentrations of cytosol and membrane fractions (in Buffer A) were measured by the method of Bradford [19] using bovine γ-globulin as a standard. To measure [^{3}H]phorbol dibutyrate ([^{3}H]PDBu) binding, cytosol or membrane (200 μg/mL protein) was incubated with [^{3}H]PDBu (80 nM; Amersham, Arlington Heights, IL) in Buffer B (Tris, 50 mM, pH 7.6; phosphatidylserine 100 μg/

mL; $CaCl_2$, 2 mM; bovine γ-globulin, 2 mg/mL) for 30 min at 37°. The binding reaction was terminated by precipitating proteins with 35% polyethylene glycol and the sample were centrifuged at 12,000 g, 4° for 20 min. The pellet was washed once with 35% polyethylene glycol, and the centrifugation step was repeated. The tip of the tube containing the pellet was amputated and placed in Ecolyte aqueous scintillation fluid (ICN Chemicals, Irvine, CA) and counted in a Beckman LS 1801 scintillation counter (Beckman Instruments, Irvine, CA). Specific [^{3}H]PDBu binding was calculated as total [^{3}H]PDBu bound less the nonspecific binding measured in parallel samples also containing unlabeled PDBu (80 μM). Under all conditions, nonspecific binding did not exceed 30% of the total.

Esterolytic assay for PA and PA inhibitor activities.

Plasminogen activator activity was measured with the esterolytic assay of Coleman and Green, with minor adaptations for use in 96-well plates [20]. Conditioned media (10 μL) were mixed with an optimal amount of plasminogen in 0.67 M glycine/ 0.17% bovine serum albumin (BSA)/1.7 μM Tris/ 0.02% Triton X-100 (50 μL) and incubated at 37° for 60 min. Plasmin generated during this step was quantified by the addition of a plasmin substrate, thiobenzyl benzyloxycarbonyl-L-lysinate (0.2 mM; Calbiochem, La Jolla, CA), and 5,5′-dithiobis-2-nitrobenzoic acid (2.2 mM; Calbiochem) in 200 μL of 0.2 M phosphate/0.2 M NaCl/1% Triton X-100. After 30–60 min at 37°, optical absorbance was read at 414 nm with a Titertek Plus multichannel spectrophotometer (Flow Laboratories, McLean, VA). PA activity was determined from a standard curve generated with commercially prepared urokinase (Calbiochem), and expressed in milliPloug units (mPU). As this assay relies on the conversion of plasminogen to plasmin, prourokinase could not be distinguished from active urokinase by this method [4]. To measure PA inhibitor activity, serial dilutions of the test samples were co-incubated with 2 mPU of commercial urokinase, and the residual PA activity was measured in standard fashion. PA inhibitor activity was calculated from a plot of the sample concentration (reciprocal of the dilution factor) versus residual PA activity, and expressed in PAI units/mL (1 PAI unit = 1 mPU PA inhibited).

mRNA analysis. For these experiments, U937 cells were cultured for 4 hr with A23187 (0.1 μM), calcitriol (50 nM) for 24 hr, or ethyl alcohol (0.05%) for 24 hr. Cells were dislodged by gentle scraping, washed with RPMI, and flash frozen for storage at −70°. To extract cellular RNA, cell pellets were sonicated on ice in urea (5 M), LiCl (3 M), and heparin (14 units/mL) [21]. RNA was pelleted after overnight precipitation at −20° and extracted repeatedly with phenol/chloroform. The aqueous layer was precipitated with ethanol/sodium acetate at −20°. The RNA pellet was suspended in RNAase-free water and concentration determined by spectroscopy at 260 nm. The RNA was size fractionated electrophoretically on 1% agarose gels (10 μg/lane) containing 0.22 M formaldehyde and 0.4 μg/mL ethidium bromide [22]. Visualization of ribosomal bands under UV light provided internal size markers for each lane and also confirmed that

Table 1. Effects of calcitriol on expression of protein kinase C by U937 cells

Treatment with 50 nM Calcitriol (hr)	Specific binding of [³H]PDBu (% control)
6	88.4 ± 12.2*
12	96.7 ± 4.93
24	122.2 ± 8.0†‡
48	110 ± 19.5
72	109.3 ± 14.6

* Mean ± SEM, N = 3–7 Experiments.

† P < 0.05 compared to unstimulated control.

‡ Binding of unstimulated control cells at 24 hr was 3382 ± 424 fmol/mg protein. The increase with calcitriol stimulation was 857 ± 309 fmol/mg protein (P < 0.05).

the RNA content was equal among corresponding lanes on the gels. The RNA was transferred to Hybond nylon filters (Amersham) and fixed by exposure to UV light [22]. The cDNA clone of interest was labeled with [³²P]dCTP (Amersham) by random priming, achieving specific activities of approximately 5×10^8 cpm/µg DNA [23]. The nylon filters were then hybridized with 2×10^7 cpm of [³²P]cDNA for 18 hr at 65°, followed by serial washes of increasing stringency, with the final wash consisting of 1.5 mM sodium citrate, 15 mM sodium chloride, 0.1% sodium dodecyl sulfate, 1 mM EDTA, 10 mM sodium phosphate at 68° [20]. The filters were then developed by autoradiography using Kodak XAR-5 X-Omat AR film at −70°, and scanned by laser densitometry (LKB Ultroscan XL, Piscataway, NJ).

Statistics. Where appropriate, comparisons of group means were performed with an unpaired, two-tailed Student's *t*-test [24].

RESULTS

Effects of calcitriol on protein kinase C. To determine if calcitriol alters cellular expression of PKC, U937 cells were cultured with or without calcitriol (50 nM) for periods up to 72 hr and the cellular content of PKC was assessed by specific binding of [³H]PDBu. As shown in Table 1, binding of [³H]PDBu was unaffected by stimulation with calcitriol for 6 and 12 hr. After 24 hr, calcitriol induced a modest increase in [³H]PDBu binding, reaching 122.2 ± 8.0% of control values (P < 0.05). This corresponded to an absolute increase in binding of 857 ± 309 fmol/mg protein (P < 0.05). This effect was negated by co-stimulation with cycloheximide (5 µg/mL), indicating a requirement for protein synthesis (not shown). After incubation with calcitriol for 48 and 72 hr, [³H]PDBu binding was diminished and indistinguishable from that of control cells. The increase in [³H]PDBu binding at 24 hr was evaluated by Scatchard analysis, demonstrating that calcitriol treatment had no effect on binding affinity (K_d), but induced an increase in B_{max} comparable to the above increase in [³H]PDBu binding (not shown).

To determine if PKC activity is necessary for calcitriol to induce secretion of PA activity, cells

were incubated for 48 hr in the presence or absence of calcitriol (50 nM) or staurosporine, a potent PKC inhibitor (2 nM; Kamiya Biomedical, Thousand Oaks, CA). At the concentration used, staurosporine has been shown to express excellent specificity for PKC [25], and had no adverse effects on cell viability (not shown). After this incubation period, the cells were washed and incubated in serum-free medium for 48 hr, as described in Methods. As shown in Fig. 1, unstimulated U937 cells secreted PA inhibitor activity. Calcitriol induced the cells to express PA activity rather than PA inhibitor. As we have shown previously [3], PA activity was below the detection limits of the assay under conditions where PA inhibitor activity was demonstrable; likewise, PA inhibitor activity was not demonstrable in conditioned media containing detectable PA activity (calcitriol and calcitriol + staurosporine). Staurosporine had no effect on PA inhibitor secretion by otherwise unstimulated cells, and likewise had no effect on calcitriol-induced PA secretion, indicating that optimal expression of PKC activity was not required for the observed effects of calcitriol. We have shown that pretreatment with calcitriol amplifies secretion of PAI-2 by U937 cells in response to phorbol myristate acetate [3]. Since the principal action of PMA is to activate PKC, separate experiments were performed to determine if PKC activity was required for this priming effect of calcitriol. Cells were incubated with calcitriol in the presence or absence of staurosporine, washed extensively, and subsequently incubated for 24 hr in serum-free medium containing an optimally stimulating concentration of PMA (10 ng/mL). Staurosporine substantially reduced the resultant secreted PA inhibitor activity to 43% of control values (P < 0.05; Fig. 1). Carry-over of staurosporine into the incubation with PMA was not responsible for this effect, as pretreatment with staurosporine alone had no effect on PMA-induced PA inhibitor activity. This indicates that, in contrast to the effect of calcitriol alone, this priming effect of calcitriol requires PKC activity. PA activity was not demonstrable in any of the PMA-treated cells, likely due to the augmented secretion of PA inhibitor activity [3].

Effects of calcitriol and A23187 on secreted PA and PA inhibitor activities. Experiments were performed to determine if the effects of calcitriol on secreted PA and PA inhibitor activities could be duplicated by the calcium ionophore A23187. U937 cells were suspended in serum-free medium in the presence or absence of A23187 (0.5 µM). For comparison, cells pretreated for 24 hr with calcitriol (50 nM) were cultured in parallel. After 48 hr, conditioned media were collected and assayed for PA and PA inhibitor activities. As shown in Fig. 2, calcitriol and A23187 induced opposite changes in secreted activity. In contrast to the effect of calcitriol, A23187 induced a modest increase in secreted PA inhibitor activity, and a conversion from PA inhibitor to PA secretion was never observed in these cells. In preliminary studies, treatment with A23187 (0.01 to 1.0 µM) or ionomycin (5 µM) for periods up to 24 hr produced similar results (not shown). As in our previous study [3], the PA and PA inhibitor
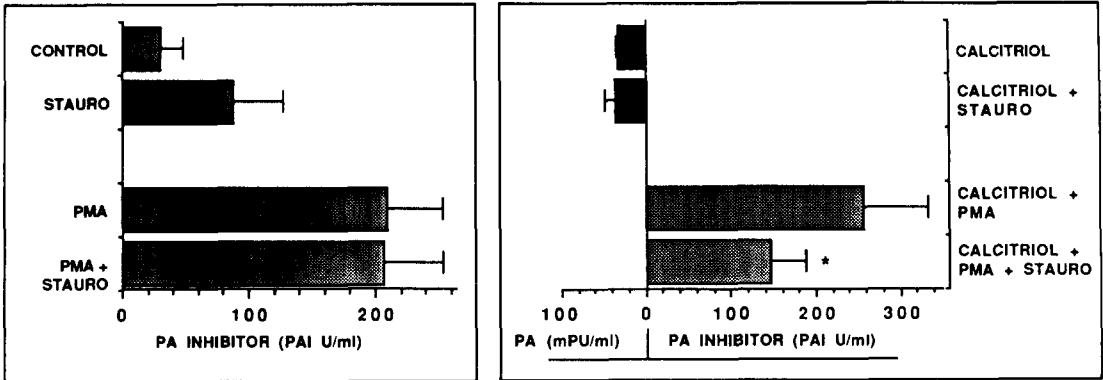
Fig. 1. Effect of staurosporine on calcitriol-induced modulation of U937 cell PA and PA inhibitor activities. U937 cells were pretreated with 50 nM calcitriol with or without 2 nM staurosporine, a protein kinase C inhibitor. Cells were subsequently cultured in serum-free medium and assayed for secreted PA and PA inhibitor activities as described in Methods (mean ± SEM, N = 5). Data are expressed in milliPloug units (mPU) and PAI units (1 PAI unit = 1 mPU PA inhibited). Cells were also stimulated with 10 ng/mL phorbol myristate acetate (PMA) during the second incubation period. Key: (*) pretreatment with staurosporine significantly (P < 0.05) reduced the PMA-induced secretion of PA inhibitor activity by calcitriol-primed cells.
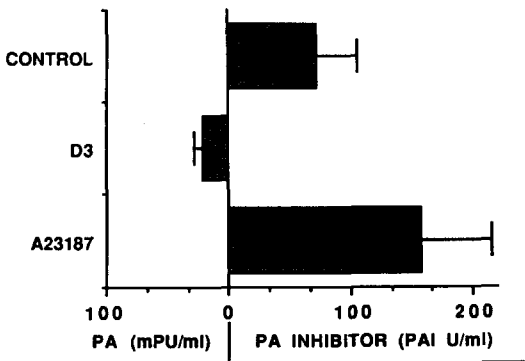

Fig. 2. Effects of calcitriol (D3) and A23187 on secretion of PA and PA inhibitor activities. Cells were pretreated with calcitriol (50 nM) and cultured in serum-free medium for 48 hr to determine secretion of PA and PA inhibitor activities, as described in Methods. For comparison, cells were stimulated with the calcium ionophore A23187 (0.5 μM) during the 48-hr incubation period. A23187 induced a modest increase in PA inhibitor activity which did not reach statistical significance relative to control cells (P = 0.2). Data are expressed as in Fig. 1.

activities of cell lysates paralleled the conditioned media under all treatment conditions (not shown).

Northern blot analysis. To expand on the comparison of the effects of calcitriol and A23187 on regulation of PA and PA inhibitor activities, Northern analysis was performed to determine the effects on the biosynthesis of the individual uPA and PAI-2 proteins. Cells were cultured in serum-supplemented medium alone, or treated with calcitriol (50 nM) for 48 hr or A23187 (0.5 μM) for 4 hr. Steady-state levels of mRNA for uPA and PAI-2 were then determined as described in Methods. Using control cells as a reference, calcitriol and

A23187 induced opposite changes in steady-state levels of mRNA for PAI-2; calcitriol consistently caused a marked reduction in PAI-2 mRNA, in contrast to an increase in message level induced by A23187 (Fig. 3). Calcitriol and A23187 also produced opposite changes in uPA mRNA. However, in this instance, calcitriol induced an increase in uPA mRNA, whereas A23187 decreased the level of uPA mRNA. Therefore, calcitriol and A23187 share an interesting property, in that they induce opposite directional changes in mRNA levels for uPA and PAI-2 and, in both cases, the relative changes in uPA and PAI-2 mRNA parallel the eventual secretion of PA or PA inhibitor activities. However, the effects of calcitriol were opposite to those of A23187 for both uPA and PAI-2 expression, effectively arguing that an increase in intracellular calcium is not the proximate mechanism by which calcitriol modulates uPA and PAI-2 expression.

DISCUSSION

Local formation of plasmin at inflammatory foci is thought to contribute to proteolytic release of proinflammatory peptides, release and activation of cytokines, and degradation of extravascular fibrin and extracellular matrix glycoproteins [2, 26–29]. While the formation and inactivation of extravascular plasmin are undoubtedly a complex multistep process, mononuclear phagocytes provide a central regulatory role in plasminogen activation by synthesizing and secreting uPA and PAI-2 [3, 4]. Secreted PAI-2 may not only prevent excessive plasmin formation, but also assist in spatially restricting this process to the pericellular environment where uPA is localized to plasma membrane receptors and is relatively resistant to inhibitors of PA or plasmin [30]. It is therefore of considerable interest to define the pathways by which expression
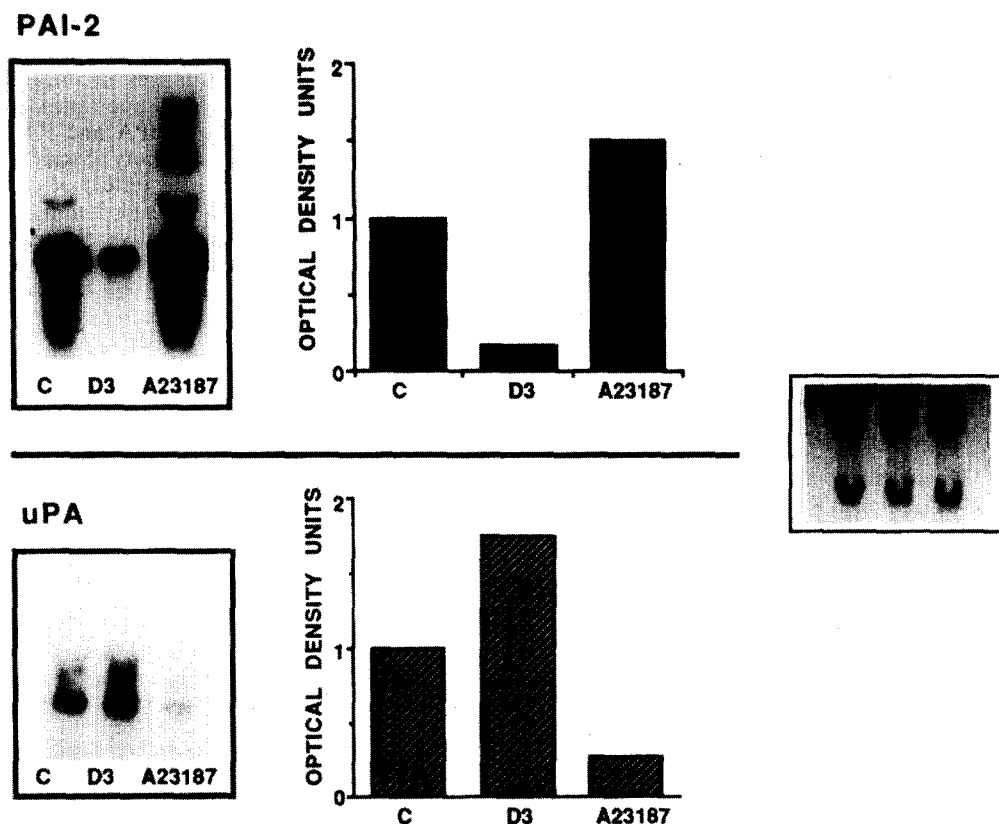
Fig. 3. Northern blot analysis of calcitriol (D3) and A23187-treated cells. Cells were treated with calcitriol or A23187 as described in Methods. The top panel shows mRNA levels for PAI-2 (major band at 2 kb). Densitometry measurements of the Northern blot are shown at the right, with optical density expressed in arbitrary units, normalized to control cells. The Northern blot for uPA is shown in the lower panel (major band at 2.4 kb). Results are expressed as in the upper panel. Ethidium bromide-stained ribosomal RNA bands are shown in the insert at the right to demonstrate equal RNA loading of the lanes.

of uPA and PAI-2 activities by macrophages are coordinately regulated.

A host of exogenous signals have been shown to stimulate expression of PA activity by macrophages in vitro, leading to common use of elevated PA activity as a functional marker of macrophage activation [31, 32]. Previous studies have shown that stimulation with phorbol ester is sufficient to increase expression of both uPA and PAI-2, presumably by activating PKC [3, 5]. Bacterial lipopolysaccharide causes similar increases in uPA and PAI-2 synthesis and secretion [30, 33]. PKC activation may be responsible for this effect as well, as lipopolysaccharide and phorbol ester produce similar patterns of protein phosphorylation in macrophages, suggesting overlapping mechanisms of action [34]. The signal transduction pathways mediating expression of uPA and PAI-2 are otherwise incompletely defined, although agents which augment levels of cyclic AMP generally suppress expression of PA activity [35, 36]. However, these studies largely relied on measurement of plasminogen-dependent proteolysis, and therefore were incapable of distinguishing whether changes in PA activity resulted from changes in expression of PA or PA

inhibitor proteins. Using both enzymatic assays and measurements of specific mRNA species, we have shown recently that calcitriol can augment uPA synthesis while concomitantly reducing synthesis of PAI-2 [3]. These changes in synthetic activity paralleled the ultimate expression of protease/inhibitor activity in cell lysates and medium. The ability of macrophages to regulate uPA and PAI-2 gene expression in either parallel or inverse fashion indicates a means by which these cells can selectively promote or suppress plasminogen activation. Moreover, this type of interplay between proteases and antiproteases may not be unique to PA activity, as similar regulation in tandem or inverse fashion has been shown for collagenase and the tissue inhibitor of metalloproteases (TIMP) [37, 38]. Therefore we have sought to characterize more fully mechanisms by which uPA and PAI-2 can regulated independently.

Experiments were first performed to examine the possibility that calcitriol modulated uPA and PAI-2 expression at least partly by inducing changes in cellular PKC activity. In our previous study, calcitriol amplified phorbol-induced expression of both uPA and PAI-2 [3]. Calcitriol-induced differentiation of HL-60 promyelocytes into a macrophage phenotype

is PKC dependent and is associated with an increase in cellular PKC activity [6, 7]. These observations further support the possibility that calcitriol alters expression of phorbol inducible genes by amplifying the PKC signal transduction pathway. Contrary to this argument, there is limited evidence that amplification of PKC activity is not required for induction of cell differentiation [39, 40]. We therefore questioned whether calcitriol altered cellular PKC activity under conditions known to modulate expression of uPA and PAI-2 proteins. Calcitriol induced an increase in cytosolic [³H]PDBu binding of 22% over control values. This effect was transient and seen after a 24-hr incubation. Importantly, the primary modulation of uPA and PAI-2 synthesis was PKC independent, as co-incubation with staurosporine had no effect on this direct effect of calcitriol. However, the ability of calcitriol to prime cells for PMA-induced PAI-2 synthesis was clearly suppressed by staurosporine and, therefore, at least partly PKC dependent.

Calcitriol-induced differentiation of HL-60 promyelocytes is associated with an increase in intracellular calcium concentration, and calcium is required for some effects of calcitriol on cellular differentiation [9–11]. In view of the known synergy between calcium inophores and PKC activation in macrophage priming [14, 15], we reasoned that calcitriol may modulate uPA and PAI-2 expression by a primary effect on intracellular calcium, and therefore compared the effects of calcitriol and the calcium ionophore A23187. A23187 shared with calcitriol the capacity to induce opposite changes in uPA and PAI-2 mRNA (Fig. 3). However, the effects were the inverse of those seen with calcitriol, as A23187 decreased uPA mRNA while increasing PAI-2 mRNA. With both stimuli, the comparative changes in the two mRNA species were consistent with the ultimate expression of PA or PA inhibitor activity (Fig. 2). Several important conclusions may be drawn from these results. First, inverse regulation of uPA and PAI-2 is not unique to calcitriol. Because altered intracellular calcium is a common event in signal transduction, its occurrence may mediate or influence the effects of many exogeneous stimuli on uPA and PAI-2 expression. Second, depending on the agonist used, the inverse regulation of uPA and PAI-2 can favor either augmented or suppressed expression of PA activity, potentially expanding the range over which macrophages can direct local plasminogen activation. Finally, it is clear that calcitriol does not affect uPA and PAI-2 expression by increasing intracellular calcium. Interestingly, we previously showed that exogenous calcium and calcitriol can have opposite effects on the proliferation of malignant cells [10]. Further studies will be necessary to determine if calcitriol may be antagonizing some effects of intracellular calcium. In addition, the presence of a calcitriol receptor in U937 cells raises the possibility that the hormone directly affects gene expression in the manner demonstrated for other steroid hormones [41].

In summary, we have demonstrated that calcitriol alters synthesis of uPA and PAI-2 by both PKC-dependent and PKC-independent pathways in U937 cells. Also, elevated intracellular calcium caused an increase in PAI-2 mRNA with a coincident reduction in uPA mRNA and ultimately, enhanced expression of PA inhibitor activity. Changes in intracellular calcium, therefore, may be an important signal in determining the expression of uPA and PAI-2 by macrophages in response to a variety of stimuli, and calcitriol may act to oppose this effect of calcium. Further characterization of the signal transduction pathways that influence comparative expression of the uPA and PAI-2 genes will be instrumental in understanding the basis for agonist-specific and differentiation-associated expression of macrophage protease and antiprotease activities.

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