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Transmembrane signaling by interferon α involves diacylglycerol production and activation of the ε isoform of protein kinase C in Daudi cells

(signal transduction/antiviral/antiproliferative/stimulated gene expression)

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ABSTRACT **The early events that occur after treatment of the highly interferon α (IFN-α)-sensitive human lymphoblastoid Daudi cell line with human leukocyte IFN-α have been examined. IFN-α treatment of Daudi cells results in a rapid and transient increase in the cellular content of diacylglycerol, which occurs in the absence of inositol phospholipid turnover, or an increase in intracellular calcium concentration. Furthermore, IFN-α treatment results in a selective, time-dependent activation of the Ca^{2+}-independent ε isoform of protein kinase C (PKC), while the α isoform is unaffected by IFN-α treatment. In contrast, IFN-α treatment of an IFN-resistant subclone of Daudi cells had no effect on the diacylglycerol content of cells and on the activation of PKC-ε. The selective PKC inhibitor staurosporine blocked the transcriptional activation of IFN-α-stimulated genes, the cytoplasmic accumulation of mRNAs for these genes, and the induction of antiviral activity by IFN-α against vesicular stomatitis virus in IFN-sensitive cells. These observations suggest that transmembrane signaling of IFN-α involves diacylglycerol production and activation of PKC-ε in Daudi cells.**

Interferons (IFNs) are cytokines that induce a variety of physiological responses, among which are induction of antiviral activity and inhibition of cell proliferation (1). IFNs elicit these effects by binding to specific cell surface receptors and transducing a signal to the nucleus that results in activation of specific gene expression. Type I IFNs (IFN-α/β) bind to receptors that are distinct from those for type II IFN (IFN-γ) (2). Although the immediate biological consequences of IFN–receptor interactions are unknown, recent evidence suggests that activation of protein kinase C (PKC) plays an important role in signaling by IFN-α/β (3, 4). For example, general inhibitors of protein kinases [aminopurine (5, 6)], as well as pharmacologic inhibitors of PKC [staurosporine and H-7 (4)], inhibit IFN-α-induced antiviral activity and transcriptional activation of IFN-stimulated genes (ISGs).

PKC is a serine and threonine kinase whose activity was originally shown to be dependent on calcium, phospholipids, and diacylglycerol (DAG). PKC has been shown to play a crucial role in the signal transduction pathway elicited by a variety of growth factors, hormones, and neurotransmitters (for review, see ref. 7). Molecular cloning analysis has shown that PKC is a family of multiple isoforms having related structures (7, 8). Thus far, eight isoforms of PKC have been deduced from brain, spleen, and epidermis (9) cDNA libraries. COS cell expression analysis has indicated that PKC isozymes may be grouped into Ca^{2+}-dependent (PKC-α, -βI, -βII, and -γ) and Ca^{2+}-independent (PKC-δ, -ε, -ζ, and -η) enzymes. Furthermore, PKC-ε has been shown to have Ca^{2+}-independent phorbol ester binding activities and exhibits substrate specificity distinct from other characterized PKC isoforms (10, 11).

We have investigated the early events that occur upon treatment of human Daudi lymphoblastoid cells with IFN-α in order to identify the biochemical pathways of transmembrane signaling. IFN-α treatment of these cell lines results in cessation of cell growth, protection against viral infection, and the rapid transcriptional activation of ISGs (12, 13). We report that the outstanding features of the signal transduction pathway of IFN-α in Daudi cells are the generation of DAG in the absence of inositol phospholipid turnover or Ca^{2+} elevation and the activation of the calcium-independent ε isoform of PKC. Furthermore, staurosporine, a potent inhibitor of PKC activity blocks the transcriptional activation of ISGs, the cytoplasmic accumulation of ISG mRNA, and the induction of antiviral activity against vesicular stomatitis virus (VSV) in Daudi cells by IFN-α.

MATERIALS AND METHODS

Cell Culture. IFN-resistant and sensitive subclones of Daudi cells were maintained in static suspension culture at densities between 2×10^5 and 1.5×10^6 cells per ml in RPMI 1640 medium supplemented with 10% defined calf serum (HyClone). Cell cultures were diluted with fresh medium every 2–3 days. Although IFN-resistant Daudi cells display a similar number of high-affinity IFN-α receptors as IFN-sensitive Daudi cells, IFN-α treatment of the IFN-resistant cell line has little effect on cell proliferation, has only slight inhibitory activity on viral replication, and results in a greatly reduced transcriptional response of ISGs (13).

IFN. Recombinant DNA-derived IFN-α (1×10^9 units per mg of protein) designated IFN-αCON_1, a consensus analog of the known IFN-α subtypes (14), was provided by L. Blatt (Amgen Biologicals) for these studies. IFN activities are expressed in terms of international reference units per ml, as assayed by protection against the cytopathic effect of VSV on human fibroblasts, using the National Institutes of Health human IFN-α standard (G-023-901-527) for reference.

Antiviral Assay. Daudi cells were suspended at 5×10^5 cells per ml and incubated overnight with IFN-α at the indicated concentrations. The cells were washed twice with medium and infected with VSV (Indiana strain) for 1.5 hr at a

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Abbreviations: IFN, interferon; PKC, protein kinase C; VSV, vesicular stomatitis virus; ISG, interferon-stimulated gene; DAG, diacylglycerol; PS, phosphatidylserine; diC_8, 1,2-dioctanoyl-*sn*-glycerol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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multiplicity of infection of 0.1 plaque-forming unit per cell. Cell suspensions were washed with medium, and 24 hr later the virus yield in the medium was assayed by plaque formation on Vero cells.

Analysis of the Distribution of PKC Isozymes in Cytosolic and Particulate Extracts of Daudi Cells. Daudi cells were lysed in a hypotonic buffer [25 mM Tris·HCl, pH 7.4/10 mM EGTA/2 mM EDTA/20 mM 2-mercaptoethanol/100 mM NaF/soybean trypsin inhibitor (0.5 mg/ml)/leupeptin (0.5 mg/ml)/10 mM benzamide], and particulate and cytosolic extracts were prepared by centrifugation of cell lysates at 100,000 × *g* for 10 min at 4°C (15). The extracts were suspended in SDS sample buffer [25 mM Tris·HCl, pH 6.8/10% SDS/10% (vol/vol) glycerol/5% 2-mercaptoethanol], boiled for 5 min and subjected to SDS/PAGE (16). Proteins were transferred to nitrocellulose, immunoblotted with specific affinity-purified antisera against PKC isozymes, and visualized with ^{125}I-labeled protein A (0.1 μCi/ml; 1 Ci = 37 GBq) (17). The nitrocellulose paper was exposed to Kodak XAR film with intensifying screens at −70°C for 1–3 days.

***In Vitro* Activation of Purified Brain PKC-ε.** PKC-ε was purified as described (18) from COS-1 cells transiently expressing the enzyme. Purified PKC-ε (15 ng) was activated in a cell-free system by phosphatidylserine (PS) and 1,2-dioctanoyl-*sn*-glycerol (diC_8) in the presence of 1 mM EDTA and using unlabeled ATP to induce autophosphorylation (19). A parallel group was incubated in absence of PS and diC_8 ("basal"). The reaction was stopped by the addition of 2× Laemmli sample buffer. Samples were subjected to SDS/PAGE followed by transfer to nitrocellulose and immunoblotting with anti-PKC-ε antiserum (17) as described above.

Assay for Cellular DAG. At various times after the addition of IFN-α (1000 units/ml), Daudi cells were extracted with chloroform/methanol/6 M HCl (200:100:1). The organic phase was washed with 1% (wt/vol) HCl and dried under N_2. The lipid samples were dissolved in 20 μl of 7.5% β-octyl glucoside/25 mM cardiolipin and incubated with 1 mM [γ-^{32}P]ATP and *Escherichia coli* DAG kinase (20). The reaction was terminated by the addition of equal volumes of chloroform/methanol/6 M HCl and distilled water. The extracted lipid was analyzed by silica gel G thin-layer plate chromatography with a chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1) solvent system (3). The spot corresponding to PA was located by autoradiography, scraped off the plate, and assayed in a β-counter. The assay was linear with respect to DAG mass from 0.05 to 10 nmol.

RNA Accumulation and Transcription Assays. Daudi cells (6×10^7 cells per variable) were pretreated for 15 min at 37°C with the protein kinase inhibitors, HA1004 [*N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide] at 30 μM, or staurosporine (at 10, 100, or 1000 nM) followed by IFN-α (200 units/ml). Some cells received IFN-α without protein kinase inhibitor pretreatment, while others received neither IFN-α nor protein kinase inhibitors. After a 2-hr IFN-α treatment at 37°C, total cytoplasmic RNA was prepared as described (21) and the level of specific transcripts was measured by solution hybridization with antisense RNA probes (22). The ISG15 probe was generated from a 400-nucleotide genomic *Taq* I second exon fragment (22). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was generated from a 550-nucleotide *Hin*dIII/*Xba* I cDNA fragment of the human gene (23). Nuclear run-on transcription assays were performed with specific DNA test plasmids of ISG15, ISG54, chicken β-actin, and pBR322 as described (24); 5×10^7 nuclei were used in each reaction and labeled nuclear RNA was hybridized to excess plasmid DNA bound to nitrocellulose.

RESULTS

IFN-α Increases Cellular DAG Content. We have previously shown that IFN-α treatment of HeLa cells results in a transient increase in cellular DAG, followed by the selective translocation of PKC-β to a particulate cell compartment (3). To determine whether IFN-α induced an increase in cellular DAG content in Daudi cells, IFN-sensitive and IFN-resistant cells were incubated with IFN-α at 37°C for various times and cellular lipids were immediately extracted for determination of DAG mass by using the DAG kinase assay. As shown in Fig. 1, a 50% increase in DAG mass is observed within 2 min after IFN-α addition to IFN-sensitive Daudi cells, and a doubling of DAG mass is observed 5 min after addition. Thereafter, the DAG mass decreases and returns to baseline levels within 30 min of IFN-α addition. In contrast, IFN-α treatment has no effect on the DAG mass in the IFN-resistant subclone of Daudi cells (Fig. 1). In $^{32}P_i$ prelabeled Daudi cells, IFN-α did not result in inositol phospholipid turnover, and the production of inositol phosphates was not detected (data not shown).

Effects of IFN-α on PKC Isozymes in Daudi Cells. The increase in DAG mass in response to IFN-α treatment of IFN-sensitive, but not of IFN-resistant, Daudi cells suggested that PKC might be important in IFN-α action in Daudi cells. To test this hypothesis, we first analyzed the PKC subspecies found in these cells by using antisera that selectively detect PKC isoforms α, β, γ, δ, and ε (17). PKC-α and -ε are the only PKC isoforms detected in Daudi cells (data not shown). PKC-α is completely cytosolic (data not shown), while PKC-ε is found in both the cytosolic (≈30%) and the particulate (≈70%) fractions of untreated Daudi cells (Fig. 2*A*). IFN-α treatment had no effect on the amount or on the subcellular distribution of PKC-α, although the phorbol ester phorbol 12-myristate 13-acetate induced its translocation from the cytosol to a particulate fraction of Daudi cells (data not shown). Immunoblotting with the anti-PKC-ε antiserum revealed that IFN-α treatment resulted in both a time-dependent increase in the cytosolic and particulate immunoreactive PKC-ε and a decreased mobility of this isozyme in SDS/PAGE (Fig. 2*A Upper*). To test whether such effects on the immunoreactivity and apparent mobility of PKC-ε could

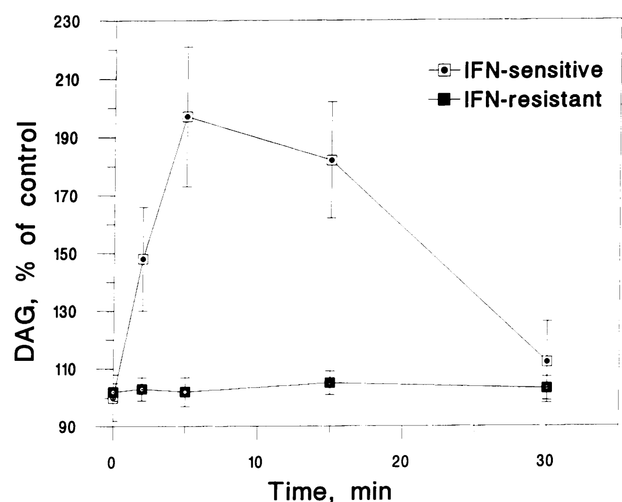

FIG. 1. Effects of IFN-α on cellular DAG content in Daudi cells. At various times after the addition of IFN-α (1000 units/ml), the DAG mass in the organic phase of an extract of IFN-sensitive and IFN-resistant Daudi cells was measured by using *E. coli* DAG kinase (20). DAG mass was obtained from the standard curve and the results (duplicate determinations in three experiments) are expressed as DAG in treated cells as a percentage of DAG in control Daudi cells (which contain ≈225 pmol of DAG per 10^6 cells). Error bars represent SEM.

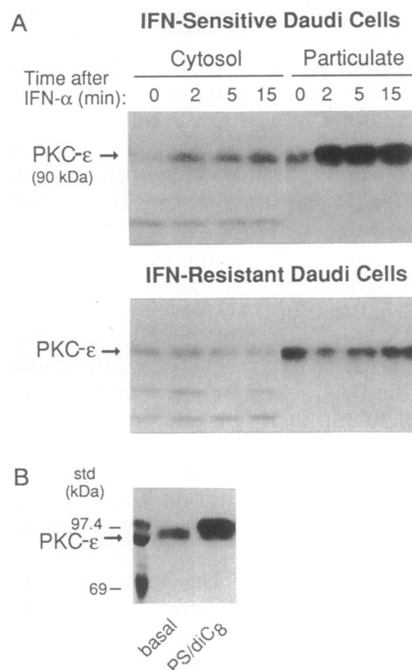

FIG. 2. Effects of IFN-α on PKC-ε in Daudi cells. (*A*) Time course of IFN-α treatment of IFN-sensitive (*Upper*) and IFN-resistant (*Lower*) Daudi cells. Cytosolic and particulate fractions were prepared as described. (*B*) Effects of PKC-ε activation on its immunoreactivity and apparent mobility in SDS/PAGE. Basal PKC-ε activity was determined as the activity of the enzyme in the absence of the allosteric activators PS and diC_8. The anti-PKC-ε antibody was used at a 1:200 dilution. The experiments were repeated three times with similar results. A representative experiment is shown.

be a result of its autophosphorylation/activation, purified brain PKC-ε was activated *in vitro* by the addition of PS, DAG, and EGTA in an unlabeled cell-free kinase reaction mixture. To ensure that during this reaction PKC-ε became autophosphorylated, experiments were performed in parallel using [γ-^{32}P]ATP (50,000 dpm/pmol) instead of unlabeled ATP (data not shown). The PKC-ε preparations that underwent unlabeled autophosphorylation were then subjected to immunoblotting with anti-PKC-ε antiserum. As shown in Fig. 2*B*, autophosphorylated PKC-ε was rendered more immunoreactive and exhibited decreased mobility in SDS/polyacrylamide gel. Thus, our findings in Fig. 2*A* can be interpreted to indicate that IFN-α induced the autophosphorylation of endogenous PKC-ε in IFN-sensitive Daudi cells. To determine whether these effects of IFN-α on PKC-ε were linked to the ability of IFN-α to induce biological responses in Daudi cells, the effect of IFN-α treatment on PKC isoforms was also examined in an IFN-resistant subclone of Daudi cells. As shown in Fig. 2*A* (*Lower*), several important differences were observed between the IFN-sensitive and resistant Daudi lines: the IFN-resistant cells exhibited overall more immunoreactive PKC-ε in the cytosolic and particulate fractions (see time point 0 for both cell lines), and IFN-α treatment of IFN-resistant cells for up to 15 min did not induce any changes. Although IFN-resistant Daudi cells seem to exhibit more immunoreactive PKC-ε, we cannot distinguish between "more" enzyme versus preactivated enzyme. Therefore, since IFN-α affects PKC-ε solely in the IFN-sensitive cell line, these data suggest that PKC-ε is involved in some of the biological actions of IFN-α in Daudi cells.

Effect of Inhibitors of PKC on the Induction of Antiviral Activity by IFN-α in Daudi Cells. A hallmark of IFN action is its ability to protect cells against a wide variety of RNA and DNA viruses. To determine whether PKC activation may play a role in specific biological actions of IFN-α on Daudi cells, the effects of agents known to affect PKC activity were assessed on the ability of IFN-α to protect cells against viral infection. IFN-α treatment (1000 units/ml) of the IFN-sensitive Daudi cell line results in a >4 $\log_{10}$ reduction in titer of VSV (data not shown). In contrast, IFN-α treatment (1000 units/ml) of the IFN-resistant Daudi subclone results in only a 1 log reduction of virus titer (Table 1). Both H-7 and staurosporine, protein kinase inhibitors that selectively act on PKC activity, resulted in a dose-dependent reduction in the ability of IFN-α to inhibit VSV replication in IFN-sensitive cells. In contrast, HA1004, a weak PKC inhibitor, had no effect on the ability of IFN-α to block VSV replication. Thus, inhibition of PKC activity by H-7 or staurosporine indicates that PKC activity is required in the induction by IFN-α of an antiviral state in Daudi cells.

Inhibitors of PKC Block the Expression of IFN-Stimulated Genes. The development of antiviral activity induced by IFN-α requires 18–24 hr. Since our results indicate that PKC-ε activation and the production of DAG occur within minutes of IFN-α addition to Daudi cells (Figs. 1 and 2*A*), we examined the effect of PKC inhibitors on early events in IFN-α action—i.e., the induction of ISG expression. IFN-α treatment of Daudi cells results in the rapid transcriptional activation of ISG15, ISG54, and ISG56, so named to denote the apparent molecular mass in kDa of the proteins they encode. These genes are transcriptionally induced in Daudi cells within 15 min after IFN-α addition, and this induction proceeds in the absence of protein synthesis (12).

Daudi cells were treated with various concentrations of staurosporine or the weak PKC inhibitor HA1004 for 15 min prior to the addition of IFN-α. After 2 hr of IFN treatment, cytoplasmic RNA was isolated and hybridized to specific antisense RNA probes for ISG15 and for GAPDH, and the

Table 1. Effect of protein kinase inhibitors on the inhibition of VSV replication in IFN-α-treated Daudi cells

Daudi cell line	Inhibitor (μM)	Virus titer: Without IFN-α	Virus titer: With IFN-α	IFN-induced -fold reduction in viral titer
IFN sensitive	None (0)	6.75	2.15	40,000
	Staurosporine (0.03)	6.71	2.71	10,000
	Staurosporine (0.1)	6.62	3.63	280
	Staurosporine (0.3)	6.51	4.62	80
	Staurosporine (1.0)	6.23	6.04	1.5
	H-7 (10)	6.61	2.11	32,000
	H-7 (30)	6.55	4.74	65
	HA-1004 (30)	6.71	2.09	42,000
IFN resistant	None (0)	6.45	5.55	8

Daudi cells were incubated with protein kinase inhibitors 15 min prior to the addition of IFN-α (100 or 1000 units/ml for IFN-sensitive or IFN-resistant cells, respectively). After overnight incubation, the cells were infected with VSV, and the virus yield was assayed by plaque formation (expressed as the $\log_{10}$ virus yield per ml of medium).

RNase-resistant products were displayed on sequencing gels. At 1 μM, staurosporine was found to completely block the accumulation of ISG15 mRNA (Fig. 3*A*), while it was only slightly decreased by pretreatment with 30 μM HA1004. As a control, we also examined the effect of staurosporine and HA1004 on the expression of GAPDH, a housekeeping gene. Pretreatment of Daudi cells with staurosporine had only a slight inhibitory effect on the level of GAPDH mRNA, while HA1004 had no effect on the expression of GAPDH.

Nuclear run-on transcription assays were performed to determine whether PKC activity was required for transcriptional activation by IFN-α. Nuclei were isolated from untreated Daudi cells or from Daudi cells treated with IFN-α for 45 min in the absence or presence of staurosporine or HA1004. Nascent RNA was radiolabeled *in vitro* by incubating the nuclei in the presence of [α-^{32}P]UTP for 10 min and was hybridized to DNA samples fixed to nitrocellulose. The addition of 1 μM staurosporine completely blocked the transcriptional response of ISG15 and ISG54 to IFN-α (Fig. 3*B*), while HA1004 was ineffective in this respect (data not shown). Staurosporine (1 μM) had no effect on the transcription of the control actin gene. The inhibitory effect of staurosporine on ISG15 mRNA accumulation (Fig. 3*A*) is therefore the result of transcriptional inhibition. A 5- to 10-fold higher concentration of staurosporine (1 μM) than was previously reported in HeLa cells (4) is required to block the transcriptional response of ISGs in Daudi cells and may reflect cell type differences in the uptake of staurosporine (data not shown).

DISCUSSION

The experiments described here provide evidence that PKC activation is an important early step in the pathway that leads to the pleiotropic effects of IFN-α on Daudi lymphoblastoid cells. First, IFN-α treatment of IFN-sensitive Daudi cells results in enhanced DAG production in a time-dependent manner. The changes in the immunoreactivity and apparent mobility of PKC-ε, which we interpret as activation of the enzyme, follow the same time course. In addition, IFN-α treatment of the IFN-resistant subclone of Daudi cells does not result in DAG production or PKC-ε activation, suggesting a direct link between enhanced DAG production, PKC-ε activation, and the biological action of IFN-α. Previous studies have established that the primary defect in nonresponsive Daudi cells is in their signal transduction pathway because of an inability to activate ISG transcription despite normal expression of high-affinity IFN-α receptors (13). Moreover, studies with protein kinase inhibitors show that staurosporine (a potent inhibitor of PKC) selectively blocks the accumulation of ISG mRNAs in the cytoplasm of Daudi cells and the IFN-α-mediated induction of antiviral activity in Daudi cells. These results suggest that PKC activity is required for ISG expression and for the induction of an antiviral state in Daudi cells.

Although treatment of human fibroblasts, HeLa cells, and Daudi cells with selective PKC inhibitors or chronic pretreatment with phorbol esters inhibits the IFN-induced cytoplasmic accumulation of mRNA for IFN-responsive genes, PKC activation by a brief treatment with phorbol esters alone does not induce the expression of these genes (24, 25). Therefore, PKC activation is implicated in posttranscriptional mechanisms that control the accumulation of ISG mRNA in cells. However, it has not been determined whether IFN-α directly activates PKC in Daudi cells or whether PKC activation plays a role in the transcriptional response of ISGs.

To address whether IFN-α directly activates PKC in Daudi cells, we determined whether IFN-α activates selective isoforms of PKC and/or induces the production of DAG, an intracellular activator of PKC. We have shown that IFN-α leads to a rapid and transient increase in cellular DAG levels in Daudi cells. Furthermore, our results indicate that IFN-α induces the rapid and selective activation of PKC-ε.

DAG, through its association with PKC, is now recognized as an important cellular messenger. In previous studies, it was reported that IFN-α induces a transient increase in DAG production in Daudi cells resulting from the turnover of inositol phospholipids (26). However, IFN-α does not induce an increase in free intracellular calcium concentration in Daudi cells as determined with the fluorescent indicators quin-2 and fura-2 (26, 27). Intracellular calcium mobilization is mediated by the production of inositol trisphosphate, a product of inositol phospholipid hydrolysis along with DAG. In the present study, we were unable to define the lipid source for DAG. Increased DAG production in response to IFN-α treatment could result from an activation of inositol phospholipid hydrolysis, phosphatidylcholine hydrolysis, or other less defined mechanisms. From our evidence, it is clear that DAG does not derive from inositol phospholipid turnover, as we cannot detect an IFN-induced increase in inositol phosphates, nor do we observe an IFN-induced calcium transient in Daudi cells (27). The classical scheme involving inositol phospholipid hydrolysis as the sole source of DAG has been challenged by accumulating evidence that phosphatidylcholine is an alternative source. Accordingly, in HeLa cells IFN-α increases DAG mass and phosphatidic acid production through the turnover of phosphatidylcholine (3).

Thus, it is clear that the generation of non-inositol phospholipid-derived DAG is a common cellular event in the signaling pathway initiated by IFN-α binding to its cell surface receptors, albeit DAG production may be governed by distinct avenues. A number of criteria suggest that the

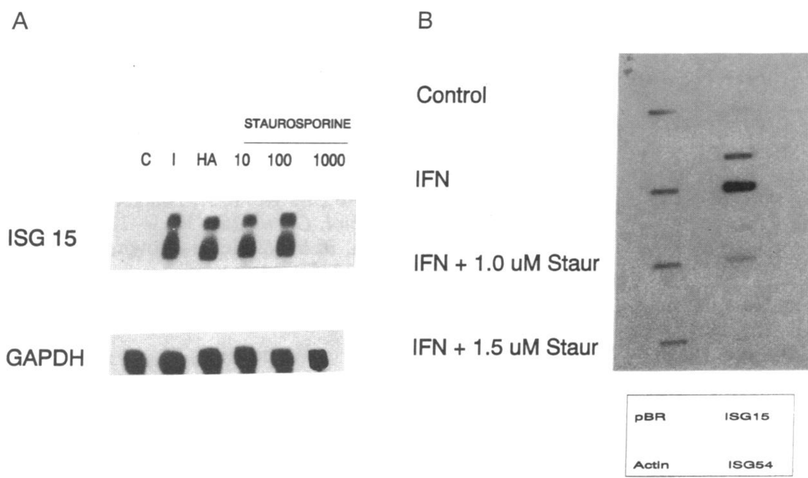

FIG. 3. Suppression of IFN-stimulated gene expression by staurosporine. (*A*) Cytoplasmic levels of ISG15 and GAPDH mRNAs. Cells were untreated (lane C), or treated with IFN-α (1000 units/ml for 2 hr) in the absence (lane I) or presence (lane HA) of 30 μM HA1004 or staurosporine (10, 100, or 1000 nM, as noted). mRNA analyses were performed by hybridization to specific antisense probes and gel electrophoresis. (*B*) Transcriptional induction by IFN-α. Nuclear run-on experiments were performed with control cells or IFN-treated cells (45 min) in the absence or presence of staurosporine. (*Lower*) Pattern of test DNAs.

PKC isoform activated by IFN-α in Daudi cells may be PKC-ε. First, IFN-α treatment induces an increase in endogenous DAG production without a measurable increase in Ca^{2+}, suggesting that a Ca^{2+}-independent PKC might be involved in the signaling pathway of IFN-α in Daudi cells. Indeed, although these cells have PKC-α and -ε, it is PKC-ε that is preferentially activated in response to IFN-α. Moreover, this PKC isotype-specific activation takes place in the Daudi cell line that is IFN-α sensitive, but not in IFN-resistant Daudi cells.

PKC-ε belongs to a recently discovered family of related enzyme subspecies designated PKC-δ, -ε, -ζ (28), and -η (9). The mechanism(s) of its activation in intact cells by ligands under physiological conditions is poorly understood. Recently, PKC-ε has shown to be the predominant PKC isoform in chicken neurons (29), where PKC-ε may be coupled to the neuronal response to insulin stimulation. The enzyme is also present in large amounts in the $CD4^+/CD8^+$ subset of murine thymocytes, where it becomes activated by plant lectins or calcium ionophores (17). Furthermore, the fact that PKC-ε is resistant to down-regulation by chronic exposure to phorbol esters in many cell systems tested (17, 29), including Daudi cells (data not shown), and that it exhibits substrate specificity distinct from the other, better characterized PKC isoforms (9, 10) suggests that this enzyme may play a unique role in signal transduction. Indeed, although Daudi cells have both PKC-α and -ε, it is PKC-ε that seems to be linked to the biological effects of IFN-α. The mechanism of PKC-ε activation by IFN-α treatment is unclear. IFN-α may induce either autophosphorylation of the enzyme (30) or, alternatively, it may activate a PKC-specific kinase. Our data at present cannot discriminate between these two possibilities. It is somewhat puzzling that the phosphorylated form of PKC-ε is found in both the cytosolic and the particulate form in Daudi cells, since redistribution of PKC is believed to be a prerequisite for enzyme activation. However, recent data have shown that phosphorylation of endogenous or exogenous substrates can occur or be sustained in the absence of significant increases in membrane-associated PKC (31). Furthermore, insulin stimulates PKC-ε activity by a mechanism associated with a change in mobility of cytosolic PKC-ε in SDS/PAGE, but not involving PKC translocation (29).

Why might cells have more than one PKC isoform and, in particular, isoforms of PKC that differ in relation to their calcium requirement? One reason may be that multiple isoforms of PKC permit selective responses to different stimuli or confer a cell-specific response to a given agonist. We have shown that IFN-α selectively activates the PKC-β in HeLa cells and PKC-ε in Daudi cells. Epidermal growth factor, which stimulates HeLa cell proliferation and has been shown to activate PKC activity, has no effect on the distribution of PKC-β (3). Specificity may reside in the particular lipid species that is hydrolyzed after ligand binding. The outstanding feature of the signal transduction pathway of IFN-α is the generation of DAG in the absence of inositol phospholipid turnover or calcium elevation.

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