Evidence for Common Mechanisms in the Transcriptional Control of Type II Nitric Oxide Synthase in Isolated Hepatocytes

REQUIREMENT OF NF-κB ACTIVATION AFTER STIMULATION WITH BACTERIAL CELL WALL PRODUCTS AND PHORBOL ESTERS*

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Incubation of primary cultures of rat hepatocytes with lipopolysaccharide (LPS), S-[2,3-bis(palmitoyloxy)-(2-R,S)-propyl]-N-palmitoyl-(R)-Cys-Ser-Lys₄ (TPP), a synthetic lipopeptide present in bacterial cell wall lipoproteins, or with phorbol 12,13-dibutyrate (PDBu) induced an increase in nitric oxide synthesis through the expression of type II nitric oxide synthase (iNOS). Transfection of hepatocytes with a HindII fragment corresponding to the promoter region of the murine iNOS gene (from nucleotide −1588 to +165) resulted in the expression of the reporter gene when cells were stimulated with these factors. The transcription factors activated by these stimuli involved an increase in the nuclear content of proteins that bind to κB, AP-1, GAS, and SIE sequences. Inhibition of NF-κB activation by pyrrolidine dithiocarbamate eliminated the expression of iNOS in hepatocytes stimulated with LPS, TPP, or PDBu. In addition to this, transfection of hepatocytes with promoter mutants in which a sequential 2-base pair change within the κB sites was introduced (position −971 to −961 and −85 to −75, respectively), resulted in approximately 17 and 35%, respectively, of the activity of the naïve promoter. Simultaneous mutation of both κB sites abolished the promoter activity. Analysis of the proteins involved in κB binding showed the presence of p50/p65 dimers in the nuclei of activated cells at the time that an important decrease of IκB-α was observed soon after cell stimulation with LPS, TPP, or PDBu. However, only LPS was able to decrease the amount of IκB-β. These results suggest that LPS, TPP, and PDBu, although activating different signal transduction pathways, use a common mechanism mediating iNOS expression in cultured hepatocytes.

The involvement of nitric oxide in different pathophysiological pathways is a subject of current research (1, 2). Three different nitric oxide synthase species have been identified in mammalian tissues, each exhibiting an important degree of tissue-specific expression as well as significant differences in their regulatory properties (1–3). The species recognized as type II nitric oxide synthase (iNOS)¹ is induced in a number of different cell types in response to cytokines involved in inflammation and host defense as well as by bacterial cell wall products and some pharmacological agents (3–6). The promoter region of iNOS has been characterized in different species, including humans and mice (7, 8). A 1753-base pair fragment of the promoter region of iNOS has been cloned and characterized from the murine RAW 264.7 macrophage cell line (8–10). Sequence analysis of this promoter revealed the presence of at least 24 consensus motifs for binding of transcription factors, including 2 copies for nuclear factor κB (NF-κB), 2 copies for activator protein-1 (AP-1), 10 copies of IFN-γ response elements (γ-IRE), 3 copies of the γ-activated site (GAS), 2 copies of the IFN-stimulated response element (ISRE), and 2 copies of the tumor necrosis factor-α responsive element, among others (8–11). Functional analysis in RAW 264.7 cells using deletional mutants of the iNOS promoter revealed the presence of two important regulatory regions, each one containing a κB binding site for NF-κB and exhibiting an important interaction of their roles in activation of iNOS transcription (8, 9). These regions cover 200 base pairs upstream of the start site of transcription and positions −913 to −1020 and have been referred to as the proximal and distal regulatory regions, respectively (8, 9). In addition to the cooperation between these two regions, a concerted synergism among nuclear factor binding sites exists in each region. This is the case for macrophages, where the interaction elicited by suboptimal concentrations of LPS and IFN-γ on NO synthesis has been demonstrated to be the result of a cooperative interaction between the IFN regulatory factor 1 and NF-κB sites in the distal region (12, 13).

Apart from macrophages, iNOS is induced in a variety of other cell types such as neural cells, keratinocytes, myocytes, mesangial cells, tumor cells, and hepatocytes in response to a wide array of either physiological or pathological cellular stresses (1). In the case of the liver, both hepatocytes and Kupffer cells express iNOS in response to different cell stimuli such as septic shock, cirrhosis, hyperdynamic circulation, or after partial hepatectomy (14–16). Moreover, primary cultures of hepatocytes retain their ability to express iNOS after exposure to LPS, TPP, or after treatment with phorbol esters that are pharmacological activators of protein kinase C (5, 6). All of these molecules induce by themselves the expression of a functional iNOS as deduced by the large amounts of NO released to the medium (5, 6, 14–16). Hepatocytes also constitute an interesting experimental model because these cells have an extremely high transcriptional rate in combination with a very low proliferative capacity, and, therefore, most of the transcriptional factors required for commitment to cell growth are switched off (17). In this regard, we have investigated the

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The abbreviations used are: iNOS, type II nitric oxide synthase; LPS, lipopolysaccharide; TPP, S-[2,3-bis(palmitoyloxy)-(2-R,S)-propyl]-N-palmitoyl-(R)-Cys-Ser-Lys₄; PDBu, phorbol 12,13-dibutyrate; IFN-γ, interferon-γ; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyltransferase; GAS, γ-activated site; PBS, phosphate-buffered saline.
Transcriptional Control of iNOS in Hepatocytes

factors involved in the transcriptional activation of hepatic iNOS in response to three defined effectors that, because of its unrelated chemical structure, act on the hepatocyte through different transduction pathways; LPS acts through the engagement of CD14 (18), whereas the TPP receptor remains elusive (6, 19). Phorbol esters are potent activators of protein kinase C subspecies containing a tandem of two zinc-finger domains and whose expression pattern varies between tissues (20). The ability of cells to express iNOS in response to phorbol esters appears to be restricted to some cell types, including rat hepatocytes and peritoneal macrophages (5, 21). For this reason we investigated the pattern of transcriptional factors relevant to iNOS expression in hepatocytes incubated with these three stimuli.

Our results show that treatment of primary cultures of hepatocytes with LPS, TPP, and phorbol esters trigger a similar pattern of transcription factor activation, including members of the NF-κB, AP-1, GAS, and SIF binding proteins. This transcriptional activation was also evident in hepatocytes transfected with plasmids encoding the murine iNOS promoter or consensus sequences for the binding of NF-κB and AP-1 linked to a CAT reporter gene. These results suggest that the expression of iNOS in hepatocytes in response to LPS, TPP, and phorbol esters is mediated through the engagement of a similarly regulated transcriptional mechanism.

MATERIALS AND METHODS

Reagents—Cytokines and biochemicals were from Sigma. Materials and chemicals for electrophoresis were from Bio-Rad. 3-[2,3-bis(palmitoyloxy)-2-\(R,S_{\text{h}}\)-propyloxy)-(2-\(R,S\)\)-toyloxy]-N-palmitoyl-(R)-Cy5-Ser-Lys (TPP) was from Boehringer Mannheim. LPS was from *Salmonella typhimurium*. Plas- mids were purified by extensive washing using Qiagen columns (Hilden, FRG). The endotoxin content in cytokines and plasmid preparations was determined using the *Limulus polyphemus* test (Sigma) and was below 0.1 ng/ml protein and 30 pg/ml plasmid dilution, respectively, at the dose used for transfection. Serum and media were from BioWhittaker (Walkersville, MD).

Isolation and Culture of Hepatocytes—Isolation of hepatocytes was carried out from 3-month-old male rats by perfusion with collagenase in Krebs bicarbonate buffer under continuous gassing with carbogen (O\(_2\)/car- bon\(_2\)) at 1 atm. Collagenase (L-1365, Worthington, Orangeburg, NY) was from Boehringer Mannheim. The hepatocytes (3 \times 10^6) were maintained overnight with incubation medium supplemented with 10% fetal calf serum. The hepatocytes (3 \times 10^6) were washed twice with ice-cold PBS, and the hepatocytes were collected with a 1 ml pipet. Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22). Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22). Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22). Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22). Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22).

Transfection of Hepatocytes and CAT Assays—Freshly isolated hepatocytes were transfected by electroporation (22). Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22). Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22). Cells (4 \times 10^9 in 0.8 ml of PBS) were kept at 4 °C and electrophoresed (22).

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FIG. 1. iNOS mRNA levels and NO synthesis in cultured hepatocytes stimulated with LPS, TPP, and PDBu. Hepatocytes (3 × 10^6 cells) were cultured in the presence of 1 μg/ml LPS, 5 μg/ml TPP, or 40 μM PDBu, and samples were collected at 6 (open bars) and 18 h (hatched bars) to determine the NO release to the culture medium (A) and the mRNA levels of iNOS (B), respectively. Results show the mean ± S.E. of three experiments. *, p < 0.005 versus the control condition.

iNOS promoter (8), 5′-tgcaACATGGGAGGCTCCCTTGG-GAAC-A3′ and 3′-GTTGACCCCTGAGAGGGAAACCCTTGTagct-5′; NF-κB (corresponding to the distal NF-κB motif (nucleotides 978 to -952) of this promoter), 5′-tgcaTGCTAGGGGATTCCCCCTTTCTCTGT-3′ and 3′-ACGATCCCCCTAAAAGGGAGAGAGACAagct-5′; GAS (corresponding to the Ly-6E promoter GAS site (29)), 5′-catgTTATGC-c-5′-gtc-3′; SIE (corresponding to the high affinity SIE m67 site (29), 5′-GACAGTTCCCGTCAATC-3′ and 3′-tcgaTTCCAAAGAGTCATCAG-3′; promoter (30)), 5′-GGTTGACCCCTGAGAGGGAAACCCTTGTagct-5′; AP-1 (consensus) (corresponding to the AP-1 motif of the albumin promoter (30), 5′-tgcaATTTTCCGTAATTG-3′ and 3′-AATACCTATAAGGACATTACgct-3′; SIE (corresponding to the high affinity SIE m67 site (29), 5′-gtc-GAGAGTTCCCGTCAATC-3′ and 3′-GCTAGGGGCGTTAAGG-5′; AP-1 (consensus) (corresponding to the AP-1 motif of the albumin promoter (30), 5′-tgcaTCTCAAGAGGACTCAG-3′ and 3′-AAGGTGTT-GCTTTTCTCTTGAGTAgct-5′.

The oligonucleotides were annealed after incubation for 5 min at 85 °C in 10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 10 mM MgCl₂, and 1 mM dithiothreitol. Aliquots of 50 ng of these annealed oligonucleotides were end-labeled with the Klenow enzyme fragment in the presence of 50 μCi of [α-32P]dCTP and the other unlabeled dNTPs in a final volume of 50 μl. The oligonucleotides were precipitated in ethanol, extracted with phenol/chloroform to remove the unincorporated nucleotides, and 10 μg/ml LPS, 5 μg/ml TPP, or 1 μg/ml LPS CAT activity was determined after 24 h of culture. The amount of acetylated chlorphenol was determined by counting the radioactivity of the corresponding spots, and CAT activity was expressed as the percentage of the activity of cells transfected with kSV₂-CAT plasmid (A). The effect of PDBu, TPP, and LPS on the activity of a control promoter construct (kSV₂-CAT) is shown (B). Results show the mean ± S.E. of three experiments. *, p < 0.01 versus nonstimulated cells transfected with p1iNOS-CAT.

Absence of iNOS mRNA levels and NO synthesis resulted when the hepatocytes were incubated with α-phorbol 12,13-didecanoate, an inactive phorbol ester (data not shown). To investigate whether the iNOS expression elicited by TPP and PDBu is mediated by the regulatory elements identified in the murine iNOS promoter, hepatocyte suspensions were transfected by electroporation with a plasmid (p1iNOS-CAT) containing a 1.8-kilobase fragment of the iNOS promoter region linked to a CAT gene, and CAT activity was measured after treatment with LPS, TPP, or PDBu. As Fig. 2A shows, hepatocytes transfected with this promoter construct exhibited a significant capacity to express CAT activity in response to these stimuli (7.2- and 14-fold increase in cells treated with TPP or PDBu and with LPS, respectively). Stimulation of hepatocytes transfected with a kSV₂-CAT vector with PDBu, TPP, or LPS did not affect CAT activity (Fig. 2B), suggesting a specificity in the response of the iNOS promoter to these factors. The release of NO and the expression of CAT activity by rat hepatocytes transfected with p1iNOS-CAT and stimulated with LPS, TPP, and PDBu suggest that the organization of the analyzed region of the iNOS promoter in murine and rat cells might be equivalent and therefore makes it possible to investigate the mechanism by which iNOS is induced by LPS, TPP, and PDBu on the basis of the known structure of the murine promoter.

**Characterization of Transcriptional Activation by Electrophoretic Mobility Shift Assays**—The 1.8-kilobase murine iNOS promoter region contains at least 24 consensus sequences for the binding of transcriptional factors, and some of them have an important role in the control of iNOS expression (8–12). We have investigated whether the sequences corresponding to the distal and proximal NF-κB motifs of the iNOS promoter, as well as GAS, SIE, and AP-1 motifs, bind nuclear factors in response to hepatocyte activation by LPS, TPP, and PDBu. As Fig. 3A shows, when the proximal NF-κB sequence was used for EMSA, an enhanced binding of nuclear proteins was observed in hepatocytes at 1 h after stimulation. In samples assayed at 6 h, the band corresponding to the b complex (p50/p65, see below) was significantly lower in hepatocytes incubated with PDBu but not when they were treated with LPS or TPP. Similar results were obtained when the distal NF-κB sequence was
assayed. The nature of the proteins that bind to the \( \kappa B \) sequence were characterized using supershift assays. As Fig. 3B shows, the protein complexes retained in hepatocytes stimulated with 5 \( \mu \)g/ml TPP for 1 h corresponded to p50/p50 dimers (band a) and p50/p65 heterodimers (band b), respectively. Treatment of the nuclear extracts with anti-p52 or anti-c-Rel antibodies did not modify the pattern of bands, indicating the absence of the corresponding NF-\( \kappa B \)/c-Rel proteins in the nuclei of control or stimulated hepatocytes. Identical supershift results were obtained using the distal \( \kappa B \) sequence or when nuclear extracts were prepared from cells treated for 1 h with LPS or PDBu. In addition to \( \kappa B \), binding to AP-1, GAS, and SIE sequences reflected a similar pattern of engagement of these response elements (1 h after activation) in the mechanism of action of LPS, TPP, and PDBu (Fig. 4).

To ensure that the nuclear factors that bind \( \kappa B \) and AP-1 sequences in EMSA operate effectively in intact cells and were not the result of nonspecific binding or cytosolic contamination of the nuclear extracts, hepatocytes were transfected with plasmids harboring a tandem of three \( \kappa B \) ((\( \kappa B \))\(_2\)ConACAT vector) or two AP-1 motifs ((AP-1)\(_2\)ConACAT vector) linked to a minimal promoter (corresponding to the conalbumin A gene). As Fig. 5, A and B show, CAT activity increased at least 6-fold upon stimulation with PDBu, TPP, or LPS of hepatocytes transfected with (\( \kappa B \))\(_2\)ConACAT or (AP-1)\(_2\)ConACAT vectors. Transfection with a ConACAT plasmid failed to respond to these stimuli (data not shown).

Characterization of the Proteins Involved in NF-\( \kappa B \) Activation—In order to characterize the proteins involved in the activation of \( \kappa B \) motifs, the amount of p50 and p65 in the cytosolic and nuclear extracts from activated hepatocytes was assessed by Western blot. As Fig. 6 shows, p65 was translocated from the cytosol to the nuclear compartment in stimulated cells. The amount of p65 present in nuclear extracts of untreated cells was negligible, confirming the results observed by EMSA (Fig. 3). Regarding p50, the protein was immunodeTECTED in the nuclear extracts of control cells and the levels increased upon translocation from the cytosol to the nucleus after cell stimulation with either LPS, TPP, or PDBu.

Dissociation and degradation of the IкB moieties of the cytosolic NF-\( \kappa B \)/Rel complexes is an important mechanism for the control of the translocation to the nucleus of members of the Rel family (32). Since the amount of NF-\( \kappa B \)/Rel proteins that become free to access the nucleus is supposed to be proportional to the degradation of IкB, we measured the IкB-\( \alpha \) and IкB-\( \beta \) protein levels in the cytosol by Western blot. As Fig. 7 shows, the levels of IкB-\( \alpha \) 1 h after stimulation of hepatocytes with LPS, TPP, or PDBu decreased by 64, 73, or 42\%, respectively.
Fig. 7. Western blot analysis of IκB-α and IκB-β. Cytosolic extracts (30 μg of protein/lane) from cells treated for 1 or 6 h with 1 μg/ml LPS, 5 μg/ml TPP, or 40 nM PDBu were analyzed by Western blot using murine anti-IκB-α or IκB-β antibodies. Results show the mean ± S.E. of three blots. * and ** are given for p < 0.05 and p < 0.001 of the differences between control and stimulated cells at each sampling time.

However, the levels of IκB-α at 6 h fully recovered in the cytosol independently of the treatment of the cells. Regarding IκB-β, this protein only decreased in cells treated with LPS for 1 h, and control levels were restored 6 h after stimulation. At the mRNA level, an important up-regulation of IκB-α was observed in stimulated cells for 6 h with LPS and TPP and to a lesser extent in cells treated with PDBu (Fig. 8). In hepatocytes stimulated for 18 h with LPS an important increase of the IκB-α mRNA levels was still evident.

Role of NF-κB Activation on iNOS Expression—To investigate further the importance of nuclear binding of proteins from the Rel family to the κB sites, experiments were undertaken in which cells were treated for 1 h prior to exposure to LPS, TPP, or PDBu with pyrrolidine dithiocarbamate (25 μM), an inhibitor of IκB degradation. As Table 1 shows, pyrrolidine dithiocarbamate abrogated the NO synthesis elicited by hepatocytes incubated with LPS, TPP, or PDBu to the same extent. Under these conditions, an important reduction in the nuclear content of p50/p65 complexes was observed, reflecting the fact that NF-κB activation is required for iNOS expression in response to either LPS, TPP, or PDBu in hepatocytes.

The relative contribution of each κB site to the activity of the iNOS promoter was analyzed using constructs in which the proximal and/or distal sequences were mutated to abolish the binding of NF-κB/Rel proteins (substitution of the GGG motif by the CCG sequence). As Fig. 9 shows, mutants of the proximal site (pNOS-κBp− vector) still retained 27–35% of the original promoter activity, whereas mutation of the distal κB site (pNOS-κBd− vector) resulted in 82–84% inhibition of the promoter activity, regardless of treatment of hepatocytes with LPS, TPP, or PDBu. As predicted on the basis of the data reported in Table 1, simultaneous mutation of both κB sites (pNOS-κBp−,d− vector) eliminated the promoter activity.

The relative contribution of the two AP-1 binding sites to the promoter activity was investigated using deletional mutants. As Fig. 9 also shows, promoter activity of the vector p1NOS(Δ1), which contains both the distal and proximal AP-1 sites, was roughly similar to the activity of the pNOS-κB vector, which lacks the distal AP-1 site, independently of the stimulation with bacterial products or phorbol esters. The same behavior was observed when the promoter activity of p1NOS(Δ2), which contains the proximal AP-1 and κB sites, was compared with the activity of the vector p1NOS(Δ3), which lacks the proximal AP-1 site (Fig. 9). These results agree with the data reported by other authors using different approaches and suggest that the activity of the promoter is totally independent of AP-1 activation.

DISCUSSION

The expression of iNOS in response to LPS and TPP is well established among different cell types; however, the ability of phorbol esters to induce iNOS seems to be restricted to some cell types such as hepatocytes, peritoneal rat macrophages, and astrocytes (5, 6, 33, 34). For this reason, primary cultures of rat hepatocytes were used to investigate the transcriptional control mediating iNOS expression in response to bacterial products and phorbol esters.

The promoter region of the rat iNOS gene is still unknown, but it is likely to be closely related to the cloned murine sequence in view of the similarities in the transactivation mechanism of iNOS (8, 9, 25, 33). We first confirmed that rat hepatocytes transfected with a plasmid harboring an heterologous murine iNOS promoter displayed a functional behavior that was equivalent to the response described in murine cells (8, 9) and paralleled the release of NO by the endogenous enzyme,
sequences we have confirmed the involvement of NF-κB mutants in which both the NO synthesis mediated in response to LPS, TPP, and dithiocarbamate, an inhibitor of iNOS, has been widely recognized as an absolute requirement (10, 11), and in this regard, incubation of hepatocytes with pyrrolidine dithiocarbamate decreased the amount of IκB-β, which is in agreement with previous results (38). It is possible that the regulation of binding to κB motifs in activated hepatocytes is even more complex, because NO seems to be an important modulator of IκB-α function (39). However, NO has no effect on IκB-β levels or over other transcription factors such as AP-1 or GATA (39). The importance of the role of IκB-α in the regulation of NF-κB functioning is underlined by the observation that the transrepression of NF-κB by glucocorticoids is due to the control exerted by these hormones on IκB-α levels (40).

The 1.7-kilobase murine iNOS promoter region that has been characterized contains two AP-1-related binding sequences (8, 9), which prompted us to investigate the effect of this transcription factor upon hepatocyte stimulation. Fos and Jun form part of the dimeric complex recognized as AP-1, and this transcription factor participates in the regulation of the basal or the inducible activity of several genes (41). As expected from previous work (41–43), PDBu and LPS stimulated the binding of proteins to AP-1 consensus sequences at the time that induced CAT activity in hepatocytes transfected with a (AP-1)2ConA-CAT plasmid. The ability of TPP to activate AP-1 was previously unrecognized. However, deletion of the AP-1 binding sites in the iNOS promoter did not affect the reporter activity in hepatocytes stimulated with LPS, TPP, or PDBu. These results confirm the low contribution of the AP-1 sites to the activity of the iNOS promoter in macrophages stimulated with cytokines (8, 9). Current view about transcriptional activation through the AP-1 complex stresses the importance of the phosphorylation at distinct residues of c-Jun as a critical requirement for the stability and transcriptional activity of AP-1 (41, 43, 44). Therefore, since several kinases can independently regulate AP-1 activity, the mechanisms that maintain a functionally active AP-1 complex in hepatocytes stimulated with LPS, TPP, or PDBu might be different.

IFN-γ has proved to be an important cytokine for iNOS induction, synergistically acting with LPS and tumor necrosis factor-α or with phorbol esters in a more exclusive fashion (1, 11, 12, 34, 45, 46). These effects of IFN-γ on iNOS transcription have been attributed to the presence in the murine promoter region of this gene of a sequence for binding of interferon regulatory factor-1 (IRF-1), and this motif acts in combination with the distal NF-κB site, giving rise to IFN-κB-dependent potentiation when macrophages are activated with suboptimal doses of LPS (8–11). In addition to this, two important targets of IFN-γ action are the GAS and SIE motifs to which STAT proteins bind (29). GAS and SIE sequences are activated not only by IFN-γ but also by other cytokines such as epidermal growth factor, platelet-derived growth factor, and interleukin-6, the latter being an important cytokine that participates in the hepatic acute phase response (14, 47, 48). Moreover, a GAS site has been identified in the promoter region of the IRF-1 gene, suggesting a feedback up-regulation of this impor-

### Table I

**Effect of PDTC on NO synthesis and p50/p65 nuclear complexes in activated hepatocytes**

| Stimulus     | None                                       | LPS 1 μg/ml | TPP 5 μg/ml | PDBu, 40 nM |
|--------------|--------------------------------------------|-------------|-------------|-------------|
| NOx− (nmol/mg protein) | 2.2 ± 0.3                                  | 17 ± 3      | 15 ± 3      | 14 ± 2      |
| + PDTC       | 0.5                                        | 2 ± 0.3     | 3 ± 0.1     | 2 ± 0.2     |
| p50/p65 intensity (arbitrary units) | 3.1 ± 0.2                                  | 56 ± 6      | 45 ± 4      | 39 ± 5      |

**Fig. 9. Role of NF-κB and AP-1 on the activity of the iNOS promoter in stimulated hepatocytes.** Primary cultures of hepatocytes were transfected with the indicated vectors. Cells were stimulated for 24 h with LPS (1 μg/ml), TPP (5 μg/ml), or PDBu (40 nM), and CAT activity was measured. The activity of the different promoters for each treatment was referred to the pNOS-κB vector taken as 100%. The activity of the iNOS promoter region contains regulatory sequences activated upon hepatocyte stimulation. Fos and Jun form part of the dimeric complex recognized as AP-1, and this transcription factor participates in the regulation of the basal or the inducible activity of several genes (41). As expected from previous work (41–43), PDBu and LPS stimulated the binding of proteins to AP-1 consensus sequences at the time that induced CAT activity in hepatocytes transfected with a (AP-1)2ConA-CAT plasmid. The ability of TPP to activate AP-1 was previously unrecognized. However, deletion of the AP-1 binding sites in the iNOS promoter did not affect the reporter activity in hepatocytes stimulated with LPS, TPP, or PDBu. These results confirm the low contribution of the AP-1 sites to the activity of the iNOS promoter in macrophages stimulated with cytokines (8, 9). Current view about transcriptional activation through the AP-1 complex stresses the importance of the phosphorylation at distinct residues of c-Jun as a critical requirement for the stability and transcriptional activity of AP-1 (41, 43, 44). Therefore, since several kinases can independently regulate AP-1 activity, the mechanisms that maintain a functionally active AP-1 complex in hepatocytes stimulated with LPS, TPP, or PDBu might be different.

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Transcriptional Control of iNOS in Hepatocytes

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Abstract: In hepatocytes, the transcription factor for iNOS expression is NF-κB/Rel. The presence of activated NF-κB in the nucleus of hepatocytes is a prerequisite for iNOS expression. The role of NF-κB in the control of iNOS expression in hepatocytes is well documented. The NF-κB pathway is a complex network of signaling molecules that play a crucial role in the regulation of iNOS expression in hepatocytes.

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