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Cross-talk between cyclooxygenase and nitric oxide pathways: Prostaglandin E_2 negatively modulates induction of nitric oxide synthase by interleukin 1

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ABSTRACT **The inflammatory cytokine interleukin 1β (IL-1β) induces both cyclooxygenase (COX) and nitric oxide synthase (NOS) with increases in the release of prostaglandin (PG) and nitric oxide (NO) by mesangial cells. Recently, activation of the COX enzyme by NO has been described. However, the effects of COX products (PGs) on the NO pathway have not been fully clarified. Thus we determined the effect of COX inhibition and exogenous PGs on NO production and NOS induction in rat mesangial cells. A COX inhibitor, indomethacin, enhanced IL-1β-induced steady-state level of the inducible NOS (iNOS) mRNA and nitrite production. The effect of indomethacin was dose dependently reversed by the replacement of endogenous PGE_2 with exogenous PGE_2, which is the predominant product of the COX pathway in rat mesangial cells. In contrast to PGE_2, a stable analog of PGI_2, carba prostacyclin, enhanced IL-1β-induced iNOS mRNA levels and nitrite production. Forskolin, an activator of the adenylate cyclase, mimicked the effect of carba prostacyclin but not PGE_2. These data suggest that (*i*) endogenous PGE_2 downregulates iNOS induction, (*ii*) this inhibitory effect of PGE_2 on iNOS induction is not mediated by activation of adenylate cyclase, and (*iii*) exogenous PGI_2 stimulates COX induction possibly by activation of adenylate cyclase.**

Interleukin 1 (IL-1) and tumor necrosis factor (TNF) are cytokines that mediate a variety of processes in host defense, such as inflammation and the response to injury (1). During glomerular inflammation, cytokines from infiltrating macrophages and activated mesangial cells may act to sustain and promote glomerular damage.

IL-1 and TNF increase prostaglandin (PG) release in mesangial cells. IL-1 has been shown to induce the cyclooxygenase (COX) (2–5) and secretory phospholipase A_2 (6, 7) in mesangial cells. Two isoforms of COX are now recognized. One is constitutive (COX-1), and the other (COX-2) is inducible by IL-1β, TNF-α, and phorbol esters (8). We have recently demonstrated that IL-1β induces COX-2 but not COX-1 followed by increases in PG production in rat mesangial cells (4, 5). The released PGs, in turn, modulate mesangial cell and macrophage function. PGs regulate mesangial cell contraction and proliferation (9). PGs also regulate numerous aspects of macrophage cell function, including cytokine gene expression and differentiation (10).

IL-1 and TNF increase nitric oxide (NO) release in macrophages (11, 12) and mesangial cells (13). The free radical NO has emerged as an important signal and effector molecule in mammalian physiology (11, 12), including neurotransmission, vasodilation, and inflammation. NO is synthesized from the guanidino nitrogen of L-Arg by the catalytic reaction of NO synthase (NOS) (11, 12, 14, 15). At least two families of NOS enzymes have been identified. One is the Ca^{2+}/calmodulin-dependent constitutive form predominantly expressed in brain and vascular endothelial cells. The other is the Ca^{2+}/calmodulin-independent cytokine-inducible form (iNOS) expressed in macrophages (16–18), vascular smooth muscle cells (19, 20), and mesangial cells (13). Recently, two isoforms of constitutive NOS have been cloned from rat brain (21) and vascular endothelium (22–24). The cDNA for iNOS has also been cloned from a macrophage cell line (16–18).

Thus inflammatory cytokines including IL-1 and TNF drive both COX and NOS pathways. These pathways share a number of similarities. A variety of cells and tissues that produce PGs simultaneously release NO in response to cytokines or other activators. Both of them are paracrine modulators of the cell functions and mediate intracellular signals via cyclic nucleotides (i.e., cAMP or cGMP). NO and some PGs dilate vascular smooth muscle and inhibit platelet aggregation (11, 12). In addition, NOS and COX require heme as a cofactor (25–27) and have constitutive and cytokine-inducible forms.

Many effects of NO are mediated by cGMP. NO activates the soluble guanylate cyclase and increases a second messenger, cGMP, by binding to the heme moiety of the enzyme (11, 12). However, it is likely that guanylate cyclase is not the only molecular target of NO. NO inhibits several enzymes in the mitochondrial electron transport system and aconitase in the citric acid cycle by interacting with iron–sulfur centers of these enzymes (12). Likewise, NO has been shown to stimulate COX activity possibly via the heme component, which binds to the active site of the COX enzyme (28–31). Thus, a close interaction between NOS and COX pathways has become evident. However, the effect of PGs on the NOS pathway has not been fully explored. Thus we determined the effects of COX inhibition and PGs on iNOS induction and expression in rat mesangial cells.

MATERIALS AND METHODS

Materials. Human recombinant IL-1β (50 half-maximal units/ng) and restriction enzymes were purchased from Boehringer Mannheim. Primers and cDNA [for the polymerase chain reaction (PCR)] for mouse iNOS and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were from Clontech. N^G-Monomethyl-L-arginine (LNMMA), aminoguanidine (AG), indomethacin (Indo), PGE_2, forskolin (FSK), sulfanilamide, and naphthylethylenediamine dihydrochloride were from Sigma. A stable analogue of PGI_2, carba prostacyclin, was from Cayman Chemicals (Ann Arbor, MI).

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Abbreviations: COX, cyclooxygenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1, interleukin 1; NO, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible NOS; PG, prostaglandin; TNF, tumor necrosis factor; LNMMA, N^G-monomethyl-L-arginine; AG, amino guanidine; Indo, indomethacin; FSK, forskolin.

Cell Culture and Treatment. Primary mesangial cell cultures were prepared from male Sprague–Dawley rats as described (3, 4). Cells were grown in RPMI 1640 medium supplemented with 15% (vol/vol) heat-inactivated fetal calf serum, insulin (0.6 international unit/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), amphotericin B (250 μg/ml), and 15 mM Hepes (pH 7.4). Experiments were performed with confluent cells grown in 75-cm^2 flasks for Northern blot analysis or in 6-well plates for nitrite and PGE_2 measurements. Cells were used between passages 2 and 6. Serum was reduced to 5% on the day of experiments. Cells were stimulated with IL-1β in combination with other drugs and harvested as indicated.

Preparation of cDNA Probes by PCR. cDNA probes of iNOS and GAPDH for Northern blot analysis were prepared by the PCR. Sense and antisense primers used for PCR were as follows: murine iNOS, 5′-CCCTTCCGAAGTTTCTGGCAGC-3′ and 5′-GGCTGTCAGAGCCTCGTGGCTTTGG-3′ [corresponding to aa 982–990 and 1139–1144 (17)]; rat GAPDH, 5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′ and 5′-CATGTAGGCCATGAGGTCCACCAC-3′ [corresponding to aa 2–10 and 322–329 (32)]. The PCR products for iNOS were amplified using mouse iNOS cDNA templates. PCR products for GAPDH were amplified using reverse transcriptase–PCR from total RNA of cultured mesangial cells. PCR products of the expected size were subcloned into pCR-II plasmid vector (Invitrogen). Nucleotide sequences of subcloned cDNAs were confirmed by sequencing by the dideoxynucleotide chain-termination method. Inserts were excised by *Eco*RI digest and used for Northern blot analysis.

Northern Blot Analysis. Northern blot analysis was performed as described (4). Confluent cells grown in 75-cm^2 flasks were washed twice with phosphate-buffered saline (PBS). Cells were incubated in RPMI 1640 medium containing 5% fetal calf serum with IL-1β and/or pharmacological agents and harvested as indicated. Total RNA was isolated by using the acid guanidium thiocyanate/phenol/chloroform method (RNA-STAT 60, Tel-Test "B," Friendswood, TX) from confluent mesangial cells in 75-cm^2 flask. Total RNA (20 μg) was fractionated by 1% agarose/formaldehyde gel electrophoresis, transferred onto nylon membranes (GeneScreen, DuPont) and immobilized with UV-cross-linking. cDNAs for iNOS and GAPDH (as an internal standard) were radiolabeled with [^{32}P]dCTP by the random-primed labeling method. The membrane was hybridized with radiolabeled cDNA probes at 42°C for 18–24 hr. The membrane was washed for two 5-min periods at room temperature in 2× SSC and for two 30-min periods at 60°C in 2× SSC/0.1% SDS and autoradiographed with an intensifying screen at −80°C overnight.

Measurement of Nitrite and PGE_2. Confluent mesangial cells in 6-well plates were washed twice with PBS and incubated in RPMI 1640 medium containing 5% fetal calf serum with IL-1β and/or pharmacological agents as indicated. The conditioned incubation medium was collected and the nitrite content in the supernatant was measured by the addition of Griess reagent [1% sulfanilamide/0.1% naphthylethylenediamine dihydrochloride in 2% (vol/vol) phosphoric acid] (33). The A_{550} was measured and the amount of nitrite was obtained by extrapolation from a standard curve using sodium nitrite as a standard. PGE_2 in the medium was determined by stable-isotope gas chromatography/mass spectrometry as described (2, 4). Nitrite and PGE_2 production were corrected for protein as determined by the method of Bradford (34).

Statistical Analysis. Data were expressed as the mean ± SEM. Statistical analysis was performed by using a paired or unpaired Student's *t* test. A difference with a *P* value of <0.05 was considered statistically significant.

RESULTS

Effect of Actinomycin D and Cycloheximide on Nitrite Production. The stable metabolite of NO, nitrite, in the medium was measured by the Griess reaction. IL-1β increased nitrite production in a dose-dependent manner and reached maximum at 250 units/ml (data not shown). A submaximal concentration of IL-1 (50 units/ml) was used for experiments, unless otherwise stated. To determine whether *de novo* protein synthesis is required for IL-1β-induced NO production, the effects of actinomycin D and cycloheximide were studied. Both actinomycin D (5 μg/ml) and cycloheximide (10 μg/ml) abolished IL-1β-induced nitrite production (data not shown), indicating *de novo* synthesis of protein(s) is required for IL-1β-induced nitrite production.

Effect of NOS Inhibitors on Nitrite and PGE_2 Production. To determine the effect of endogenous NO on COX pathway, the effects of NOS inhibitors on nitrite and PGE_2 production were determined. As shown in Fig. 1, LNMMA (1 mM) significantly decreased both nitrite and PGE_2 production induced by IL-1β. A 10-fold excess of L-Arg (10 mM) reversed the inhibition by LNMMA. AG (1 mM), a selective inhibitor of iNOS (35), completely abolished the IL-1β-induced nitrite production and resulted in a decrease in PGE_2 production. These data suggested that IL-1β induced the iNOS enzyme and confirmed that endogenous NO produced by iNOS enhances COX activity. These observations are unlikely to be a result of endotoxin contamination, since the added agents by themselves had no effects on nitrite production or iNOS expression (see Fig. 4).

Effect of Indo and PGE_2 on Nitrite Production. To determine the effect of endogenous PGs on the NO pathway, the effect of a nonselective COX inhibitor, Indo, on nitrite production was examined. Indo (10 μM) significantly enhanced the effect of IL-1β on nitrite production (Fig. 2) but inhibited IL-1β-induced PGE_2 release by <99% (375.3 ± 14.9 to 1.1 ± 0.2 ng; $P < 0.001$). As shown in Fig. 2 *Inset*, the replacement of endogenous PGE_2 by exogenous PGE_2, at concentrations similar to IL-1β-induced endogenous PGE_2 (10–1000 ng/ml), reversed the effect of Indo in a dose-dependent manner with an IC_{50} of 5×10^{-8} M. In the absence of Indo, exogenous PGE_2 did not affect nitrite production by IL-1β (Fig. 2). Exogenous PGE_2 or Indo alone did not affect basal nitrite production. These data suggest that endogenous PGE_2 negatively modulates the NO pathway.

Effect of FSK and PGI_2 on Nitrite Production. To determine whether the inhibitory effect of endogenous PGE_2 on IL-1β-

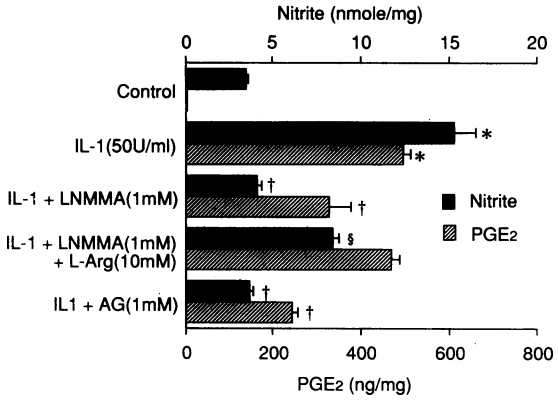

FIG. 1. Effect of LNMMA and AG on IL-1β-induced nitrite and PGE_2 production. Mesangial cells were incubated in RPMI 1640 medium containing 5% fetal calf serum with IL-1β [IL-1 (50 U/ml); U, unit(s)], LNMMA (1 mM), and AG (1 mM) for 24 hr as indicated. Nitrite in the culture medium was determined by the Griess reaction. Results are the mean ± SEM ($n = 3$). *, $P < 0.001$ vs. control; †, $P < 0.05$ vs. IL-1β alone; §, $P < 0.05$ vs. IL-1β/LNMMA.

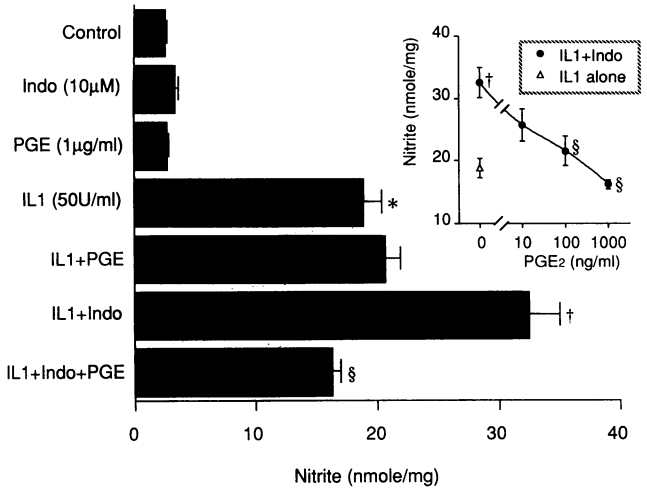

FIG. 2. Effect of Indo and exogenous PGE_2 on IL-1β-induced nitrite production. (*Inset*) Exogenous PGE_2 reverses the stimulatory effect of Indo on IL-1β-induced nitrite production in a dose-dependent manner. Mesangial cells were incubated in RPMI 1640 medium containing 5% fetal calf serum with IL-1β [IL-1 (50 U/ml); U, unit(s)], Indo (10 μM), and PGE_2 (1 μg/ml) for 24 hr as indicated. Results are the mean ± SEM (n = 9 to 12). *, $P < 0.001$ vs. control; †, $P < 0.001$ vs. IL-1β alone; §, $P < 0.01$ vs. IL-1β/Indo (without PGE_2).

induced nitrite production is mediated by activation of the adenylate cyclase, the effect of an adenylate cyclase activator, FSK, on IL-1β-induced nitrite production was tested. As shown in Fig. 3*A*, FSK (10 μM) increased IL-1β-induced nitrite production. The stimulatory effects of FSK and Indo on IL-1β-induced nitrite production were additive. Thus, FSK did not mimic the effect of PGE_2. Carba prostacyclin (1 μg/ml), a stable analogue of PGI_2, increased IL-1β-induced nitrite production (Fig. 3*B*). Neither carba prostacyclin nor FSK by themselves affected basal nitrite production.

Regulation of iNOS mRNA by Indo and PGs. To determine whether the alterations in NO production by PGs occur because of changes in iNOS mRNA levels, the steady-state level of iNOS mRNA was determined by Northern blot analysis. The peak steady-state level of iNOS mRNA (≈4.0

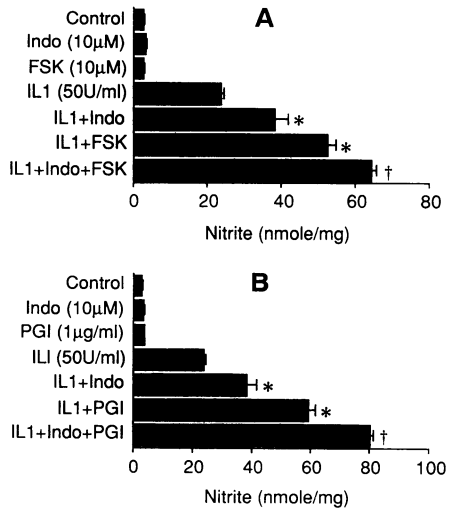

FIG. 3. Effect of FSK (*A*) and a PGI_2 analogue, carba prostacyclin (*B*), on IL-1β-induced nitrite production. Mesangial cells were incubated in RPMI 1640 medium containing 5% fetal calf serum with IL-1β [IL-1 (50 U/ml); U, unit(s)], FSK (10 μM), carba prostacyclin (PGI; 1 μg/ml), and Indo (10 μM) as indicated. Results are the mean ± SEM (n = 3 to 6). *, $P < 0.001$ vs. IL-1β alone; †, $P < 0.01$ vs. IL-1β/FSK or carba prostacyclin.

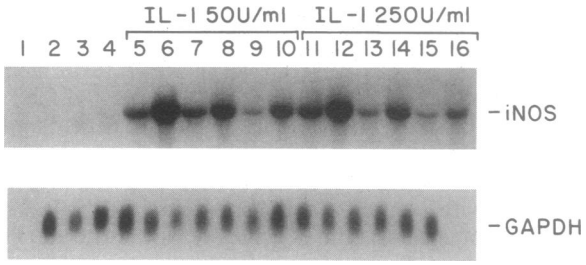

FIG. 4. Effect of Indo, PGE_2, and FSK on IL-1β-induced iNOS mRNA expression. Mesangial cells were incubated in RPMI 1640 medium containing 5% fetal calf serum with IL-1β [IL-1 at 50 or 250 units (U)/ml], (10 μM), PGE_2 (1 μg/ml), and FSK (10 μM) for 12 hr as indicated below. Total RNA was isolated and analyzed on Northern blots with ^{32}P-labeled iNOS and GAPDH cDNA probes. Lanes: 1, control; 2, Indo; 3, PGE_2; 4, FSK; 5, IL-1 (50 units/ml); 6, IL-1 (50 units/ml)/Indo; 7, IL-1 (50 units/ml)/Indo/PGE_2; 8, IL-1 (50 units/ml)/Indo/FSK; 9, IL-1 (50 units/ml)/PGE_2; 10, IL-1 (50 units/ml)/FSK; 11, IL-1 (250 units/ml); 12, IL-1 (250 units/ml)/Indo; 13, IL-1 (250 units/ml)/Indo/PGE_2; 14, IL-1 (250 units/ml)/Indo/FSK; 15, IL-1 (250 units/ml)/PGE_2; 16, IL-1 (250 units/ml)/FSK.

kb) occurred 12 hr after IL-1β stimulation (data not shown). Therefore, the effect of Indo and PGs on IL-1β-induced steady-state level of iNOS mRNA was determined at 12 hr. As with nitrite production, Indo (10 μM; Fig. 4, lanes 6 and 12) enhanced IL-1β-induced (Fig. 4, lanes 5 and 11) steady-state level of iNOS mRNA. This stimulatory effect of Indo was reversed by addition of exogenous PGE_2 (1 μg/ml; Fig. 4, lanes 7 and 13). FSK (10 μM) enhanced IL-1β-induced iNOS mRNA expression (Fig. 4, lanes 10 and 16). Carba prostacyclin (1 μg/ml) also enhanced IL-1β-induced iNOS mRNA expression (data not shown). Carba prostacyclin or FSK partially attenuated the effect of Indo on IL-1β-induced iNOS mRNA level rather than enhanced it, whereas carba prostacyclin and FSK additively increased nitrite production with Indo. Individually Indo, PGE_2, and FSK had no effect on iNOS mRNA levels in the absence of IL-1β.

DISCUSSION

Mesangial cells serve multiple functions within the glomerulus, including the regulation of glomerular filtration, elaboration of extracellular matrix, and phagocytosis of immune complexes. Coincubation experiments have shown that NO release from glomerular endothelial cells increases cGMP in mesangial cells and inhibits angiotensin II-induced mesangial cell contraction (36, 37). Thus NO synthesized by endothelial constitutive NOS might be an important signaling molecule in cross-communication between glomerular cells and thus regulate normal glomerular function. While mesangial cells may be a target for NO, mesangial cells themselves have been shown to express iNOS in response to inflammatory cytokines such as IL-1β and TNF-α (13). These cytokines have also been shown to induce COX-2 in mesangial cells (3–5). Recent published experiments have demonstrated closer interactions between these two signaling pathways. NO can activate COX in macrophage cell lines, rat islet cells of pancreas, rat uteri, and experimental hydronephrotic kidneys (28–31). In agreement with these studies, the present study showed that NOS inhibition by LNMMA or AG decreased IL-1β-induced PGE_2 release, indicating endogenous NO enhances COX activity in rat mesangial cells.

Since mesangial cells require serum for full COX-2 induction by IL-1β (2), the serum requirement for iNOS induction was studied. In the presence of 15% serum, IL-1β-induced iNOS mRNA expression was weak and the peak occurred 12 hr after IL-1β stimulation. Surprisingly, in mesangial cells

starved 24 hr for serum, IL-1β strongly induced iNOS mRNA expression after 6 hr, while PGE_2 production was blunted under these conditions (unpublished observation). In the current experiments, we evaluated the effects of PGE_2 on iNOS induction in cells cultured with serum. The present study has demonstrated that endogenous PGE_2 negatively modulates IL-1β-induced NO release and steady-state iNOS mRNA levels in rat mesangial cells. COX inhibition by Indo decreased PGE_2 production and resulted in enhancement of IL-1β-induced steady-state iNOS mRNA levels and NO production. Since PGE_2 is known to be the predominant product of isolated rat glomeruli and cultured rat mesangial cells (9), the replacement of endogenous PGE_2 by exogenous PGE_2 was examined in Indo-treated cells. Exogenous PGE_2 reversed the stimulatory effect of Indo on IL-1β-induced iNOS mRNA level and NO production. Thus Indo reversed the inhibitory effect of endogenous PGE_2 on iNOS expression and NO release.

PGE_2 is known to function through a variety of second messenger pathways. The PGE receptor is pharmacologically subdivided into three subtypes: EP_1, EP_2, and EP_3 (38). These subtypes are believed to utilize different signal transduction mechanism. EP_1, EP_2, and EP_3 receptors are presumably coupled to activation of the Ca^{2+}/inositol phospholipid pathway, stimulation of adenylate cyclase, and inhibition of adenylate cyclase, respectively. In the present study, FSK, a stimulator of adenylate cyclase, did not mimic the effect of PGE_2. FSK synergistically increased IL-1β-induced NO production and iNOS mRNA level; however, endogenous PGE_2 inhibited iNOS induction. Thus, it is likely that the regulatory mechanism by which PGE_2 downregulates IL-1β-induced iNOS induction is distinct from the EP_2-mediated signaling pathway. In a recent study using *in situ* hybridization, Sugimoto *et al.* (39) showed that the glomerulus is dominated by EP_2 receptors. However, the study in cultured rat mesangial cells by Mene *et al.* (40) showed that PGE_2, $PGF_{2\alpha}$, and the thromboxane A_2/endoperoxide analog U-46619 increased intracellular free Ca^{2+} concentration and inositol phosphates, suggesting the existence of EP_1 receptors in mesangial cells. Recent studies in vascular smooth muscle cells have demonstrated that angiotensin II or protein kinase C activation downregulates iNOS induction by IL-1β (20). Thus, it is possible that activation of Ca^{2+}/inositol phospholipid pathways via EP_1 receptor accounts for inhibition of iNOS induction by PGE_2. Kreisberg *et al.* (41) showed that PGE_2 attenuated catecholamine-induced elevations in intracellular cAMP in cultured rat mesangial cells, suggesting the existence of EP_3 receptors in mesangial cells. Namba *et al.* (42) have described alternatively spliced EP_3 isoforms that differ only in the C-terminal sequence, couple to different G proteins, and activate different second messengers. This molecular diversity in PGE receptors and changes in the expression of these receptors might play a pivotal role in signal transduction by PGE_2. Thus, further studies will be required to clarify the regulatory mechanism of iNOS induction by PGE_2. Regardless of the precise mechanisms, this report demonstrates that endogenous PGE_2 negatively modulates cytokine-stimulated iNOS induction.

In contrast to PGE_2, a stable analogue of PGI_2, carba prostacyclin, increased IL-1β-induced NO production and the iNOS mRNA level. This stimulatory effect on iNOS induction was similar to the effect of FSK. Thus, the effect of PGI_2 is likely to be mediated by activation of the adenylate cyclase. These results are consistent with the observations that cAMP elevating agents synergistically enhance the cytokine-induced iNOS induction in vascular smooth muscle cells and mesangial cells (7, 19). Although the effects of Indo and FSK or PGI_2 on IL-1β-induced NO production were additive, FSK and PGI_2 did not enhance the effect of Indo on the steady-state levels of iNOS mRNA. Thus, the regulation of mRNA levels and posttranslational control might be involved in the regulation of cAMP-mediated iNOS activation. The brain type of constitutive NOS has been demonstrated to be phosphorylated by cAMP-dependent protein kinase, protein kinase C, or Ca^{2+}/calmodulin protein kinase (43). Thus phosphorylation of iNOS by cAMP-dependent protein kinase might be involved in this regulation, although, to our knowledge, there is no report for phosphorylation of iNOS by protein kinases. Alternatively, the availability of cofactors for NOS (tetrahydrobiopterin, heme, flavin mononucleotide, and flavine adenine dinucleotide) (14, 15) might explain the discrepancy between NO production and iNOS mRNA level.

The data presented here do not address the molecular mechanism by which PGE_2 and/or PGI_2 modulate iNOS induction. Changes in transcription or iNOS mRNA stability may account for the changes in steady-state level of iNOS mRNA. iNOS mRNA has the "AUUUA" motif in its 3′ untranslated region, which is considered a mRNA instability determinant. We have shown (5) that IL-1β stabilizes the COX-2 mRNA by phosphorylation of cytosolic factors that bind to the AUUUA-rich 3′ untranslated region in rat mesangial cells (5). Similarly PGE_2 and/or PGI_2 might interact with some factors that modulate iNOS mRNA stability.

Fig. 5 presents our model for interactions between COX and NOS systems in rat mesangial cells. IL-1β induces both iNOS and COX-2 mRNA by transcriptional activation and/or stabilizing mRNA. These mRNAs are translated into proteins. The iNOS enzyme produces NO and NO activates COX enzymes. The predominant product of the COX pathway PGE_2 downregulates iNOS mRNA expression and decreases NO production. This interaction between PGE_2 and iNOS expression requires the appropriate PGE_2 receptor isotype coupled to the appropriate signal transduction mechanisms. Because of these requirements, this phenomenon may not be applicable to all cell systems.

Both NO and PGs have been implicated in the regulation of normal glomerular function and in the mediation of a

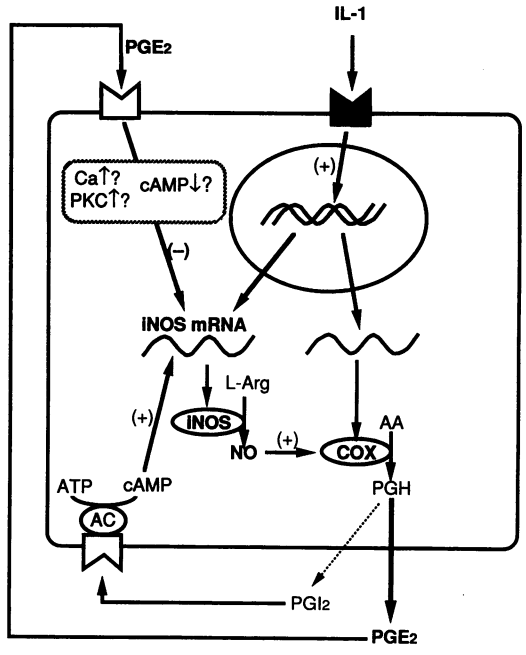

FIG. 5. Model for the interaction between COX and NOS pathways in rat mesangial cells. IL-1 induces both iNOS and COX-2 mRNA expression. NO produced by iNOS enzyme activates COX enzyme. Activated COX enzyme produces a large amount of PGE_2 and a smaller amount of PGI_2. The predominant COX product PGE_2 downregulates iNOS mRNA induction. AC, adenylate cyclase; PKC, protein kinase C; AA, arachidonic acid.

number of disorders, including glomerular injury, acute and chronic renal failure, diabetic nephropathy, and essential hypertension (9, 11, 12, 44). Since NO and some PGs have antiproliferative effects, they might be cell-protective in part. However, the excessive production of these autacoids might lead to cellular dysfunction and tissue injury.

In conclusion, NOS and COX pathways interact with each other and the cross-talk between these two pathways might be important in fine tuning glomerular function and the regulation of the inflammatory process.

1. Le, J. & Vilcek, J. (1987) *Lab. Invest.* **56,** 234–248.
2. Coyne, D. W. & Morrison, A. R. (1990) *Biochem. Biophys. Res. Commun.* **173,** 728–724.
3. Coyne, D. W., Nickols, M., Bertrand, W. & Morrison, A. R. (1992) *Am. J. Physiol.* **263,** F97–F102.
4. Rzymkiewicz, D., Leingang, K., Baird, N. & Morrison, A. R. (1994) *Am. J. Physiol.* **266,** F39–F45.
5. Srivastava, S. K., Tetsuka, T., Daphna-Iken, D. & Morrison, A. R. (1994) *Am. J. Physiol.* **267,** F504–F508.
6. Nakazato, Y., Simonson, M. S., Herman, W. H., Konieczkowski, M. & Sedor, J. R. (1991) *J. Biol. Chem.* **266,** 14119–14127.
7. Schalwijk, C., Pfeilschifter, J., Märki, F. & Van den Bosch, H. (1992) *J. Biol. Chem.* **267,** 8846–8851.
8. Kujubu, D. A., Fletcher, B. S., Varnum, B. C., Lim, R. W. & Herschman, H. R. (1991) *J. Biol. Chem.* **266,** 12866–12872.
9. Badr, K. F. & Jacobson, H. R. (1991) in *The Kidney*, eds. Brenner, B. M. & Rector, F. C. (Saunders, Philadelphia), pp. 584–619.
10. Kunkel, S. & Chensue, S. (1985) *Biochem. Biophys. Res. Commun.* **128,** 892–897.
11. Moncada, S., Palmer, R. & Higgs, E. (1991) *Pharmacol. Rev.* **43,** 109–142.
12. Nathan, C. (1992) *FASEB J.* **6,** 3051–3064.
13. Pfeilschifter, J., Rob, P., Mülsch, A., Fandrey, J., Vosbeck, K. & Busse, R. (1992) *Eur. J. Biochem.* **203,** 251–255.
14. Marletta, M. A. (1993) *J. Biol. Chem.* **268,** 12231–12234.
15. Knowles, R. G. & Moncada, S. (1994) *Biochem. J.* **298,** 249–258.
16. Lowenstein, C. J., Glatt, C. S., Bredt, D. S. & Snyder, S. H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6711–6715.
17. Lyons, C. R., Orloff, G. J. & Cunningham, J. M. (1992) *J. Biol. Chem.* **267,** 6370–6374.
18. Xie, Q. W., Cho, H. J., Calaycay, J., Mumford, R. A., Swiderek, K. M., Lee, T. D., Ding, A., Troso, T. & Nathan, C. (1992) *Science* **256,** 225–228.
19. Koide, M., Kawahara, Y., Nakayama, I., Tsuda, T. & Yokoyama, M. (1993) *J. Biol. Chem.* **268,** 24959–24966.
20. Nakayama, I., Kawahara, Y., Tsuda, T., Okuda, M. & Yokoyama, M. (1994) *J. Biol. Chem.* **269,** 11628–11633.
21. Bredt, D. S., Hwang, P. M., Glatt, C. E., Lowenstein, C., Reed, R. R. & Snyder, S. H. (1991) *Nature (London)* **351,** 714–718.
22. Janssens, S. P., Shimouchi, A., Quertermous, T., Bloch, D. B. & Bloch, K. D. (1992) *J. Biol. Chem.* **267,** 14519–14522.
23. Lamas, S., Marsden, P. A., Li, G. K., Tempst, P. & Michel, T. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6348–6352.
24. Sessa, W. C., Harrison, J. K., Barber, C. M., Zeng, D., Durieux, M. E., D'Angelo, D. D., Lynch, K. R. & Peach, M. J. (1992) *J. Biol. Chem.* **267,** 15274–15276.
25. DeWitt, D. L., el-Harith, E. A., Kraemer, S. A., Andrews, M. J., Yao, E. F., Armstrong, R. L. & Smith, W. L. (1990) *J. Biol. Chem.* **265,** 5192–5198.
26. Shimokawa, T. & Smith, W. L. (1991) *J. Biol. Chem.* **266,** 6168–6173.
27. McMillan, K., Bredt, D. S., Hirsch, D. J., Snyder, S. H., Clark, J. E. & Masters, B. S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11141–11145.
28. Salvemini, D., Misko, T. P., Masferrer, J. L., Seibert, K., Curie, M. G. & Needleman, P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7240–7244.
29. Corbett, J. A., Kwon, G., Turk, J. & McDaniel, M. L. (1993) *Biochemistry* **32,** 13767–13770.
30. Franchi, A. M., Chaud, M., Rettori, V., Suburo, A., McCann, S. M. & Gimeno, M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 539–543.
31. Salvemini, D., Seibert, K., Masferrer, J. L., Misko, T. P., Currie, M. G. & Needleman, P. (1994) *J. Clin. Invest.* **93,** 1940–1947.
32. Fort, P., Piechaczyk, M., El Sabrouty, S., Dani, C., Jeanteur, P. & Blanchard, J. M. (1985) *Nucleic Acids Res.* **13,** 1431–1442.
33. Misko, T. P., Schilling, R. J., Salvemini, D., Moore, W. M. & Currie, M. G. (1993) *Anal. Biochem.* **214,** 11–16.
34. Bradford, M. (1976) *Anal. Biochem.* **72,** 246–254.
35. Misko, T. P., Moore, W. M., Kasten, T. P., Nickols, G. A., Corbett, J. A., Tilton, R. G., McDaniel, M. L., Williamson, J. R. & Currie, M. G. (1993) *Eur. J. Pharmacol.* **233,** 119–125.
36. Marsden, P. A., Brock, T. A. & Ballermann, B. J. (1990) *Am. J. Physiol.* **258,** F1295–F1303.
37. Shultz, P. J., Schorer, A. E. & Raij, L. (1990) *Am. J. Physiol.* **258,** F162–F167.
38. Coleman, R. A., Kennedy, I., Humphrey, P. P. A., Bunce, K. & Lumley, P. (1990) in *Comprehensive Medicinal Chemistry*, eds. Hansch, C., Sammes, P. G., Taylor, J. B. & Emmet, J. C. (Pergamon, Oxford), Vol. 3, pp. 643–714.
39. Sugimoto, Y., Namba, T., Shigemoto, R., Negishi, M., Ichikawa, A. & Narumiya, S. (1994) *Am. J. Physiol.* **266,** F823–F828.
40. Mene, P., Dubyak, G. R., Scarpa, A. & Dunn, M. J. (1987) *Biochem. Biophys. Res. Commun.* **142,** 579–586.
41. Kreisberg, J. I., Venkatachalam, M. A. & Patel, P. (1984) *Kidney Int.* **25,** 874–879.
42. Namba, T., Sugimoto, Y., Negishi, M., Irie, A., Ushikubi, F., Kakizuka, A., Ito, S., Ichikawa, A. & Narumiya, S. (1993) *Nature (London)* **365,** 166–170.
43. Bredt, D. S., Ferris, C. D. & Snyder, S. H. (1992) *J. Biol. Chem.* **267,** 10976–10981.
44. Pfeilschifter, J., Kuntz, D. & Mühl, H. (1993) *Nephron* **64,** 518–525.