Expression profile of a human inducible nitric oxide synthase promoter reporter in transgenic mice during endotoxemia

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Expression profile of a human inducible nitric oxide synthase promoter reporter in transgenic mice during endotoxemia. Am J Physiol Renal Physiol 288: F214–F220, 2005. First published October 26, 2004; doi:10.1152/ajprenal.00258.2004.—Inducible nitric oxide synthase (iNOS) is involved in many physiological and pathophysiological processes, including septic shock and acute kidney failure. Little is known about transcriptional regulation of the human iNOS gene in vivo under basal conditions or in sepsis. Accordingly, we developed transgenic mice carrying an insertional human iNOS promoter-reporter gene construct. In these mice, the proximal 8.3 kb of the human iNOS 5′-flanking region controls expression of the reporter gene of enhanced green fluorescent protein (EGFP). Patterns of human iNOS promoter/EGFP transgene expression in tissues were examined by fluorescence microscopy and immunoblotting. Endogenous murine iNOS was basally undetectable in kidney, intestine, spleen, heart, lung, liver, stomach, or brain. In contrast, EGFP from the transgene was basally expressed in kidney, brain, and spleen, but not the other tissues of the transgenic mice. Bacterial lipopolysaccharide induced endogenous iNOS expression in kidney, intestine, spleen, lung, liver, stomach, and heart, but not brain. In contrast, human iNOS promoter/EGFP transgene expression was induced above basal levels only in intestine, spleen, brain, stomach, and lung. Within kidney, human iNOS promoter/EGFP fluorescence was detected most prominently in proximal tubules of the outer cortex and collecting ducts and colocalized with endogenous mouse iNOS. Within the collecting duct, both endogenous iNOS and the human iNOS promoter/EGFP transgene were expressed in cells lacking aquaporin-2 immunoreactivity, consistent with expression in intercalated cells. Although it remains possible that essential regulatory elements reside in remote locations of the gene, our data concerning this 8.3-kb region provide the first in vivo evidence suggesting differential transcriptional control of the human iNOS gene in these organs and marked differences in transcriptional regulatory regions between the murine and human genes.

Nitric oxide (NO) is a short-lived biological mediator that plays an important role in many physiological and pathophysiological conditions, including neurotransmission, vasodilation, inflammation, autoimmunity, and acute kidney failure (20). NO is produced by one of three NO synthases (NOS): neuronal NOS, endothelial NOS, and inducible NOS (iNOS). iNOS-mediated NO release plays a key role in the pathogenesis of septic shock. Administration of iNOS inhibitors to mice reduces LPS-induced shock and NO synthesis in vivo (18), and iNOS knockout mice similarly have improved outcomes after LPS administration compared with wild-type mice (7, 23). In vivo and in vitro investigations have demonstrated that inhibition of iNOS expression or activity, or scavenging of iNOS-generated NO can mitigate or prevent NO-mediated injury to multiple organs, including the kidney, during endotoxemia (6).

The human iNOS (hiNOS) gene on chromosome 17 spans 37 kb and contains 26 exons, with translation initiation encoded by exon 2 (5). hiNOS is tightly regulated at the level of transcription, and the mechanisms of iNOS induction and suppression are cell, species, and stimulus specific (9, 12). For example, the hiNOS promoter differentially responds to mixtures of the same two cytokines in different cell lines (8). Moreover, depending on the cell type studied, different regions of the 5′-flanking sequence mediate cytokine induction of the hiNOS gene (8, 22, 30). All of these findings suggest that cell- and stimulus-specific control of the hiNOS promoter may be important in determining its functional roles in different tissues. Little is known, however, about the mechanisms underlying this specificity or the signaling pathways that converge on iNOS gene activation.

The bulk of analysis of iNOS gene expression has focused on the murine gene in transient transfection-reporter gene assays. In rodent cells, single cytokines (IL-1β, IFN-γ, or TNF-α) are sufficient to induce iNOS gene transcription. However, in most human cells, combinations of cytokines are required to induce maximal iNOS expression. We have demonstrated key roles of the transcription factors CCAAT/enhancer binding protein (15, 16), upstream stimulatory factor-1 and -2 (17), nuclear factor-κB (NF-κB) (35, 36), and STAT3 (35, 37), and for direct interactions between NF-κB and STAT3 and histone deacetylases (35–37) in controlling cytokine-induced murine iNOS transcription in murine mesangial cells. However, there is much less known about the cis-elements and trans-acting factors responsible for hiNOS transcription, and only limited analysis of hiNOS transcriptional activation in the context of native chromatin has been performed. From the published data, the regulation of the hiNOS gene regulation appears to differ in some aspects from its rodent counterparts, and the specific enhancers appear to be cell specific. A restricted analysis of the 5′-flanking sequence of the hiNOS gene done by transient transfection of promoter-reporter gene constructs in cell lines from liver (10, 14, 31), lung (24), and colonic epithelium (22, 25) has shown that, compared with the murine iNOS promoter, a much larger
Expression of human iNOS Promoter-Reporter Transgene in Mice

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region of the 5′-flanking sequence of hiNOS is required for maximal cytokine induction, and the presence and position of key control elements are different. Moreover, the published data suggest important tissue-specific differences in hiNOS transcriptional regulation and highlight the gaps in our understanding of its control.

Given that iNOS plays a role in many disease states and that the regulation of iNOS is primarily through induction of gene expression, one approach to study the role of iNOS in disease development and progression in animal models is to use a reporter system to report on the transcriptional activity of the iNOS gene. Such a reporter system would be useful for better understanding the role of iNOS in disease and also for understanding the response of iNOS to therapy. Recently, Zhang et al. (38) developed such a model by generating transgenic mice harboring a transgene containing a 1.24 kb of the murine iNOS promoter fused to the luciferase gene transgene. In the present study, we describe a transgenic mouse model in which an extended stretch of the hiNOS 5′-flanking region drives expression of enhanced green fluorescent protein (EGFP) to monitor induction of the hiNOS gene in vivo, and we tested its utility in monitoring the transcriptional response of the hiNOS gene to acute endotoxemia.

Materials and Methods

Generation of transgenic mice. A 8.3-kb fragment of the hiNOS promoter (Gene Bank no. AF017634) containing nucleotides −8,296 to +86 was obtained by PCR using human genomic DNA (Clontech, Palo Alto, CA) as template. This fragment was inserted into the BgII and SalI sites of the EGFP-reporter plasmid pEGFP-1 (Clontech) upstream of the EGFP open-reading frame to obtain plasmid construct −8,296/+86 hiNOS/EGFP (Fig. 1). In this construct, the reporter gene EGFP is under the transcriptional control of the hiNOS 5′-flanking region. The proper orientation and sequence authenticity of the construct were confirmed by restriction enzyme digestion mapping and DNA sequence analysis. The −8,296/+86 hiNOS/EGFP plasmid was then digested with BglII and SalI to liberate the −8,296/+86 hiNOS/EGFP sequence from vector sequences, and the −8,296/+86 hiNOS/EGFP DNA was gel purified with the GENE CLEAN Turbo kit (BIOgene, Carlsbad, CA) for generation of transgenic mice. Microinjections were performed in the Transgenic Facility at the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases at The University of Texas Health Science Center at Houston using standard techniques. Briefly, purified DNA (1–2 ng/μl) was microinjected into the male pronucleus of fertilized one-cell embryos from C57BL/6N/Hsd mice. Embryos surviving micoinjection were reimplanted into the oviducts of pseudopregnant Institute of Cancer Research female mice on the same day. Both the donor and recipient mice were obtained from Harlan, Houston, TX.

All animal used in this study were bred, housed, and monitored in accordance with the standards set by the Animal Care and Use Committee at The University of Texas Health Science Center at Houston.

Southern blotting and PCR to screen transgenic mice. Transgenic mice were identified by Southern blotting, using genomic DNA recovered from tail biopsies. Genomic DNA was isolated from the lysate of the tail using the DNeasy Tissue Kit (Qiagen, Valencia, CA). Ten micrograms of the genomic DNA were digested with BamHI overnight and electrophoresed through a 0.8% agarose gel, transferred to Hybond-XL nylon membranes (Amersham, Piscataway, NJ), and ultraviolet cross-linked. The DNA blots were hybridized with [32P]dCTP-labeled 466 bp Stul/SacI fragment and a 309 bp Stul/StuI fragment from −8,296/+86 hiNOS/EGFP to detect the hiNOS 5′-flanking region. The intensity of these two bands from the same mouse on the same blot was determined using the KODAK Image Station 1000. The significant differences in DNA between the endogenous mouse iNOS promoter and the hiNOS promoter transgene precluded the design of a common probe to recognize both endogenous and transgene sequences to estimate copy number of the transgene.

PCR was used for identification of transgenic mice to detect the EGFP region as further confirmation of the integration of the transgene, using the primer set (forward) 5′-GTGAGCAAGGCGAGGAGCTTG-3′ and (reverse) 5′-GCTTCTACTCTGACCTGTC-3′ to amplify nucleotides +4 to +723 of EGFP. The genomic template DNA was used in the range of 200–300 ng per reaction. The PCR conditions consisted of a 3-min incubation at 95°C followed by 35 cycles of 45 s at 95°C, 45 s at 55°C, 2 min at 72°C, and a final elongation step for 20 min at 72°C. The PCR product was detected on a 0.8% agarose gel as a 720-bp fragment. Founder mice carrying the −8,296/+86 hiNOS/EGFP transgene were bred with wild-type C57BL/6 mice to obtain hemizygous F1 progeny. F1 offspring were used for subsequent histological and immunoblot analyses.

Model of acute endotoxemia. To induce a systemic inflammatory response, mice were injected intraperitoneally with Escherichia coli (strain O111:B4, Sigma) LPS (10 mg/kg) dissolved in 0.9% NaCl. Control animals were injected with a similar volume of 0.9% NaCl without LPS. After an ~16-h exposure to LPS or vehicle, groups of mice were anesthetized for harvesting tissue specimens for further analysis.

Cell culture and transient transfection. Mouse mesangial cells (ATCC CRL-1927) were maintained in Ham’s F12 plus Dulbecco’s modified Eagle’s medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5% FBS. Mesangial cells were seeded in 24-well plates and grown to 90–95% confluency. IFN-γ (100 U/ml), mouse recombinant IFN-γ (10 ng/ml; R & D Systems, Minneapolis, MN), LPS (1 μg/ml), mouse recombinant IFN-γ (100 U/ml; BioSource, Camarillo, CA), or LPS + IFN-γ as indicated in the text and figure legends. Twenty-four hours later, cell lysates were prepared for further analysis by immunoblotting of EGFP expression.

Preparation of cytosol and nuclear extracts and immunoblot analysis. Samples of lung, stomach, small intestine, kidney, brain, heart, liver, spleen, and skeletal muscle from the transgenic mice and their nontransgenic littermates were homogenized in cold PBS and then extracted with the nuclear/cytosol fractionation kit (BioVision, Mountain View, CA) according to the manufacturer’s protocol. Twenty-micromg samples of cytosolic extracts were resolved by 4–15% SDS-PAGE, and the proteins were electrophoretically transferred to

Fig. 1. Transgene construct. Schematic representation of the DNA construct used to generate the −8,296/+86 human inducible nitric oxide synthase (hiNOS) promoter-enhanced green fluorescent protein (EGFP) mouse transgenic lines. The 5′ untranslated region (UTR) contains 86 bp from hiNOS exon 1. Restriction endonuclease sites and hiNOS promoter-specific probes 1 and 2 used for Southern blot analyses are shown (not drawn to scale). Positions of forward (F1) and reverse (R1) PCR primers to amplify full-length EGFP are also indicated.

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polyvinylidene difluoride membranes (Hybond enhanced chemiluminescence, Amersham). The blots were probed overnight at 4°C with an anti-iNOS antibody (Upstate Biotechnologies, Charlottesville, VA) that recognizes the endogenous murine iNOS protein, BD living Colors AV Monoclonal Antibody JL-8 (Clontech), which detects EGFP, or anti-α-tubulin, as a control for sample integrity, loading, and blotting. The antigen-antibody complexes were detected using the enhanced chemiluminescence method.

**Fluorescence and immunofluorescence microscopy.** Transgene-positive mice and negative littersmates were anesthetized with ethyl ether and perfused via the heart with 4% paraformaldehyde in PBS. Lung, stomach, intestine, kidney, brain, heart, liver, spleen, and skeletal muscle tissues were isolated and immersed overnight in the same fixative at 4°C. The tissues were immersed in 20% sucrose in PBS at 4°C and then frozen in liquid nitrogen. Frozen 4-μm sections were cut with a cryostat. Separate tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. The sections of the same tissue from a transgene-positive mouse or a negative littersmate were placed onto the same slides. Frozen and paraffin 4-μm sections were cut with a cryostat and frozen at −20°C until analyzed.

Formalin-fixed, paraffin wax-embedded sections were processed for immunofluorescence using the microwave antigen retrieval method. The slides were washed in 0.3% Tween-PBS for 5 min. Slides were blocked with 1% normal sheep serum in ×1 antibody dilution buffer (1% BSA in 0.3% Tween-PBS) for 1 h and incubated with 1:200 dilution of anti-iNOS antibody overnight at 4°C in a humidified chamber to prevent evaporation of the antibody solution. After washing three times each in 0.3% Tween-PBS for 10 min, the sections were reprobed with goat anti-rabbit Alexa 594 (1:1,000; Molecular Probes, Eugene, OR) for 1 h at room temperature in a dark, humidified chamber and then washed four times in 0.3% Tween-PBS for 10 min/wash and rinsed in deionized water. Slides were mounted with gel mount (Biomeda, Foster, CA) and visualized. Samples without primary antibody were used as control. Images were acquired with a Nikon TE2000-U microscope outfitted with epifluorescence and a Cascade digital camera (Roper Scientific, Tucson, AZ), and images were acquired and processed using Metamorph Software version 6.0 (Universal Imaging, Downingtown, PA).

**RESULTS**

**Expression of the hiNOS/EGFP transgene by transfection in mouse mesangial cells.** Before using the hiNOS/EGFP transgene to generate transgenic mice, the functional activity of the −8,296/+86 hiNOS/EGFP transgene was tested by transiently transfecting mouse mesangial cells with the transgene in pEGFP-1, treating the cells with vehicle, IL-1β, LPS, IFN-γ, or LPS + IFN-γ, and analyzing subsequent EGFP expression on immunoblots. Our previous work established that endogenous mouse iNOS is not expressed under basal conditions but is induced by IL-1β and LPS + IFN-γ to a much greater extent than LPS alone. As seen in Fig. 2, mouse mesangial cells transfected with the −8,296/+86 hiNOS/EGFP transgene exhibited EGFP immunoreactivity under basal conditions, and this was enhanced by treatment with IL-1β, LPS, or LPS + IFN-γ, but not significantly with IFN-γ alone.

**Identification of transgenic mice.** The hiNOS promoter sequence significantly differs from the mouse iNOS promoter sequence so that species-specific probes and primer sets could be used to analyze directly transgene incorporation using Southern blotting and PCR as detailed in MATERIAL AND METHODS. Five founders were identified to carry the −8,296/+86 hiNOS/EGFP transgene by Southern blot and PCR analysis. A BamHI-digested Southern blot is shown in Fig. 3. BamHI digests the transgene uniquely in the hiNOS promoter region and the EGFP region to produce two fragments (−1.6 and 5.75 kb, respectively). Therefore, there are two fragments that can be detected by two hiNOS promoter-specific probes (see Fig. 1). These two bands were present in mice 2, 5, 6, 7, and 9 and the positive control (−8,296/+86 hiNOS/EGFP plasmid) but were absent in all other mice (Fig. 3). These founders were further confirmed to carry the transgene by Southern blot analysis using SphI digestion and by PCR (data not shown). Collectively, these results indicate that the five founder mice carry the full-length −8,296/+86 hiNOS/EGFP transgene. F1 transgenic mice from mating of founders with wild-type C57BL/6 mice were screened by Southern analysis in the same way and used for organ harvesting. The −8,296/+86 hiNOS/EGFP transgenic mice exhibited Mendelian inheritance in these lines as expected for a single autosomal integration event. Gross anatomic and histological examination of major organs, including lung, stomach, intestine, kidney, brain, heart, liver, spleen, and skeletal muscle, revealed that these were phenotypically normal.

**In vivo expression of the −8,296/+86 hiNOS/EGFP transgene in mice under basal conditions and during endotoxemia.** To determine the pattern of expression of the −8,296/+86 hiNOS/EGFP transgene, lung, stomach, intestine, kidney, brain, heart, liver, and spleen were harvested from multiple positive F1 mice, heterozygous for the transgene, from five founders along with their negative littermates for detection of EGFP and endogenous mouse iNOS proteins. Immunoblot analysis revealed that treatment with LPS induced endogenous iNOS protein in the lung, intestine, spleen, stomach, heart, kidney, and liver of transgenic mice and nontransgenic mice, whereas basal iNOS expression was not detected in any of these tissues (Fig. 4) in accord with previous observations (2, 4, 19). In contrast, basal EGFP expression was observed in kidney, spleen, and brain of the transgenic mice. Moreover, significant LPS induction of EGFP expression was only ob-
served in lung, intestine, brain, spleen, and stomach but not in kidney, liver, or heart (Fig. 4).

In lung, LPS-induced −8,296/+86 hiNOS/EGFP EGFP fluorescence colocalized with immunofluorescence of endogenous iNOS in the airway epithelium (Fig. 5). Similarly, in kidney, EGFP fluorescence precisely colocalized with mouse iNOS expression (Fig. 6A). LPS treatment induced the majority of EGFP expression in the proximal tubular epithelial cells in the outermost parts of cortex; however, in non-LPS treatment transgenic mice, the majority of EGFP expressed in the tubular epithelial cells of the outer medulla/inner cortex. In the collecting duct, aquaporin-2-positive cells did not express the transgene, suggesting that the minority population of collecting duct cells that expressed the transgene were intercalated cells (Fig. 6B).

DISCUSSION

Studies of hiNOS transcriptional regulation have largely been restricted to transient transfection of promoter-reporter gene constructs in cultured cells. Although several cis- and trans-regulatory factors have been characterized using this approach, the local changes in chromatin structure that participate in the activation of the iNOS gene have not been extensively considered. In vivo binding conditions may be difficult to recreate in vitro; for example, binding of proteins that require DNA secondary structure to bring two distal binding sites into close proximity cannot be studied by in vitro methods. To overcome these limitations, we used an insertional transgenic approach in mice to analyze the functional promoter activity, expression profile, and response to endotoxemia of the hiNOS gene 5′-flanking region. These studies represent the first analysis of the hiNOS promoter in vivo and highlight the regulatory differences between the human and murine 5′-flanking regions of the iNOS gene.

Endotoxin is a potent inducer of iNOS transcription directly and indirectly through its cytokine mediators TNF-α, IFN-γ, and IL-1β. Studies of hiNOS promoter-reporter constructs transfected into various cell lines indicate that the proximal 0.35 kb of the promoter region contains cytokine-enhancer elements that bind NF-κB (21), KLF6 (34), and C/EBPβ (14). Another enhanceosome resides beyond −5.0 kb and contains

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Fig. 3. Southern analysis of transgenic mice. Southern blots of BamHI-digested genomic DNA isolated from tail biopsies of mice were prepared and probed with 32P-labeled 466-bp SmaI/SacI plus the 309-bp StuI-StuI fragments specific for the hiNOS promoter region. Because there are 2 BamHI sites in the hiNOS promoter and 1 BamHI site in the EGFP region, respectively, enzyme digestion yields 2 bands (5.75 and 1.6 kb, respectively) that can be detected by probes in mice with successful integration of the transgene. These 2 bands were present and apparently identical in transgenic mice 2, 5–7, and 9 and absent in all other mice. P, mixture of plasmid DNA of −8,296/+86 hiNOS/EGFP and genomic DNA isolated from a C57BL/6 mouse.

Fig. 4. Tissue distribution of −8,296/+86 hiNOS/EGFP transgene expression in response to endotoxemia. A: immunoblot of extracts from a panel of tissues harvested from transgenic mice treated with vehicle or LPS (10 mg/kg) probed with BD Living Colors AV monoclonal antibody JL-8 directed against EGFP, an anti-iNOS polyclonal antibody directed against endogenous iNOS, or anti-α-tubulin antibody. Blot is representative of results obtained from all founders. No EGFP immunoreactivity was observed in any of these tissues in the nontransgenic littermates. B: immunoblot for EGFP and endogenous iNOS of whole kidney lysates obtained from wild-type and transgenic mice that had been treated with vehicle or LPS (10 mg/kg) for 16 h.
NF-κB-like sequences (31), inducible activator protein-1 (21) and STAT1 binding sites, and a bifunctional motif that binds either STAT1 and/or NF-κB (11). Chromatin structure analysis in A549 and AKN-1 cells demonstrated cytokine-inducible DNase I hypersensitive sites and in vivo footprints in region −5 to −5.5 kb (24). We found that under basal conditions the hiNOS transgene was expressed in spleen, brain, and kidney, whereas endogenous mouse iNOS protein was not detected in these same tissues. Significant LPS induction of the hiNOS promoter EGFP transgene was observed in lung, intestine, brain, spleen, and stomach but not in kidney, liver, or heart, even though the murine iNOS protein was induced at significantly higher levels in all the tissues examined after LPS injection. The findings of LPS induction of endogenous iNOS are in keeping with the report of Kan et al. (19), who found induction in heart, liver, spleen, lung, gut, and kidney in endotoxin shock. The expression patterns of hiNOS among tissues has not been well characterized. Consistent with our findings of transgene expression, basal and induced iNOS expression have been shown in human lung (13), intestine (27), brain (29), spleen (26), and stomach (3). The discrepancy between the expression of EGFP controlled by the hiNOS promoter and the endogenous mouse iNOS protein in these settings and tissues presumably reflects the significant sequence differences and cis-acting control elements between the murine iNOS and hiNOS promoters. These differences are further highlighted by comparison of the present results with those of Zhang and co-workers (38), who developed a transgenic mouse model in which the luciferase gene reporter was driven by the activity of only a 1.24-kb stretch of the mouse iNOS promoter. In their study, treatment of these animals with LPS and IFN-γ resulted in transgene induction predominantly in mouse liver. Again, a limited stretch of 5′-flanking region of the mouse iNOS promoter is required for maximal LPS and cytokine induction, whereas the hiNOS promoter requires a much more extensive stretch of 5′ gene control region for similar induction. It is also possible that the 16-h time point we selected for analysis of transgene expression may have missed earlier, transient increments in iNOS transcription in the liver or other tissues. For example, iNOS expression in rodent liver is rapidly induced ~4 h after stimulation with LPS and then decays (1).

Within the kidney, hiNOS promoter-EGFP transgene expression colocalized with endogenous iNOS, with most prominent expression in the proximal tubules and the length of the collecting duct. In the collecting duct, expression of both the transgene and endogenous iNOS were absent from cells that were immunoreactive for aquaporin-2, a marker of principal cells in this segment, suggesting that the transgene and endogenous iNOS are expressed in intercalated cells, in agreement with the results of Tojo et al. (32) in rats.

Endotoxemia-related acute kidney failure is a common clinical problem that involves a complex sequence of events and is associated with considerable morbidity and mortality. Endotoxia-stimulated iNOS expression leads to NO-mediated arterial vasodilatation, which ultimately promotes renal sympathetic and angiotensin II activities and vasoconstriction. Additionally, the reaction of NO with newly generated reactive oxygen species during sepsis promotes peroxynitrite-related tubular injury and suppression of renal endothelial NOS (28, 33). On the other hand, endotoxemia increases renal cortical iNOS, with a resultant increase in NO that may protect against glomerular microthrombi and counteract renal vasoconstriction (28). Our transgenic model will allow investigators to test many of these hypotheses, which were primarily formulated using rodent models, in the context of hiNOS transcriptional control mechanisms.

As with any transgenic model, the present results could have been affected by not having the promoter-reporter gene construct integrated at the native site in the genome, by transgene copy number, by secondary gene mutation unrelated to the transgene (which is estimated to occur infrequently), or by position effects related to the influence of neighboring chromosomal elements and disrupted relationships of the transgene with locus control regions and boundary elements of the chromosome. It is possible that paracrine, autocrine, or endocrine mediators are necessary for transcriptional competency of the hiNOS promoter in the in vivo setting. In addition to the linear order of cis-regulatory elements in the promoter, nuclear matrix, nucleosome organization, chromatin structure, epigenetic pathways, and the ordered recruitment of transcription factors and coregulatory proteins all contribute to the accurate expression of a gene and may contribute to any lack of fidelity of transgene expression in certain tissues. It is also possible that the transgene did not include remotely positioned and essential positive or negative regulatory promoter elements. Accordingly, a BAC- or YAC-based transgenic construct containing large stretches of genomic DNA might be required to address this issue in the future.

In summary, we identified a 8.3-kb linear region of the hiNOS gene 5′-flanking region that directs specific basal expression of an EGFP reporter gene in the kidney, brain, and lung, and LPS-inducible expression in the intestine, spleen, brain, stomach, and lung with an intrarenal expression profile that mirrors that of the endogenous mouse iNOS protein. These transgenic hiNOS promoter-reporter lines offer advantages...
over other means to monitor iNOS activity in vivo, such as measurement of circulating levels of nitrate and nitrite, which does not distinguish the specific NOS producing NO nor does it provide information about the anatomic site of iNOS induction. In addition, this transgenic model permits the in vivo tracking of localized inflammation and injury and the screening of anti-inflammatory compounds that act through inhibition of iNOS induction, and allows dissection of signaling cascades.

Fig. 6. Intrarenal distribution of −8.296/+86 hiNOS/EGFP transgene expression. Sections of kidney from transgenic mice that had been treated with vehicle or LPS (10 mg/kg) for 16 h were fixed, and images of the EGFP fluorescence (A), EGFP and endogenous iNOS (B), or aquaporin-2 (C) immunofluorescence were obtained by fluorescence microscopy and image analysis as described in MATERIALS AND METHODS. Representative images are shown. B: magnification ×40. C: magnification ×60.
that impact iNOS transcription. Finally, our data also indicate that caution should be exercised in extrapolating data concerning iNOS transcriptional control obtained in other species to the hiNOS gene.

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