Inhibition of Nitric-oxide Synthase-I (NOS-I)-dependent Nitric Oxide Production by Lipopolysaccharide plus Interferon-γ Is Mediated by Arachidonic Acid

EFFECTS ON NFκB ACTIVATION AND LATE INDUCIBLE NOS EXPRESSION*

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Received for publication, November 21, 2003, and in revised form, May 11, 2004

The Journal of Biological Chemistry Vol. 279, No. 29, Issue of July 16, pp. 29895–29901, 2004

Previous results have indicated that lipopolysaccharide (LPS) plus interferon-γ (IFNγ) inhibits nitric-oxide synthase (NOS-I) activity in glial cells. We report here that arachidonic acid (AA) plays a pivotal role in this response, which was consistently reproduced in different glial cell lines and in primary rat astrocytes. This notion was established using pharmacological inhibitors of phospholipase A2 (PLA2), cytosolic PLA2 (cPLA2), antiense oligonucleotides, and AA, and add-back experiments. This approach not only allowed the demonstration that AA promotes inhibition of NOS-I activity but also produced novel experimental evidence that LPS/IFNγ itself is a potential stimulus for NOS-I. Indeed, LPS/IFNγ fails to generate nitric oxide (NO) via NOS-I activation simply because it activates the AA-dependent signal that impedes NOS-I activity. Otherwise, LPS/IFNγ promotes NO formation, sensitive to exogenous AA, in cells in which cPLA2 is pharmacologically inhibited or genetically depleted. Because NO suppresses the NFκB-dependent NOS-II expression, inactivation of NOS-I by the LPS/IFNγ-induced AA pathway provides optimal conditions for NFκB activation and subsequent NOS-II expression. Inhibition of cPLA2 activity, while reducing the availability of AA, consistently inhibited NFκB activation and NOS-II mRNA induction and delayed NO formation. These responses were promptly re-established by addition of exogenous AA. Finally, we have demonstrated that the LPS/IFNγ-dependent tyrosine phosphorylation of NOS-I and inhibition of its activity are mediated by endogenous AA.

Nitrergic oxide (NO) is generated in different cell types by the conversion of l-arginine into citrulline mediated by at least three distinct isoforms of the enzyme NO synthase (NOS). Two enzymes, the neuronal (NOS-I) and the endothelial (NOS-III) isoforms, are Ca2+-dependent and constitutively expressed. The third enzyme is an inducible Ca2+-independent isofrom (NOS-II), expressed after stimulation with Escherichia coli lipopolysaccharide (LPS) and/or different cytokines, such as interferon-γ (IFNγ), interleukin-1β, or tumor necrosis factor-α (1). NOS-II induction occurs at the transcriptional level and is mediated by the early activation of some nuclear transcriptional factors, including NFκB (2). A large body of experimental evidence suggests that physiological levels of NO, similar to those produced by the basal activity of NOS-I or NOS-III, prevent induction of NOS-II mRNA expression through the suppression of NFκB activation (3, 4). As a consequence, NOS-II gene expression takes place after LPS/cytokine stimulation, provided that the NOS-I or NOS-III-generated NO is reduced below a threshold value in a short time (5). We have recently reported (6,7) that NOS-II inducers (e.g. LPS and IFNγ) consistently elicit a rapid inactivation of NOS-I by tyrosine phosphorylation, an event leading to a decrease of basal NO levels.

In a recent study (8), we reported that inhibition of NOS-I can be achieved via activation of cytosolic phospholipase A2 (cPLA2), a large molecular mass member of the family of PLA2 enzymes. The activities of NOS-I and cPLA2 are both regulated by increases in the intracellular concentration of free Ca2+ ([Ca2+]i) (9); not surprisingly, enhancing the [Ca2+]i was found to cause a parallel increase in both activities and accumulation of respective products, NO and arachidonic acid (AA). Interestingly, however, critical levels of AA were eventually reached that reduced or even suppressed formation of NO (8). Thus, sustained activation of cPLA2 is expected to promote a rapid inactivation of NOS-I via the AA-dependent inhibitory signaling. It is intriguing that both LPS and IFNγ stimulate the activity of cPLA2 and promote the release of AA (10, 11).

In the present study, glial cell lines as well as rat primary astrocytes were utilized as cellular models to demonstrate that the mechanism whereby LPS and IFNγ impair NOS-I activity indeed involves an AA-dependent tyrosine phosphorylation of the enzyme. The inhibitory signaling triggered by AA therefore allows downstream events leading to NFκB activation, NOS-II mRNA expression, and NOS-II-dependent NO production. However, when the release of AA was prevented by pharmacological inhibition or genetic depletion of cPLA2, the mixture LPS/IFNγ stimulated NOS-I activity and failed to elicit the above NFκB-dependent delayed responses.

EXPERIMENTAL PROCEDURES

Materials
AA, A23187, LPS, Nω-nitro-l-arginine methyl ester (l-NAME), and other reagent grade biochemicals were from Sigma. IFNγ (specific ac-
tivity: 2 × 10^19 international units/mg of protein) and AACOCF₃ were purchased from Biogen Sà (Geneva, Switzerland) and Calbiochem, respectively. [3H]IAA or [3H]arginine were from Amersham Biosciences. 4,5-diaminofluorescin diacetate (DAF-2DA) was purchased from Alexis Italia (Florence, Italy). Mouse monoclonal CLPLA₂ and fluorescein isothiocyanate-conjugated secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). LipofectAMINE and PLUS reagent were from Invitrogen.

Methods

Cell Culture and Treatment Conditions—Primary cultures of cortical astrocytes derived from neonatal 1- or 2-day-old Sprague-Dawley rats (Charles River, Calco, Italy), were prepared as described (12). At this age, the progenitor cells have lost the ability to differentiate into neuronal cells (13), giving rise to a pure glial cell preparation that was confirmed by immunocytochemistry using antibodies directed to glial fibrillary acidic protein. Primary astrocytes, human A172 astrocytoma, or C6 glioma cells were cultured in Eagle’s minimal essential medium (primary astrocytes) or Dulbecco’s modified Eagle’s medium (A172 or C6 cells) supplemented with either 10% horse serum and 10% fetal bovine serum (HyClone Laboratories, Logan, UT) (primary astrocytes) or 10% fetal bovine serum (A172 or C6 cells), penicillin (50 units/ml), streptomycin (50 μg/ml) (HyClone) at 37 °C in 35-mm Primaria dishes (primary astrocytes) or T-75 tissue culture flasks (A172 or C6 cells) gassed with an atmosphere of 95% air-5% CO₂. For experiments, cells were used 2 weeks (primary astrocytes) or 18–24 h (A172 or C6 cells) after plating into 35-mm culture dishes.

Stock solutions of IFNγ, LPS, t-NAME, and AA were freshly prepared in distilled water. AACOCF₃ and A23187 were dissolved in Me2SO. At the treatment stage, the final concentration of Me2SO was never higher than 0.05% (v/v).

NO Detection System—The production of NO was assayed using the DAF-2DA detection system, as described elsewhere (8). Briefly, the cells were loaded with 10 μM DAF-2DA for 10 min in saline A (8.122 g/liter NaCl, 0.372 g/liter KCl, 0.336 g/liter NaHCO₃, and 0.9 g/liter glucose) at 37 °C. After washings, the cells were treated (15 min at 37 °C), and cell membranes were imaged using a confocal laser microscope (Bio-Rad DVC 250) equipped with a Hamamatsu 5985 (Milan, Italy) chilled CCD camera. Confocal images were digitally acquired and processed for fluorescence determination at the single cell level on a Macintosh G4 computer using the public domain NIH Image 1.61 program (developed at the U.S. National Institutes of Health and available on the Internet at rsb.info.nih.gov/nih-image/).

Immunocytochemical Detection of cPLA₂—The cPLA₂ protein was detected using an immunocytochemical technique following the manufacturer’s instructions, as previously described (8). Briefly, the cells were fixed with ethanol/acetic acid and blocked in phosphate-buffered saline containing bovine serum albumin (2%, w/v). Mouse monoclonal cPLA₂ antibodies were used as primary antibodies. After 18 h at 4 °C, the cells were washed and exposed to fluorescein isothiocyanate-conjugated secondary antibodies for 2 h in the dark. Stained cells were analyzed using a confocal microscope, and the resulting images were processed for fluorescence determination as described above.

Transfection of Sense or Antisense Oligonucleotides into C6 Cells—The antisense and sense oligonucleotides for rat cPLA₂ were transfected into the cells using the LipofectAMINE PLUS method as detailed elsewhere (8). Treatments were performed 24 h after transfection.

Measurement of Extracellular Release of [3H]IAA—Cells were subcultured in 6-well plates at 2 × 10⁶ cells/well with [3H]IAA (0.5 μCi/mM) and grown for 18 h. Before treatments, the cells were washed twice with saline A supplemented with 1 mg/ml fatty acid-free bovine serum albumin and then exposed to LPS/IFNγ in the absence or presence of 75 μM AACOCF₃ alone or associated with either AA or t-NAME. The cells were then treated for an additional 15 min with 0 or 2.5 μM A23187. DAF-2DA-preloaded cells were incubated for 5 min with LPS/IFNγ in the absence or presence of 75 μM AACOCF₃ alone or associated with either AA or t-NAME. The cells were then treated for an additional 15 min with 2.5 μM A23187. After treatments, the cells were observed with a confocal microscope; the resulting images were digitally acquired and analyzed as detailed under “Experimental Procedures.” Data points are the means ± S.E. from three to five separate experiments, each performed in duplicate. *p < 0.05, **p < 0.001 versus untreated cells; ***p < 0.001 versus A23187/LPS/IFNγ-treated cells (unpaired t test).

4 °C. Immunoprecipitated samples were subjected to electrophoresis in 7.5% polyacrylamide gel and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 3% bovine serum albumin for 1 h and incubated overnight at 4 °C with 1 μg/ml monoclonal anti-phosphotyrosine antibody (Upstate Ltd., Milton Keynes, UK). Secondary peroxidase-labeled anti-mouse IgG antibody was from Amersham Biosciences. Detection was performed using ECL Western blotting detection reagents (Amersham Biosciences).

NFB Activation Assay—NF-κB activation was quantified using the TransAM NFκB kit (Active Motif, Rixensart, Belgium). Briefly, whole-cell extracts were prepared from 5 × 10⁶ C6 cells according to the manufacturer’s instructions. Protein concentrations of cell extracts were determined according to Bradford (14). Ten micromg/well of cell extracts were incubated in a 96-well plate on which have been immobilized double-stranded oligonucleotides containing the consensus NFκB DNA binding site (5′-AGTTGAGGGCATTCCCAGGC-3′). The primary antibody used to detect NFκB recognized an epitope on p65 subunit that is accessible only when NFκB is activated and bound to its target DNA. After incubation with a horseradish peroxidase-conjugated secondary antibody and the developing solution, absorbance was read at 450 nm with a reference wavelength of 655 nm.

Reverse Transcriptase-PCR—Reverse transcriptase-PCR was carried out on total cellular RNA purified from 1 × 10⁶ rat C6 glioma cells and reverse-transcribed into cDNA as previously reported (15). cDNA was amplified for the NOS-II gene (450 bp) using rat NOS-II-specific primers as described elsewhere (16). The mRNA for the constitutive GAPDH enzyme was examined as the reference cellular transcript. GAPDH mRNA amplification products (195 bp) were present at equivalent levels in all cell lysates. Estimates of the relative NOS-II mRNA amounts were obtained dividing the area of the NOS-II band by the area of the GAPDH band (Bio-Rad MultiAnalyser).

Statistical Analysis—Statistical analysis of the data for single comparisons was performed by Student’s t test.

RESULTS AND DISCUSSION

We recently reported that exposure of C6 cells to concentrations of A23187 in the 0.5–2.5 μM range leads to a progressive increase in NO formation and that this response is reduced, or even abolished, when the ionophore is utilized at 5 or 7.5 μM (8). These results were readily explained by the
observation that high A23187 concentrations cause a cPLA2-dependent release of AA, which then promotes a NOS-I inhibitory signaling pathway. On the other hand, exogenous AA suppressed formation of NO mediated by lower concentrations of A23187 (8).

The results illustrated in Fig. 1A confirm and extend these findings by showing that the DAF-2 fluorescence response elicited by 2.5 μM A23187 (15 min), although sensitive to both l-NAME (1 mM) and exogenous AA (30 nM), is also inhibited by a pretreatment (5 min) of the cells with the mixture LPS (1 μg/ml)/IFNγ (1000 units/ml). At these concentrations, LPS and IFNγ promote maximal activation of NFκB and NOS-II expression (data not shown), and as we previously showed (6, 7) also cause suppression of basal NO production, an effect readily detected by measuring conversion of l-arginine to l-citrulline in cell homogenates supplemented with cofactors for NOS-I activity. Under our experimental conditions, however, LPS/IFNγ failed to affect the basal DAF-2 fluorescence, and similar results were obtained using AA or l-NAME (Fig. 1A). This represents a limitation of the assay, most likely associated with the significant NO-independent DAF-2 fluorescence signal, which does not allow detection of small changes mediated by basal NO levels generated by NOS-I in the absence of stimuli. The time of DAF-2 exposure is indeed too short to allow formation of NO levels promoting detectable changes in the DAF-2 fluorescence signal. However, as indicated above, such variations are readily detected in cells stimulated with A23187, and LPS/IFNγ was found to suppress this response, thus confirming the LPS/IFNγ-mediated NOS-I inactivation.

Further analyses revealed that the LPS/IFNγ-induced inhibition of the A23187-stimulated NO formation is because of AA-dependent NOS inhibition. Three separate lines of evidence support this inference. First, as shown in Fig. 1B, the effects mediated by LPS/IFNγ were prevented by the cPLA2 inhibitor A23187, and AA or L-NAME (Fig. 2A), responded to 2.5 μM A23187 with formation of NO insensitive to LPS/IFNγ, albeit sensitive to either AA or l-NAME (Fig. 2B). In marked contrast, the experiments performed with cPLA2 sense oligonucleotide-transfected cells (Fig. 2C) led to outcomes identical to those obtained using non-

TABLE I

| Treatment | Non-transfected cells | cPLA2 antisense oligonucleotide-transfected cells |
|-----------|-----------------------|-----------------------------------------------|
|           | Senset | Antisenset |
| Untreated | 295 ± 50 | 305 ± 62 |
| LPS/IFN   | 1778 ± 381** | 1670 ± 289** |
| +AACOCF3  | 595 ± 80a | 580 ± 59ab |
| Untreated | 295 ± 50 | 305 ± 62 |
| LPS/IFN   | 1778 ± 381** | 1670 ± 289** |
| +A23187   | 595 ± 80a | 580 ± 59ab |

* [3H]AA-labeled cells were exposed for 5 min to 75 μM AACOCF3 and then treated for a further 15 min with LPS/IFNγ. After treatments, the [3H]AA release was quantified as described under “Experimental Procedures.”

** The cells were labeled with [3H]AA after a 6-h incubation with the cPLA2 sense or antisense oligonucleotides. After an additional 18 h, the cells were treated as described above.
transfected cells (Fig. 1, i.e. AA, l-NAME as well as LPS/IFNγ abolishing formation of NO).

These results confirm that AA promotes inhibition of NOS-I (8) and provide experimental evidence indicating that LPS/IFNγ/H9253 inhibits A23187-induced NOS-I activation by triggering the AA-dependent mechanism. The antisense oligonucleotide experiments imply that the cPLA2 isoform is specifically involved in the LPS/IFNγ-induced formation of AA. Consistently, LPS/IFNγ caused an extensive release of AA, sensitive to AA-COCF3 and remarkably lower in cells transfected with cPLA2 antisense oligonucleotides (Table I). Experiments using cells transfected with cPLA2 sense oligonucleotides produced results identical to those obtained with non-transfected cells. It should be noted that AACOCF3 is not efficiently taken up in some cell types. By monitoring its intrinsic fluorescence, we found that this was the case for C6 cells (not shown), which is the reason a high concentration of AACOCF3 was employed in this study. Thus, although a minor contribution of an AACOCF3-insensitive (e.g. group II) PLA2 in the LPS/IFNγ-induced AA release cannot be ruled out, the inability of the inhibitor to completely suppress the release of AA might be dependent on its low cellular uptake.

The above results indicate that LPS/IFNγ potently stimulates the activity of cPLA2 and that the ensuing release of AA promotes inhibition of NOS-I activity. Interestingly, we found that incubation of LPS/IFNγ-stimulated cells with the PLA2 inhibitor AACOCF3, although reducing AA release (Table I), caused a remarkable increase of DAF-2 fluorescence sensitive to either l-NAME or AA (Fig. 3A). Consistently, LPS/IFNγ, in the absence of additional treatments, caused a similar DAF-2 fluorescence signal in cells transfected with cPLA2 antisense oligonucleotides (Fig. 3B). This response, although insensitive to AACOCF3, was suppressed by l-NAME or AA. The results obtained using cells transfected with cPLA2 sense oligonucleotides were identical to those generated with non-transfected cells (Fig. 3C). The above findings are consistent with the possibility that LPS/IFNγ is paradoxically a potential activator of NOS-I and that it fails to do so simply because the prevailing

FIG. 3. LPS/IFNγ is a potential stimulus for NOS-I activity that is, however, prevented by parallel AA-dependent NOS-I inhibitory signaling. A, DAF-2DA-preloaded cells were incubated for 5 min in the absence of AACOCF3 or AA. Cells were then treated with LPS/IFNγ and analyzed as described in the legend to Fig. 1. B and C, cells were transfected with cPLA2 antisense (B) or sense (C) oligonucleotides and loaded with DAF-2DA. cPLA2 antisense oligonucleotide-transfected cells were incubated for 5 min in the absence or presence of AACOCF3, l-NAME, or AA. Cells were then treated for an additional 15 min with LPS/IFNγ. cPLA2 sense oligonucleotide-transfected cells were treated as detailed in panel A. Cells were analyzed as described in the legend to Fig. 1. Data points are the means ± S.E. of three to five separate experiments, each performed in duplicate. *, p < 0.001 versus untreated cells; (†), p < 0.001 versus LPS/IFNγ-treated cells (unpaired t test).

FIG. 4. Exogenous AA inhibits NO formation stimulated by LPS/IFNγ in PLA2 inhibitor-supplemented primary astrocytes. Representative micrographs of DAF-2DA-preloaded primary astrocytes incubated for 5 min in the absence (B) or presence of AACOCF3 alone (C) or associated with either l-NAME (D) or AA (E) and then treated for an additional 15 min with LPS/IFNγ. The DAF-2 fluorescence response observed in untreated cultures is shown in panel A; the experimental values obtained under these conditions were used to normalize the fluorescence response detected under the remaining treatment conditions (F). The micrographs are representative of at least three separate experiments with similar outcomes. Quantitative analysis was performed in at least 70 cells/treatment condition/experiment.

AA-dependent inhibitory signaling suppresses NOS-I activity. In other words, LPS/IFNγ may produce effects superimposable on those previously observed using a high A23187 concentration (7.5 μM) (8). This notion was established in additional glial
cell lines (not shown), including human A172 astrocytoma cells (see below) as well as primary rat astrocytes (Fig. 4).

Thus LPS/IFNγ, under conditions not permissive for AA release, promotes formation of NO. Under physiological conditions, however, the LPS/IFNγ-mediated NOS-I activation is prevented by the parallel NOS-I inhibitory signaling pathway(s) triggered by AA.

Because it is well established that suppression of NOS-I activ-

**Fig. 5.** A critical role for AA in LPS/IFNγ-induced NFκB activation, NOS-II mRNA accumulation, and delayed NO formation. Cells were incubated for 5 min in the absence or presence of AACOCF3 alone or associated with AA and then treated for increasing time intervals with LPS/IFNγ. A, cells were analyzed after 0.5 h for NFκB activation as detailed under “Experimental Procedures.” B, cells were analyzed after 4 h for NOS-II mRNA induction. The PCR product for the GAPDH gene was taken as the reference cellular transcript. Estimates of the relative NOS-II mRNA amounts were obtained dividing the peak densitometry of the NOS-II band by the peak densitometry of the GAPDH band. Setting equal to 1 unit of the value of NOS-II mRNA from untreated cells (lane 1); values for the other samples, i.e. LPS/IFNγ alone (lane 2) or associated with AACOCF3 (lane 3) or AACOCF3 plus AA (lane 4) were calculated relative to it. MW is the 100-bp DNA ladder. The experiment was repeated three times; the histogram and the gel show a representative result. C, cells were analyzed after 8 h for NO formation. DAF-2DA was added to the cultures in the last 10 min of incubation. Data points shown in panels A and C are the means ± S.E. from three to five separate experiments, each performed in duplicate. *, p < 0.001 versus untreated cells (unpaired t test).

**Fig. 6.** LPS/IFNγ fails to induce NOS-II mRNA expression and NOS-II-dependent NO formation in cPLA2 antisense oligonucleotide-transfected cells; this response is promptly recovered upon addition of exogenous AA. The cells were transfected with cPLA2 antisense oligonucleotides, incubated for 5 min in the absence or presence of AA alone or associated with L-NAME, and treated for increasing time intervals with LPS/IFNγ. A, cells were analyzed after 4 h for NOS-II mRNA induction. The PCR product for the GAPDH gene was taken as the reference cellular transcript. Estimates of the relative NOS-II mRNA amounts were obtained dividing the peak densitometry of the NOS-II band by the peak densitometry of the GAPDH band. Setting equal to 1 unit of the value of NOS-II mRNA from untreated cells (lane 1); values for the other samples, i.e. LPS/IFNγ alone (lane 2) or associated with AACOCF3 (lane 3) or AACOCF3 plus AA (lane 4) were calculated relative to it. MW is the 100-bp DNA ladder. The experiment was repeated three times, and the histogram and the gel show a representative result. B, cells were analyzed after 8 h for NO formation. DAF-2DA was added to the cultures in the last 10 min of incubation. Data points shown are the means ± S.E. from three to five separate experiments, each performed in duplicate. *, p < 0.001 versus untreated cells; (*), p < 0.001 versus LPS/IFNγ-treated cells (unpaired t test).
Fig. 7. LPS/IFNγ induces tyrosine phosphorylation of NOS-I and inhibition of its activity via an AA-dependent mechanism. A, DAF-2DA-preloaded A172 cells were incubated for 5 min with AACOCF₃ or genistein (300 μM) in the absence or presence of either L-NAME or AA. Cells were then treated for an additional 15 min with LPS/IFNγ. After treatments, the DAF-2 fluorescence response was determined as described above. Data points are the means ± S.E. of three to five separate experiments, each performed in duplicate. *, p < 0.001 versus LPS/IFNγ-treated cells; (†), p < 0.001 versus LPS/IFNγ + AACOCF₃-treated cells; §, p < 0.001 versus LPS/IFNγ + genistein-treated cells (unpaired t test). B, NOS-I was immunoprecipitated from A172 cells using a polyclonal anti-NOS-I antibody. Immunoprecipitated NOS-I was Western-blotted using a specific antiserum against phosphotyrosine. NOS-I from untreated cells was weakly and partially tyrosine-phosphorylated (lane 1). Treatment of cells with LPS/IFNγ for 10 min increased tyrosine phosphorylation of NOS-I (lane 2). Preincubation of LPS/IFNγ-treated A172 cells with AACOCF₃ (5 min) caused a reduction of tyrosine phosphorylation of NOS-I (lane 3). This effect was reverted in the presence of AA (lane 4).

Arachidonic Acid Mediates NOS-I Inhibition by LPS plus IFNγ

A possible candidate of the downstream kinase leading to NOS-I phosphorylation and inactivation is a tyrosine kinase, because AA is a known activator of this pathway (20) and our previous work showed that LPS/IFNγ-induced inhibition of NOS-I activity is mediated by tyrosine phosphorylation (7). To address this question, experiments were performed using the human A172 astrocytoma cell line, which expresses higher levels of NOS-I than C6 cells. As illustrated in Fig. 7A, A172 cells responded to LPS/IFNγ as C6 cells (e.g. NO being formed only in the presence of AACOCF₃ via an AA- or L-NAME-sensitive mechanism). Interestingly, however, formation of NO was also promoted by the tyrosine kinase inhibitor genistein (300 μM), and this response, although prevented by L-NAME, was only slightly reduced by AA. Genistein produced identical effects in C6 cells (not shown); therefore, tyrosine kinase-dependent phosphorylation appears to be a likely mechanism of the AA-dependent NOS-I inhibitory signaling. To obtain more information in this direction, immunoprecipitation experiments were performed using A172 cells. We found under basal conditions NOS-I is partially tyrosine-phosphorylated and that LPS/IFNγ (10 min) further enhances this response (Fig. 7B). Interestingly, however, tyrosine phosphorylation, although inhibited by genistein (not shown), was also markedly reduced by AACOCF₃ via an AA-sensitive mechanism. Taken together, these results strongly suggest that the downstream target of AA-dependent NOS-I inhibitory signaling is indeed a tyrosine kinase.

In conclusion, the present study underscores a novel mechanism whereby stimulation by LPS/IFNγ elicits NFκB activation and NOS-II expression. Our previous studies indicated that these events were preceded by, and causally linked to, removal of the NFκB block mediated by NO generated under conditions of basal NOS-I activity (5). We have demonstrated here that inhibition of NOS-I is achieved via AA-dependent inhibitory signaling and that the downstream kinase leading to NOS-I phosphorylation and inactivation is a tyrosine kinase.

As a final note, it is important to keep in mind that AA is extensively released during inflammation and that, under these conditions, autocrine or paracrine AA may cause profound effects on cytokine-induced NFκB activation and NOS-II expression. Growing experimental evidence suggests that reduced availability of constitutive NO levels is detrimental in a variety of conditions, including neurodegenerative disorders associated with inflammation (21). One possible explanation for these observations is that physiological levels of NO might promote cytoprotection (22–24). In addition and/or as an alternative, the possibility exists that AA-dependent suppression of NOS-I activity might facilitate cytokine-mediated activation of astrocytes and/or other cell types with the concomitant generation of an array of toxic species.

Acknowledgments—We thank Prof. Valeria Bruno for help in setting up the cultures of rat primary astrocytes and Dr. Zulema Percario for helpful technical support.
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J. Biol. Chem. 2004, 279:29895-29901.
doi: 10.1074/jbc.M312768200 originally published online May 17, 2004

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