Role of Inducible Nitric Oxide Synthase and Cyclooxygenase-2 in Endotoxin-Induced Cerebral Hyperemia

Hirotsugu Okamoto, MD, PhD; Osamu Ito, MD, PhD; Richard J. Roman, PhD; Antal G. Hudetz, BMD, PhD

Background and Purpose—Bacterial lipopolysaccharide (LPS), an endotoxin, has been reported to induce the expression of inducible isoforms of both nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) in various cell types. LPS is also known to dilate systemic vasculature, including cerebral vessels. This study aimed to determine to what extent LPS induces iNOS and COX-2 expression in the brain and whether NO and/or cyclooxygenase metabolites derived from iNOS and/or COX-2 contribute to the LPS-induced cerebral hyperemia.

Methods—Regional cerebral blood flow (rCBF) was measured by laser-Doppler flowmetry in halothane-anesthetized, artificially ventilated rats for 4 hours after intracerebroventricular administration of LPS.

Results—LPS at doses of 0.01 mg/kg to 1 mg/kg caused dose-dependent, progressive increases in rCBF at 1 to 4 hours after administration. The increase in rCBF was attenuated by systemic administration of the selective iNOS inhibitor aminoguanidine (100 mg/kg IP) or the selective COX-2 inhibitor NS-398 (5 mg/kg IP), and it was abolished by preventing induction of these isoforms with dexamethasone (4 mg/kg IP). LPS significantly increased iNOS and COX-2 mRNA, iNOS protein, and iNOS and cyclooxygenase enzyme activity. The increases in iNOS and cyclooxygenase enzyme activity were eliminated by aminoguanidine and NS-398, respectively. Dexamethasone also prevented the increase in iNOS and cyclooxygenase activity.

Conclusions—These results indicate that induction of iNOS and COX-2 expression and the increased production of NO and vasodilator prostanoids in the brain contribute to the elevation in CBF after intracerebroventricular administration of LPS. (Stroke. 1998;29:1209-1218.)

Key Words: cerebral blood flow ● endotoxins ● lipopolysaccharides ● nitric oxide synthase ● prostaglandins ● rats
inhalation of 0.6% halothane (Anaquest Inc). Body temperature was maintained at 37 ± 1°C with the use of a water-circulated heating pad. One of the femoral arteries was cannulated to facilitate the measurement of arterial pressure and arterial blood gases. Arterial Po2, Pco2, and pH were measured with a blood gas/pH analyzer (ABL-300, Radiometer). A femoral vein was cannulated for the infusion of drugs. Arterial blood pressure, end-tidal carbon dioxide tension, inspired and expired oxygen, and halothane concentrations were continuously monitored (POET II, Criticare Systems, Inc) and recorded on an eight-channel polygraph recorder (Astro-Med, Inc). As previously reported, a 30-gauge stainless steel cannula (HTX-50, Small Parts) was placed into the left lateral ventricle for intracerebroventricular injection with the bregma chosen as the stereotaxic point (anteroposterior, −0.3 mm; lateral, +1.2 mm; dorsoventricular, −4.5 mm). Intracerebroventricular infusions were performed at the rate of 1 μl/min with the use of a microinfusion pump (model 55–2222, Harvard Apparatus) in a volume of 10 μl. The solutions used for semiquantitative comparisons of the amount of mRNA could be used for the RT reaction. The sequences of the primers used (Operon) have been reported previously and were as follows: iNOS forward, 5'-ACACGTTGGGAGAGTGCTC-3'; iNOS reverse, 5'-CCCCAGGTG-3'; iNOS reverse, 5'-ACACGTCGCGGCATCGAA-GACC-3'; COX-2 forward, 5'-GAAGTGGGTTGTTAGGAT-CATC-3'; COX-2 reverse, 5'-CTCTTACCTCCGGATAACCA-3'; GAPDH forward, 5'-CAGCGGAAATGTTCAATGGCACA-3'; GAPDH reverse, 5'-GAATGTGAGGGAGAGTGCTC-3'. The primers chosen amplified across several large interspersed introns to avoid the possibility of amplification of genomic DNA. The iNOS reactions were cycled 35 times at 96°C for 30 seconds, 60°C for 60 seconds, and 72°C for 90 seconds and yielded a single band corresponding to a 565-bp cDNA fragment. The COX-2 reactions were cycled 35 times at 96°C for 30 seconds, 60°C for 60 seconds, and 72°C for 90 seconds and yielded a single band corresponding to a 381-bp cDNA fragment. The GAPDH reactions were cycled under the same conditions as iNOS or COX-2 and produced a single band corresponding to a 565-bp cDNA fragment. RNA extracted from spleen of rats treated with LPS (10 mg/kg IP) was used as a positive control expression of iNOS or COX-2 mRNA. Twenty microliters of the RT-PCR reactions was electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining under UV light. The ratios of the intensities of iNOS or COX-2 to GAPDH bands were assessed by a fluororager (Vistra) and normalized with the intensity of GAPDH band, as previously reported. Cloning and Sequencing of Rat Brain iNOS and COX-2 PCR Products

The specificity of the RT-PCR reactions was verified by cloning and sequencing the 385-bp band amplified by the COX-2 primers and the
Expression of iNOS and COX-2 Proteins
(Western Blot Analysis)

After careful removal of the pial vessels, the cerebral cortex was homogenized and centrifuged at 3000g for 5 minutes and 9000g for 5 minutes at 4°C. The concentration of protein was determined with the use of the Bio-Rad Protein Assay system (Bio-Rad Laboratories). An aliquot of protein (20 μg) was separated by 7.5% sodium dodecyl sulfate–polyacrylamide gel (150 V for 100 minutes) and transferred to a nitrocellulose membrane (100 V for 60 minutes). After transfer, nonspecific binding was blocked by incubation in 10% nonfat dry milk in Tris-buffered saline solutions (50 mmol/L Tris HCl, 0.25 mol/L NaCl, 0.08% Tween 20, Sigma) followed by a 2-hour incubation at room temperature with monoclonal antibody for iNOS (1:2000 dilution, Transduction Laboratories) or polyclonal antibody for COX-2 (1:1000 dilution, Cayman Chemical Corp). The antibody for iNOS cross-reacts with nNOS.24 The bands at molecular weights of 131 and 155 kd correspond to iNOS and nNOS, respectively. The membranes were incubated with a 1:1000 horseradish peroxidase–labeled secondary antibody (Bio-Rad). Immunoblots were detected by chemiluminescence (ECL, Amersham) on x-ray film, and optical density was scanned by a scanning laser densitometer (Vistra). LPS-stimulated murine macrophage lysate (Transduction Laboratories) was used as a positive control for the expression of iNOS or COX-2 protein. A monoclonal antibody raised against the structural protein β-actin (1:1000 dilution, Sigma) was used as control (30 kd) for equal loading, and the optical density ratio of iNOS and COX-2 bands to that of β-actin was used to compare steady state levels of the various proteins.

Measurement of the Brain Calcium-Independent
(iNOS Activity)

Calcium-independent (iNOS) activity was measured by the conversion of [3H]l-arginine to [3H]l-citrulline by the high-performance liquid chromatography method originally described by Carlberg.25 Cerebral cortical tissue was homogenized in 20 mmol/L HEPES buffer (pH 7.4). After the homogenate was centrifuged twice at 4°C, aliquots of homogenate (150 μg protein) were incubated with [3H]l-arginine (0.2 μCi, 20 μmol/L, Amersham) in 100 μL of 20 mmol/L HEPES calcium-free buffer containing 0.5 mmol/L EGTA, 1 mmol/L NaDAPH, 2.5 μmol/L flavin adenine dinucleotide, 1 μmol/L flavin mononucleotide, and 100 μM tetrahydrobipterin for 5 minutes at 37°C. The reactions were stopped by adding 50 μL of 20 mmol/L EDTA solution (pH 5.5) and frozen in liquid nitrogen. Proteins were separated by reverse-phase high-performance liquid chromatography on an LC-18 DB column (Supleco). Products were monitored with an on-line radioactive flow detector (A-100, Radiomatic Instruments). Results were expressed as picomoles citrulline produced per milligram protein per minute. All chemicals used in the iNOS assay except [3H]l-arginine were purchased from Sigma.

In preliminary experiments, we compared the conversion of l-arginine to l-citrulline in cerebral homogenates prepared from a control rat in the presence or absence of 0.5 mmol/L EGTA in the reaction. Addition of 0.5 mmol/L EGTA to the reactions reduced the conversion rate by 50-fold to levels that were not significantly different from the blank samples. Therefore, this concentration of EGTA included in the reactions was sufficient to completely block calcium-dependent NOS catalytic activity in control brain homogenates and allowed for the selective measurement of calcium-independent conversion.

PGE2 Levels by Enzyme Immunoassay
(Cyclooxygenase Activity)

Cyclooxygenase activity was assessed by measuring concentration of PGE2 with the use of an enzyme immunoassay (Cayman Chemical)

565-bp product amplified by the iNOS primers. PCR products were excised from the agarose gels and purified with the use of a dialysis membrane (Geno Technology). Purified PCR products were ligated into the pCRII vector (Invitrogen). Then 250 ng purified PCR product was added to a 10-μL ligation reaction containing 6 mmol/L Tris (pH 8.3), 5 mmol/L NaCl, 6 mmol/L MgCl2, 5 mmol/L dNTPs, 0.1 mg/mL BSA, 7 mmol/L β-mercaptoethanol, 0.1 mmol/mL ATP, 2 μmol/L dithiothreitol, 1 mmol/L spermidine, 30 ng vector, and 4 U of T4 DNA ligase (Invitrogen). The reactions were incubated at 14°C for 16 hours. Escherichia coli strain TOP10F (50 μL) was transformed by heat shock with 2 μL ligation reaction. The cells were placed in 1 mL or 250 μL super optimal catalyolyte medium and incubated at 37°C for 1 hour, then plated on lauria broth agar plates with 50 mg/mL ampicillin, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (0.8 mg per plate), and isopropylthio-β-D-galactoside (0.5 mg per plate). Subsequent white colonies were screened for the presence of an insert by PCR in a 50-μL reaction under conditions described above with the use of M13 forward and reverse primers (Gibco-BRL). Positive colonies were grown in 10 mL lauria broth medium with 50 μg/mL ampicillin overnight at 37°C. Plasmid DNA was then extracted with the use of alkaline lysis and silica-gel membrane-based purification (Qiagen), resuspended in 10 mM Tris EDTA buffer (pH 7.4), and stored at 4°C. Sequencing of plasmid DNA was performed by the dideoxy chain termination method with the use of an ABI model 377 sequencer.

Figure 1. A, Effects of LPS (0.01 to 10 mg/kg) on CBF. Dose-dependent, progressive increases in rCBF are seen 1 to 4 hours after LPS (0.01 to 1 mg/kg). The dose of 10 mg/kg LPS does not follow dose dependency because of the fall in MAP. *P<0.05 vs baseline (0 hour) values; †P<0.05 vs lower dose of LPS. B, Effects of LPS (0.01 to 10 mg/kg) on rCVC. Dose-dependent, progressive increases in rCVC are seen 1 to 4 hours after LPS (0.01 to 10 mg/kg). †P<0.05 vs baseline (0 hour) values; †P<0.05 vs lower dose of LPS.
Previously described by Pradelles et al.26 Cerebral cortical tissue was homogenized in HEPES buffer. Aliquots of homogenates (150 µg protein) were incubated with tracer (PGE2: acetylcholinesterase conjugate) and PGE2 monoclonal antibody in a 96-well microtiter plate precoated goat anti-mouse antibody. The plate was developed with Ellman’s reagent containing the substrate to acetylcholinesterase, and the amount of the product of this reaction was detected by plate reader (Micro reader, Bio-Tech) at 410 nm. The concentration of PGE2 in each sample was then calculated according to standard curve generated with various concentrations of PGE2 standards (1 pg/mL to 10 ng/mL).

**Data Acquisition and Statistical Analysis**

Baseline resting rCBF was taken as the average of a 15-minute control period before administration of LPS. The rCBF over time after intracerebroventricular injection was expressed as percent change from this baseline value. rCVC was estimated by dividing the amount of LPS-treated brain RNA added to the PCR reaction over the range of 0.25 to 2 mg. The specificity of the RT-PCR reactions was verified by cloning and sequencing the 385-bp product amplified by the COX-2 primers and the 565-bp product amplified by the iNOS primers. RT-PCR of RNA extracted from the brain of an LPS-treated rat yielded single bands of the expected sizes of 565, 381, and 970 bp when amplified with the iNOS, COX-2, and GAPDH primers. The iNOS and COX-2 products were sequenced according to the fluorescent dideoxynucleotide method in both directions. The results of these experiments indicate that the products exhibited 100% homology with published sequences.

The time courses of changes in MAP, heart rate, arterial pH, PO2, and PCO2 in five experimental groups are presented in the Table (data from LPS group treated at 1 mg/kg are shown). There were no significant differences in baseline values (0 hours) among the treatment groups. MAP did not change significantly over the course of the study in groups 1, 3, 4, and 5, while a slight increase in MAP was seen in group 2 (LPS-treated rats) 4 hours after administration of LPS. Heart rate increased in groups 2 to 5 at 2 and 4 hours after administration of LPS compared with the respective values observed in the control group (group 1). Arterial pH, PO2, and PCO2 did not change significantly in any of the groups during the 4-hour experiments. Baseline values of rCBF and rCVC were similar in each treatment group.

The specificity of the RT-PCR reactions was verified by cloning and sequencing the 385-bp product amplified by the COX-2 primers and the 565-bp product amplified by the iNOS primers. RT-PCR of RNA extracted from the brain of an LPS-treated rat yielded single bands of the expected sizes of 565, 381, and 970 bp when amplified with the iNOS, COX-2, and GAPDH primers. The iNOS and COX-2 products were cloned into a PCRII vector (Invitrogen) and sequenced according to the fluorescent dideoxynucleotide method in both directions. The results of these experiments indicate that the products exhibited 100% homology with published sequences.

The results of the experiments to verify that the PCR reactions were linear under the present experimental conditions are presented in Figure 2. We found that there was a linear relationship between the fluorescent intensity of the PCR products for iNOS, COX-2, and GAPDH and the amount of LPS-treated brain RNA added to the PCR reactions over the range of 0.25 to 2 µg.

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**Table: Time Courses of MAP, Heart Rate, Arterial pH, and Blood Gases in Five Experimental Groups of Rats**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>ACSF (n=5)</th>
<th>LPS (n=8)</th>
<th>LPS+AG (n=6)</th>
<th>LPD+NS-398 (n=5)</th>
<th>LPD+DX (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>0 h 101±5</td>
<td>105±7</td>
<td>104±12</td>
<td>103±7</td>
<td>113±5</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>0 h 366±18</td>
<td>367±17</td>
<td>378±16</td>
<td>375±20</td>
<td>375±14</td>
</tr>
<tr>
<td>pH</td>
<td>0 h 7.40±0.02</td>
<td>7.37±0.05</td>
<td>7.38±0.02</td>
<td>7.42±0.03</td>
<td>7.44±0.02</td>
</tr>
<tr>
<td>PaCO2, mm Hg</td>
<td>0 h 36±3</td>
<td>34±3</td>
<td>33±4</td>
<td>37±4</td>
<td>34±2</td>
</tr>
<tr>
<td>Pao2, mm Hg</td>
<td>0 h 154±6</td>
<td>148±6</td>
<td>130±8</td>
<td>137±17</td>
<td>143±15</td>
</tr>
</tbody>
</table>

AG indicates aminoguanidine; DX, dexamethasone. Values are mean±SD.

*P<0.05 vs ACSF-treated rats.
A photograph of representative gels comparing the RT-PCR products for iNOS, COX-2 cDNA, and GAPDH when 1 mg of RNA from LPS- and vehicle-treated brains were amplified is presented in Figure 3. The intensity of bands corresponding to iNOS or COX-2 in cerebral cortices increased after intracerebroventricular administration of LPS compared with the degree of amplification seen when RNA was extracted from the brains of ACSF-treated control rats, whereas the intensity of GAPDH bands was not significantly different. A summary of the relative levels of iNOS and COX-2 mRNA is presented in Figure 4. In ACSF-treated control rats, the intensity ratio of the iNOS/GAPDH and COX-2/GAPDH bands increased to 0.15±0.01 and 0.67±0.2, respectively. These intensities of iNOS and COX-2 bands increased further 4 hours after administration of LPS compared with levels seen in control animals. Moreover, as expected, induction of iNOS and COX-2 mRNA was significantly attenuated in the animals treated by dexamethasone (Figure 4).

The effect of LPS on the levels of iNOS and COX-2 protein in the brain of rats is presented in Figure 5. After administration of LPS, the levels of iNOS protein significantly increased threefold from 0.04±0.01 versus 0.11±0.03 (P<0.05), while COX-2 protein level expression were not
significantly different between the LPS-treated and the ACSF-treated groups (0.25 ± 0.04 versus 0.33 ± 0.1; *P < 0.1).

As shown in Figure 6A, the calcium-independent iNOS activity in the brain was 11 times greater in rats treated with LPS than the levels seen in the brains of ACSF-treated control rats. Both the iNOS selective inhibitor aminoguanidine and dexamethasone attenuated the increase in calcium-independent iNOS activity in LPS-treated rats to levels that were not significantly different from those seen in the ACSF-treated control group. Aminoguanidine had no effect on constitutive NOS activity (measured as the difference in NOS activity in the presence and absence of calcium; n=3; data not shown). Concentrations of PGE₂ in the cerebral cortical tissue samples were significantly higher (by 21 ± 5%) in the LPS-treated rats than levels seen in the ACSF-treated control group. Both NS-398 and dexamethasone abolished the increase in PGE₂ levels in the brain of LPS-treated rats (Figure 6B).

Figure 7A summarizes the effects of aminoguanidine, NS-398, and dexamethasone on LPS-induced cerebrocortical hyperemia compared with the effects of ACSF or LPS alone. The LPS-induced increases in rCBF were approximately 50% smaller in the rats treated with either aminoguanidine or NS-398 and were completely eliminated in the rats treated with dexamethasone. Similar effects of these inhibitors were observed when rCVC was used to represent the cerebrovascular effects of LPS (Figure 7B).

**Discussion**

In the present study we demonstrated that intracerebroventricular administration of LPS produces a progressive and dose-dependent increase in rCBF. This is associated with increases in the levels of iNOS and COX-2 mRNA, iNOS protein, iNOS activity, and PGE₂ levels in the cerebral cortex of the rat. These effects were attenuated by the administration of either aminoguanidine or NS-398 and were completely abolished by pretreating the rats with dexamethasone. These findings suggest that the induction of both iNOS and cyclooxygenase activity and the subsequent increase in NO and cyclooxygenase metabolites of arachidonic acid contribute to the cerebral hyperemia produced by LPS.

The systemic physiological data presented in the Table demonstrate cardiovascular stability of the preparation achieved by using the intracerebroventricular endotoxin injection protocol at the dose of 1 mg/kg. The data also indicate that ventilation of the animals was well controlled, and there was no difference in blood pressure, Po₂, pH, and PcO₂ among the experimental groups. These results suggest that the cerebral hyperemia after intracerebroventricular administration of LPS was restricted to the brain and was not secondary to systemic effects.

The approximately twofold increase in CBF seen 4 hours after LPS treatment under normoxic, normocapnic conditions is clearly outside the normal physiological values. From a pathophysiological point of view, such an increase in CBF would be expected to produce elevations in microvascular and intracranial pressures and increases in cerebrovascular permeability and intraparenchymal edema that would contribute to brain injury.
To date, three isoforms of NOS have been identified, i.e., neuronal NOS (nNOS or type I NOS), inducible NOS (iNOS or type II NOS), and endothelial NOS (eNOS or type III NOS), in the brain of rats. Among these isoforms, nNOS and eNOS are constitutively expressed. They produce NO in response to elevations of intracellular calcium concentration and mediate signal transduction in various organ systems. These enzymes play an important role in the maintenance of CBF. In contrast, iNOS is induced by inflammatory stimuli such as bacterial endotoxin, interferon-gamma, UV light, and brain ischemia. Subsequently, a large amount of NO can be produced from iNOS in many cell types, including macrophages, vascular smooth muscle and endothelial cells, astrocytes, microglia, and neurons. The overproduction of NO from iNOS has been thought to contribute to the pathogenesis of septic shock, host-defense response, cytotoxicity, and ischemia/reperfusion injury.

The bacterial endotoxin LPS is among the most important and well-documented stimuli for the induction of iNOS. It has been reported that LPS dilates rabbit cerebral arterioles after direct application through a cranial window. Since this vasodilation was accompanied by an increase in cGMP production and was attenuated by dexamethasone or amino- guanidine, these results suggested that LPS may have induced iNOS to dilate the cerebral vasculature. However, direct biochemical or molecular evidence that LPS actually increased the expression of iNOS in the brain after intracerebroventricular administration of LPS has yet to be provided in any study. We therefore designed and performed experiments to test this hypothesis and found that the levels of iNOS...
mRNA and protein and calcium-independent NOS activity increased markedly after intracerebroventricular administration of LPS in the cerebral cortex. The time course of the changes in iNOS protein and enzyme activity correlated well with the changes in CBF. Moreover, we demonstrated that dexamethasone completely attenuated the increases in iNOS protein, iNOS, mRNA, and CBF. Similar effects were seen after administration of the iNOS selective inhibitor amino-guanidine, which blocked the increases in iNOS activity and the cerebral hyperemic response to LPS. These findings provide direct biochemical and molecular evidence to support the hypothesis that induction of iNOS expression and activity contributes to LPS-induced cerebral hyperemia after intracerebroventricular administration.

The present data showing enhanced levels of iNOS protein and mRNA in the cerebral cortex after intracerebroventricular administration of LPS contrast with previous reports that were unable to document increased levels of iNOS protein or RNA in the brain when given by an intravenous or intraperitoneal route. The difference is likely due to the limited ability of LPS to cross the blood-brain barrier. Recently, Minc-Golomb et al reported that direct injection of LPS into the cerebellum could increase iNOS mRNA or protein expression in cerebellar neurons. Therefore, it appears that LPS can increase iNOS levels when it is directly applied to the brain. The cellular mechanisms of cerebral hyperemia and the identity of vascular and/or parenchymal cells in the brain that increase iNOS expression after administration of LPS remain to be elucidated. The clinical significance of this experimental animal model is that direct administration of LPS could be used to investigate the mechanisms underlying changes in CBF during inflammation caused by bacterial meningitis, encephalitis, or ischemic injury.

In the present study iNOS mRNA was induced 2 hours after LPS. Such an early induction of iNOS seen in the present study is consistent with the recent findings of Bateson et al, who reported increased levels of iNOS mRNA in the heart as little as 30 minutes after systemic administration of LPS. This rapid induction of iNOS may explain the increase in rCBF in the early phase (1 to 2 hours) after the administration of LPS in our study. However, it has also been reported that activation of the production of NO and/or peroxynitrite by eNOS may also play role in the initial hyperemic response. Further studies will be necessary to clarify the mechanism of the initial rise in rCBF after administration of endotoxin. Nevertheless, it is clear from our findings that induction of iNOS does contribute importantly to the rise in rCBF seen 2 to 4 hours after administration of LPS.

Prostaglandins have also been reported to play a role in the regulation of CBF. During endotoxemia, an increased production of prostaglandins has been suggested to contribute to pathophysiological changes in brain, i.e., fever, neuroendocrine changes, and cerebral hyperemia. Recently, inducible isoforms of prostaglandin synthase (PGS-2) or cyclooxygenase (COX-2) have been identified, and expression of COX-2 mRNA and/or protein has been reported in many cell types, including fibroblasts, macrophages, endothelial and smooth muscle cells, heart, astrocytes, and neurons after induction by LPS. We hypothesized that in addition to iNOS, COX-2 might play a role in LPS-induced cerebral hyperemia. In the present study we demonstrated that the levels of COX-2 mRNA and PGE2 levels do increase in the brain increase after administration of LPS and that the rise in CBF was attenuated by NS-398. NS-398 has been reported to selectively reduce COX-2 (inducible) activity without affecting COX-1 (constitutive) activity at doses comparable to those used in our study. COX-2 catalyzes the formation of prostaglandins, thromboxanes, and prostacyclin, and PGE2 has been reported to be the major cyclooxygenase metabolite produced in the cerebral cortex. Therefore, we measured changes in cerebral cortical PGE2 levels as an index of total cyclooxygenase activity and found that they increased after administration of LPS. The rise in PGE2 levels was blocked by NS-398 or dexamethasone. These findings suggest that induction of COX-2 and increases in the production of vasodilator prostanooids may also contribute in LPS-induced cerebral hyperemia.

Another interesting finding was the high level of expression of COX-2 protein in the brains of untreated rats. Although our finding of the constitutively expressed COX-2 in the brain is consistent with other reports, the physiological significance of constitutively expressed COX-2 in the brain remains to be determined. Since COX-2 has been reported to contribute to seizure-induced changes in synaptic activity, constitutively expressed COX-2 may also have some role in the regulation of synaptic signal transduction under certain physiological conditions. Further studies will be necessary to clarify the role of COX-2 in the regulation of CBF.

A possible cross-talk between the NO and prostaglandin systems was outside the scope of the present study, and therefore our results do not explain why COX-2 protein was not significantly altered while COX-2 activity was increased. Both stimulatory and inhibitory effects of NO on cyclooxygenase activity have been reported. We speculate that the high levels of NO after administration of LPS may have decreased COX-2 protein levels, perhaps by nitrosylating the enzyme and increasing protein degradation. Thus, the enhanced expression of COX-2 mRNA was uncoupled from the levels of COX-2 protein after LPS treatment.

The present results do not exclude the possibility that other vasodilator mediators may also contribute to the cerebrovasodilatory response to LPS. For example, calcitonin gene–related peptide is known to be a potent dilator of cerebral blood vessels. It has been reported to contribute to endotoxin-induced cerebrovasodilation and interact with NO. Non–cyclooxygenase-derived eicosanoids, eg, cytochrome P-450–derived epoxygenes, have also been reported to play a role in maintaining CBF, and the production of these eicosanoids is known to be inhibited by NO. However, the question of to what extent these other mediators are involved in the LPS-induced cerebral hyperemia and to what extent they influence the iNOS and COX-2 pathways remains to be addressed in future studies.

In summary, we have demonstrated that intracerebroventricular administration of LPS increases the levels of iNOS and COX-2 mRNA, iNOS protein and enzyme activity, and PGE2 levels in the cerebral cortex of rats and that inhibitors of COX-2 and iNOS attenuate the increase in CBF produced by LPS. Our findings suggest that enhanced expression of both...
iNOS and COX-2 followed by a rise in the production of NO and vasodilator prostanooids contribute to endotoxin-induced cerebral hyperemia.

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1218  iNOS and COX-2 in LPS-Induced Hyperemia

The inflammatory process is a very complicated cascade designed (a1) to defend against tissue injury and infection, (b2) to rid the body of injured or damaged tissues, and (c3) to subsequently regenerate the injured tissues.1-3 The process consists of two components: one is “tearing down” or destructive, and the other is “rebuilding” or regenerating. In an inflamed area, the destructive component often does not discriminate between invading pathogens, damaged cells, or healthy cells. In organs like the brain, the process can be particularly destructive since neurons may not regenerate in an orderly network of synaptic connections required for normal functioning. The inflammatory process in brain can be activated by such conditions as infections (bacterial endotoxin), damage produced by stroke or traumatic injury, and other pathological states.1–3 Okamoto and colleagues have significantly added to our understanding of the inflammatory process in brain and have shown that pharmacological inhibition of the function or expression of these enzymes can reduce the hyperperfusion after endotoxin administration. This study is an important step toward the ultimate goal of therapeutically controlling the inflammatory process in the human during pathological states in brain.

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References
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