Cyclo-Oxygenase-2 Gene Expression in Neurons Contributes to Ischemic Brain Damage

Shigeru Nogawa, Fangyi Zhang, M. Elizabeth Ross, and Costantino Iadecola

Laboratory of Cerebrovascular Biology and Stroke, Department of Neurology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Cyclo-oxygenase-2 (COX-2), a rate-limiting enzyme for prostanoid synthesis, is induced during inflammation and participates in inflammation-mediated cytotoxicity. Cerebral ischemia is followed by an inflammatory reaction that plays a role in the evolution of the tissue damage. We studied whether COX-2 is induced after cerebral ischemia and if so, whether such expression contributes to ischemic brain damage. The middle cerebral artery was occluded in rats, and the ischemic area was sampled for analysis 3–96 hr later. COX-2 mRNA was determined by the competitive reverse-transcription PCR. COX-2 mRNA was upregulated in the ischemic hemisphere, but not contralaterally, beginning 6 hr after ischemia. The upregulation reached a maximum at 12 hr, at which time a fivefold induction of the message occurred. Twenty-four hours after ischemia, the concentration of prostaglandin E₂ was elevated in the injured brain by 292 ± 57% (n = 6). COX-2 immunoreactivity was observed in neurons at the medial edge of the ischemic area. Administration of the COX-2 inhibitor NS-398 attenuated the elevation in prostaglandin E₂ in the posts ischemic brain and reduced the volume of the infarct by 29 ± 6% (p < 0.05). Thus, cerebral ischemia leads to upregulation of COX-2 message, protein, and reaction products in the injured hemisphere. The data implicate COX-2 in the mechanisms of delayed neuronal death at the infarct border and provide the rationale for neuroprotective strategies employing COX-2 inhibitors.

Key words: stroke; prostanoids; prostaglandin H₂ synthase; gene expression; NS-398; reverse-transcription polymerase chain reaction; iNOS; inflammation

There is increasing evidence that the brain damage produced by cerebral ischemia develops over a period longer than previously believed. In the center of the ischemic territory, where the flow reduction is most severe, energy failure is followed by rapid cell death. However, in the surrounding region, neurons remain viable for a prolonged period of time, perhaps days (Derenski et al., 1993; Garcia et al., 1993; Marchal et al., 1996). Most research efforts to date have focused on the acute stages of cerebral ischemia (Choi, 1994; Chan, 1996), and less emphasis has been placed on the factors that contribute to the delayed progression of the injury occurring at the periphery of the infarct. The identification of such factors is important, because it might suggest new therapeutic strategies targeted at the late phase of the damage.

One of the processes that may play a role in the delayed progression of the damage is posts ischemic inflammation (Kochanek and Hallenbeck, 1992; Feuerstein et al., 1997). Cerebral ischemia is followed by infiltration of blood-borne neutrophils in the ischemic brain, a process initiated by local expression of cytokines, chemokines, and adhesion molecules (Pozzilli et al., 1985; Liu et al., 1994; Wang et al., 1994, 1995; Kim et al., 1995; for review, see Feuerstein et al., 1997). Although there is evidence that posts ischemic inflammation is deleterious to the ischemic brain, the mechanisms of its pathogenic effect have not been clearly defined (Kochanek and Hallenbeck, 1992). Expression of cyclo-oxygenase-2 (COX-2) recently has emerged as an important determinant of the cytotoxicity associated with inflammation (for review, see Seibert et al., 1995; Smith and DeWitt, 1995). COX, also known as prostaglandin H₂ synthase, is a rate-limiting enzyme for prostanoid synthesis that is present in at least two isozymes: COX-1 and COX-2 (Smith and DeWitt, 1995). COX-1 is constitutively expressed in many cell types in which it produces prostanoids that subserve normal physiological functions (Smith and DeWitt, 1995). Although COX-2 normally is not present in most cells, its expression can be induced by endotoxins and cytokines (Smith and DeWitt, 1995). COX-2 is rapidly induced in inflamed tissues, and its reaction products are responsible for many of the cytotoxic effects of inflammation (Seibert et al., 1995).

In this study, we investigated whether cerebral ischemia is associated with upregulation of the COX-2 gene and if so, we sought to define whether COX-2 expression contributes to cerebral ischemic damage. We found that focal cerebral ischemia upregulates COX-2 in neurons at the periphery of the infarct and that a COX-2 inhibitor attenuates posts ischemic prostaglandin accumulation and reduces cerebral ischemic damage. The data provide strong evidence that COX-2 is implicated in the mechanisms of delayed neuronal death at the infarct border and suggest new neuroprotective strategies targeted at the progression of ischemic brain damage.

MATERIALS AND METHODS

Procedures for transient middle cerebral artery (MCA) occlusion. The MCA was transiently occluded in 80 Sprague Dawley rats (300–400 gm; Harlan, Indianapolis, IN) using an intravascular occlusion model (Zea Longa et al., 1989) that has been previously described in detail (Iadecola et al., 1996; Zhang et al., 1996). Under halothane anesthesia (induction, 5%; maintenance, 1%), a 4–0 nylon monofilament with a rounded tip was
inserted centripetally into the external carotid artery and advanced into the internal carotid artery until it reached the circle of Willis. Throughout the procedure, body temperature was maintained at 37 ± 0.5°C by a thermostatically controlled lamp. Two hours after induction of ischemia, rats were reanesthetized, and the filament was withdrawn (Zhang et al., 1996). Animals were then returned to their cages and closely monitored until recovery from anesthesia. In sham-operated rats, the external carotid artery was surgically prepared for insertion of the filament, but the filament was not inserted (Zhang et al., 1996). Rats were killed at different time points after transient ischemia for mRNA determination, measurement of prostaglandin E2 (PGE2), and immunocytochemistry (see below).

**Reverse-transcription PCR (RT-PCR).** mRNA for COX-1, COX-2, and inducible nitric oxide synthase (iNOS) were detected by the RT-PCR method (Kawasaki et al., 1988) as described previously (Iadecola et al., 1995; Ross and Iadecola, 1994). Animals were killed 3, 6, 12, 24, 48, and 96 hr after ischemia (n = 4 per time point) and their brains removed. Sham-operated rats served as controls (n = 4). A 4-mm-thick coronal brain slice was cut at the level of the optic chiasm, and the infarcted cortex was dissected using the corpus callosum as a ventral landmark. The corresponding region of the contralateral cortex was also sampled. Total RNA was extracted from the samples according to the method of Chomczynski and Sacchi, and reverse-transcribed to cDNA using random hexamers and Moloney murine leukemia virus reverse transcriptase (New England Biolabs) according to the manufacturer’s instructions. After heating at 95°C for 10 min, 5 µl each of the RT product was used in the PCR in 0.05 µl of oligo (dT) primer as directed (18 mer; New England Biolabs, Beverly, MA). First-strand cDNA synthesis was then performed using 0.25 µg of total RNA and M-MuLV reverse transcriptase (New England Biolabs) according to the manufacturer’s instructions.

For data analysis, the ethidium-bromide and photographed (see Fig. 2B). 

**Effect of NS-398 on ischemic brain damage and PGE2 elevation.** Under halothane anesthesia, the femoral artery was cannulated, and rats were placed on a stereotaxic frame. The arterial catheter was used for monitoring arterial pressure and other parameters at different times after MCA occlusion (see below). The MCA was occluded for 2 hr, as described above, and treatments were begun 6 hr after induction of ischemia. In one group of rats (n = 6), the COX-2 inhibitor NS-398 (Putaki et al., 1993a; Masferrer et al., 1994) was administered. NS-398 was dissolved in 1 ml of 10% ethanol and 90% saline intraperitoneally (4 mg/kg) and extracted with 100% methanol (Powell, 1982). After centrifugation, the supernatant was diluted with acidified 0.1 m phosphate buffer, pH 4, (final methanol concentration, 15%) and applied to activated ODS-silica reverse-phase columns (Sep-Pak C18, Waters Associates, Milford, MA). The columns were rinsed with 5 ml of distilled water followed by 5 ml of 1% acetic acid, 5 ml of CH3CN, and 5 ml of CH2Cl2. The extract was concentrated to 2 ml containing 2% acetic acid. The ethyl acetate fraction was evaporated and resuspended in 1 ml of buffer. The recovery rate of this extraction procedure, determined using [14C]PGE2, was 77 ± 2.1% (n = 10). PGE2 concentration was determined spectrophotometrically after incubation with tracer and PGE2 monoclonal antibody in a microplate according to the manufacturer’s instructions.

For data analysis, the ethidium-bromide and photographed (see Fig. 2B).
isons were evaluated by the paired or unpaired $t$ test, as appropriate. Multiple comparisons were analyzed by the ANOVA and Tukey’s test. Differences were considered statistically significant for $p < 0.05$.

RESULTS

Cerebral ischemia and COX-2 mRNA expression

In agreement with previous reports (Yamagata et al., 1993), low levels of COX-2 PCR product were observed in the brain of sham-operated rats ($n = 4$; Figs. 1A, 2C). After transient MCA occlusion, a marked upregulation of COX-2 mRNA was observed in the postischemic brain, but not contralaterally (Fig. 1A). The upregulation began 6 hr after ischemia, reached a maximum at 12–24 hr, and subsided at 48 hr (Fig. 1A). In contrast, COX-1 remained unchanged in the postischemic period (Fig. 1B). To determine more accurately the magnitude of the mRNA upregu-
In sham-operated rats, low levels of COX-2 signal were detected on both sides of the brain. Twelve hours after ischemia, COX-2 was upregulated in the injured brain by fivefold (Fig. 2). Therefore, cerebral ischemia results in upregulation of COX-2 message in the postischemic brain.

Cerebral ischemia also leads to iNOS expression in the injured brain (Iadecola et al., 1995, 1996). Because iNOS may be co-expressed with COX-2 (Salvemini et al., 1993; Vane et al., 1994), we compared the temporal profile of COX-2 and iNOS mRNA upregulation after ischemia. As illustrated in Figure 1C, the time courses of COX-2 and iNOS mRNA expression were similar. Therefore, iNOS and COX-2 are expressed over the same time period after cerebral ischemia.

### Cerebral Ischemia and COX-2 Immunocytochemistry

We then used immunocytochemistry to determine whether upregulation of COX-2 mRNA resulted in increased synthesis of the COX-2 protein and to identify the cells in which COX-2 is upregulated. In the brain of sham-operated rats, COX-2-immunoreactive neurons were observed sparsely in cerebral cortex, hippocampus, piriform cortex, and amygdala (Yamagata et al., 1993; Breder et al., 1995). Cerebral ischemia produced a marked upregulation of COX-2 immunoreactivity, which was first observed 6 hr after MCA occlusion. The most marked upregulation occurred 12–24 hr after ischemia. At this time, numerous COX-2-positive cells were located in the transitional region between normal and infarcted brain. These cells have an angular appearance, with a shrunken cytoplasm and nucleus. In alternate sections stained with hematoxylin and eosin, these cells correspond to neurons exhibiting distinct ischemic changes (“red neurons”). Therefore, COX-2 is expressed also in injured neurons at the periphery of the ischemic territory. The region in which these COX-2 neurons are located corresponds to the so-called ischemic penumbra. The positive cells observed within the infarct are most likely shrunken neurons. (P. Anatomical location of the ischemic penumbra; i, infarct. D. High-power view of the COX-2-immunoreactive neurons depicted in C. Notice the cell shrinkage. Scale bars: A, C, 500 μm; B, D, 150 μm.)
nucleus (Fig. 3D). An increased number of COX-2-positive neurons were observed in the ipsilateral piriform cortex (Fig. 4). COX-2-immunoreactive cells were not increased in the contralateral cerebral cortex (Fig. 3A). COX-2 immunoreactivity returned to baseline 4 d after ischemia.

**PGE2 concentration in the postischemic brain**

To determine whether the upregulation of COX-2 protein corresponded to an increase in COX-2 enzymatic activity, the concentration of PGE2 was measured in the postischemic brain 24 hr after induction of ischemia. Cerebral ischemia increased PGE2 concentration in the injured brain by 292 ± 57% (p < 0.05; n = 6; Fig. 5A). No increase was observed in the contralateral cortex or in the cortex of sham-operated rats (p > 0.05; n = 6; Fig. 5A). These data suggest that COX-2 enzymatic activity is increased in the postischemic brain.

**Effect of COX-2 inhibition on cerebral ischemic damage**

To determine whether COX-2 expression contributes to cerebral ischemic damage, we used the relatively selective COX-2 inhibitor NS-398 (Futaki et al., 1993a; Masferrer et al., 1994). Treatment with NS-398 (20 mg/kg, i.p., 2 times per day for 3 d) did not affect arterial pressure, rectal temperature, plasma glucose, arterial blood gases, and hematocrit (Fig. 6; Table 1). However, in rats treated with NS-398, the size of the infarct was smaller than that of vehicle-treated controls (Figs. 5C, 7). The reduction averaged 29 ± 6% in the cerebral cortex (Fig. 5C; p < 0.05). The area spared from infarction was located at the periphery of the ischemic lesion and involved the border zone between the vascular territories of the anterior cerebral and MCAs (Fig. 7). Infarct size was not reduced in the striatum (Fig. 3C).

To determine whether treatment with NS-398 was effective in reducing COX-2 activity in the postischemic brain, PGE2 concentration was measured 24 hr after transient MCA occlusion in rats treated with NS-398 (n = 6) or vehicle (n = 7). NS-398 attenuated the postischemic increases in PGE2 (p < 0.05 from vehicle; ANOVA and Tukey’s test) (Fig. 5B). After NS-398 treatment, the PGE2 concentration in the injured brain was not statistically different from that in the contralateral (intact) side (p > 0.05). NS-398 slightly reduced resting levels of PGE2 in the cerebral cortex contralateral to the stroke (Fig. 5B). However, such reduction did not reach statistical significance (p > 0.05). These data suggest that NS-398 inhibits postischemic COX-2 activity and ameliorates cerebral ischemic damage.

**DISCUSSION**

The development of ischemic cell death is asynchronous in the different regions of the ischemic territory (Derski et al., 1993). Whereas in the center of the lesion, severe ischemia leads to rapid pan-necrosis, in the surrounding regions, the tissue damage evolves slowly over many hours (Derski et al., 1993; Garcia et al., 1993; Marchal et al., 1996). The factors involved in the secondary progression of the injury at the infarct border have not been fully elucidated (Kochanek and Hallenbeck, 1992; Feuerstein et al., 1997). After cerebral ischemia, there is infiltration of the affected brain by inflammatory cells, a process initiated by expression of inflammation-related genes in the postischemic brain (for review, see Feuerstein et al., 1997). Although there is evidence that such inflammation contributes to the progression of cerebral ischemic damage, the mechanisms of the effect remain unclear (Kochanek and Hallenbeck, 1992; Feuerstein et al., 1997). The prostanoid-synthesizing enzyme COX-2 recently has emerged as an important factor in the cytotoxicity associated with inflammation (Seibert et al., 1995). Therefore, in this study, we investigated whether COX-2 is expressed in the postischemic brain and if so, whether its reaction products contribute to the secondary evolution of the damage. We found that focal cerebral ischemia is associated with marked upregulation of COX-2 mRNA in the affected hemisphere starting between 3 and 6 hr after cerebral ischemia. This finding is in agreement with a recent report in which COX-2 mRNA was detected by Northern analysis after transient focal ischemia (Collaco-Moraes et al., 1996). The upregulation is restricted to COX-2 and does not involve the closely related prostaglandin-synthesizing enzyme COX-1. Immunocytochemical experiments showed that COX-2 protein is also upregulated, the expression occurring in neurons located primarily at the infarct border. Some COX-2-positive neurons are devoid of pathological changes and are located in the intact brain. Other COX-2 neurons exhibit ischemic changes and reside in the transitional region between normal and infarcted brain. We did not observe cells the morphology of which is consistent with that of neutrophils, the inflammatory cell present at this time after ischemia. However, this evidence is far from conclusive, and the potential localization of COX-2 to other cell types will have to be explored further in future studies. The COX-2 message and protein upregulation is associated with increased tissue concentration of PGE2, one of the COX-2 reaction products. These observations indicate that COX-2 mRNA is translated in a functional enzyme. Thus, cere-

---

### Table 1. Arterial blood gases and hematocrit in rats treated with NS-398 after focal cerebral ischemia

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Treatment</th>
<th>pH</th>
<th>pCO2 (mmHg)</th>
<th>pO2 (mmHg)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Vehicle</td>
<td>7.35 ± 0.02</td>
<td>41 ± 1</td>
<td>86 ± 4</td>
<td>49.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>NS-398</td>
<td>7.35 ± 0.03</td>
<td>40 ± 1</td>
<td>83 ± 5</td>
<td>49.2 ± 0.5</td>
</tr>
<tr>
<td>24</td>
<td>Vehicle</td>
<td>7.39 ± 0.03</td>
<td>39 ± 2</td>
<td>99 ± 4</td>
<td>48.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>NS-398</td>
<td>7.39 ± 0.03</td>
<td>40 ± 1</td>
<td>104 ± 5</td>
<td>48.5 ± 0.4</td>
</tr>
<tr>
<td>48</td>
<td>Vehicle</td>
<td>7.37 ± 0.07</td>
<td>39 ± 3</td>
<td>90 ± 8</td>
<td>48.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>NS-398</td>
<td>7.33 ± 0.04</td>
<td>39 ± 1</td>
<td>86 ± 3</td>
<td>48.5 ± 0.6</td>
</tr>
<tr>
<td>72</td>
<td>Vehicle</td>
<td>7.34 ± 0.05</td>
<td>41 ± 2</td>
<td>88 ± 7</td>
<td>48.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>NS-398</td>
<td>7.31 ± 0.05</td>
<td>44 ± 2</td>
<td>90 ± 4</td>
<td>48.0 ± 0.7</td>
</tr>
</tbody>
</table>

No statistically significant differences were found between rats treated with vehicle or NS-398 (p > 0.05, t test).
We then sought to determine whether the COX-2 upregulation, through reaction products of the COX-2 enzymatic pathway, contributes to the progression of cerebral ischemic damage occurring in the postischemic period. Although COX reaction products have long been implicated in the mechanisms of ischemic stroke (for review, see Hsu et al., 1989), previous studies focused on the effects of prostanoids in the acute stages of cerebral ischemia. In addition, previous studies have reported the effect of nonselective COX inhibitors, e.g., indomethacin and ibuprofen, on cerebral ischemic damage. The results of these investigations have been contradictory; some report protection and others show no effect or worsening (Harris et al., 1982; Johshita et al., 1989; Cole et al., 1993). These conflicting observations are likely to result from the fact that the effects of COX-2 inhibition were confounded by effects of COX-1 inhibition, an enzyme involved in normal cellular function. Furthermore, indomethacin, one of the agents studied most extensively, has profound effects on cerebral blood flow and vascular reactivity (for review, see Busija and Heistad, 1984). Because indomethacin was administered before or shortly after induction of ischemia (Harris et al., 1982; Johshita et al., 1989), it is likely that the attendant cerebrovascular effects of this agent influenced the outcome of cerebral ischemia.

To avoid confounding effects resulting from COX-1 inhibition, we used NS-398, a relatively selective inhibitor of COX-2 (Futaki et al., 1993b; Masferrer et al., 1994). In vitro, this agent inhibits COX-2 1000-fold more potently than COX-1 (Reitz et al., 1994). In vivo, NS-398, at a dose similar to that used in the present study, inhibits COX-2 but not COX-1 (Futaki et al., 1993a; Masferrer et al., 1994). We found that delayed treatment of rats with NS-398 reduces the size of the infarct produced by MCA occlusion. The brain area spared from infarction includes the region in which COX-2-positive neurons are located. These results are consistent with the hypothesis that COX-2 reaction products contribute to the delayed progression of the tissue damage that occurs in the postischemic brain. To confirm that NS-398 inhibited COX-2 activity in brain, we studied the effect of NS-398 on the elevation in PGE$_2$ produced by cerebral ischemia. It was found that NS-398 markedly attenuates the postischemic elevation in PGE$_2$. This observation indicates that NS-398 is able to enter the postischemic brain and to inhibit cerebral COX activity. The alteration in blood–brain barrier permeability that follows focal cerebral ischemia (Anwar et al., 1993) is likely to facilitate the penetration of NS-398 into the ischemic region. The protection exerted by NS-398 is not attributable to effects on body temperature, arterial pressure, blood gases, plasma glucose, or hematocrit, because these parameters were monitored and did not differ between treated and untreated groups. It is also unlikely that NS-398 reduced cerebral ischemic damage by improving postischemic blood flow, because at the time when the NS-398 treatment was instituted, i.e., 6 hr after ischemia, vascular-hemodynamic factors no longer influence tissue outcome (Overgard et al., 1994; Zhang and Iadecola, 1994a). Effects of NS-398 on platelet aggregation are also unlikely, because platelets contain COX-1 and not COX-2 (Klein et al., 1994). However, additional studies are needed to better define the cerebrovascular effects of NS-398 and to characterize the dose–response and temporal relationships of its protective effect in cerebral ischemia.

The mechanisms responsible for postischemic COX-2 induction...
remain to be defined. In the normal brain, COX-2 is expressed in selected neurons (Yamagata et al., 1993; Breder et al., 1995). COX-2 is induced in granule neurons during high-frequency hippocampal stimulation, suggesting that COX-2 expression may be regulated by normal synaptic activity (Yamagata et al., 1993). In experimental seizures, COX-2 is upregulated in neurons, an effect blocked by the NMDA receptor antagonist MK801 (Yamagata et al., 1993). The latter observation suggests that COX-2 expression may be induced by activation of glutamate receptors. Because glutamate is also released in cerebral ischemia, it is conceivable that activation of glutamate receptors participates in postischemic COX-2 induction. However, considering that glutamate is released in the penumbra only within the first 2 hr after MCA occlusion (Takagi et al., 1993), activation of glutamate receptors is unlikely to mediate the upregulation of COX-2 observed at 12 and 24 hr after ischemia. The time course of COX-2 expression closely follows the temporal profile of inflammatory genes, including genes encoding for cytokines, adhesion molecules, and iNOS (Feuerstein et al., 1997; present study). Cytokines are well known to induce COX-2 expression in vitro (Jones et al., 1993). Therefore, cytokines could also contribute to postischemic COX-2 induction, particularly in neurons located in the ischemic territory where cytokine levels are increased (Liu et al., 1993; Wang et al., 1995). Another agent released in the ischemic brain that could also participate in COX-2 expression is the platelet-activating factor (Bazan et al., 1994). Therefore, it is likely that multiple factors are responsible for the upregulation in COX-2 observed in the postischemic period. The molecular mechanisms of COX-2 expression after ischemia are likely to involve interactions with regulatory elements in the 5' flanking region of the COX-2 gene that include NFκB and NF-IL6/C/EBP binding sequences (Sirois and Richards, 1993).

Figure 5. A, Effect of transient MCA occlusion on (PGE₂) in the post-ischemic brain. In sham-operated rats, low levels of PGE₂ are present in the brain. Cerebral ischemia increases PGE₂ concentration 24 hr after stroke only on the ischemic side (p < 0.05, t test; n = 6). B, Effect of the COX-2 inhibitor NS-398 on postischemic increase in PGE₂ in the injured brain. NS-398 (20 mg/kg, i.p.) was administered starting 6 hr after transient MCA occlusion. Rats were killed 24 hr after ischemia. At the time of death, the rats had received three doses. NS-398 attenuates the postischemic increases in PGE₂ (p < 0.05 from vehicle; ANOVA and Tukey’s test). The residual increase in PGE₂ after NS-398 did not reach statistical significance (p > 0.05). NS-398 slightly reduced resting levels of PGE₂ in the cerebral cortex contralateral to the stroke. However, such reduction did not reach statistical significance (p > 0.05). C, Effect of NS-398 on the volume of the infarct produced by transient MCA occlusion in the rat. Rats were treated for 3 d (20 mg/kg, i.p., twice per day) starting 6 hr after induction of ischemia. NS-398 reduced the volume of the infarct in the cerebral cortex but not in the striatum. The reduction in infarct volume persists after correction for ischemic swelling [Cortex (E.C.)], suggesting that the reduction in the lesion volume is not attributable to an effect of NS-398 on ischemic edema.

Figure 6. Arterial pressure, plasma glucose, and rectal temperature of the rats in which the effect of NS-398 on cerebral ischemic damage was studied (see Fig. 5C). NS-398 does not affect these parameters at any of the time points studied (p > 0.05; t test from vehicle).
Of interest is the observation that COX-2 and iNOS are induced over a similar time period after cerebral ischemia. Co-induction of iNOS and COX-2 has also been reported in other models of inflammation (Corbett et al., 1993; Salvemini et al., 1993; Vane et al., 1994). Because COX-2 is a heme-containing enzyme, its enzymatic activity is modulated by NO, a gas with high affinity for heme iron (Ignarro, 1991). In some models of inflammation, NO produced by iNOS has been shown to activate COX-2 and to increase its output of proinflammatory prostaglandins (Salvemini et al., 1993, 1995). We have demonstrated previously that iNOS induction and NO production contribute to focal cerebral ischemic damage (Iadecola et al., 1995, 1996). The finding that iNOS and COX-2 are co-induced after stroke raises the possibility that NO activates COX-2, thereby increasing the toxic output of the enzyme. Therefore, COX-2 activation could be another mechanism by which NO exerts its pathogenic effect on the ischemic brain.

Although the factors responsible for the cytotoxicity of COX-2 have not been clearly defined, it is likely that one of the mechanisms is related to production of reactive oxygen species (ROS). ROS are considered to be one of the major determinants of ischemic brain death (Chan, 1996). ROS are produced by the peroxidase step of the COX reaction in which prostaglandin G2 is converted to prostaglandin H2 (Chan and Fishman, 1980; Kontos et al., 1980; Armstead et al., 1988; Tsai et al., 1994). Cerebral ischemia results in an increase in the availability of arachidonic acid, the substrate for the COX enzymatic pathway (Chan et al., 1985). Our finding that COX-2 is markedly upregulated after cerebral ischemia suggests that the COX-2 pathway is an important route for arachidonic acid metabolism and free radical production in the postischemic brain. COX-2 enzymatic activity can also mediate tissue damage by producing proinflammatory prostaglandins (Seibert et al., 1995). A third mechanism by which COX-2 could contribute to cell death is related to induction of apoptosis. In thymocytes, the COX-2 reaction product PGE2, induces apoptosis (Juzan et al., 1992). This finding raises the possibility that COX-2 contributes to postischemic apoptosis (Li et al., 1995). However, evidence that COX-2 overexpression in intestinal cells prevents apoptosis has also been presented (Tsujii and DuBois, 1995). Additional studies are needed to define the relative contribution of these pathogenic mechanisms to ischemic cell death related to COX-2 upregulation.

The finding that the COX-2 inhibitor NS-398 reduces cerebral ischemic damage when administered 6 hr after induction of ischemia has important implications for the treatment of stroke. Most patients with ischemic stroke reach the emergency room several hours after the onset of symptoms, at a time when most experimental therapeutic interventions are no longer effective (Marshall and Mohr, 1993). Therefore, in addition to therapeutic interventions targeted to the early stages of the damage, it would be highly desirable to develop strategies aimed at the delayed phase of the injury. In this context, COX-2 inhibitors would be valuable, because they could be used to target the delayed progression of the damage. However, additional studies are required to better characterize the effect of COX-2 inhibitors on cerebral ischemic damage and to define their potential use in human stroke.

In conclusion, we have demonstrated that focal cerebral ischemia induces expression of COX-2 mRNA, protein, and reaction products in the postischemic brain. The expression occurs in neurons at the periphery of the infarct. The relatively selective COX-2 inhibitor NS-398, administered 6 hr after induction of ischemia, reduces cerebral ischemic damage at the periphery of the infarct. The findings provide evidence that COX-2 expression is deleterious to the ischemic brain. In particular, COX-2 reaction products may contribute to recruit potentially salvageable regions into infarction at the border of the ischemic territory. Inhibition of COX-2 may be a valuable therapeutic strategy targeted specifically to the delayed progression of the infarct that occurs in the postischemic period.

REFERENCES


Tsai A, Hsi LC, Kalmaz RJ, Palmer G, Smith WL (1994) Characteriza-


