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Spreading depression and focal brain ischemia induce cyclooxygenase-2 in cortical neurons through *N*-methyl-D-aspartic acid-receptors and phospholipase A_2

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ABSTRACT **Repetitive spreading depression (SD) waves, involving depolarization of neurons and astrocytes and upregulation of glucose consumption, is thought to lower the threshold of neuronal death during and immediately after ischemia. Using rat models for SD and focal ischemia we investigated the expression of cyclooxygenase-1 (COX-1), the constitutive form, and cyclooxygenase-2 (COX-2), the inducible form of a key enzyme in prostaglandin biosynthesis and the target enzymes for nonsteroidal anti-inflammatory drugs. Whereas COX-1 mRNA levels were undetectable and uninducible, COX-2 mRNA and protein levels were rapidly increased in the cortex, especially in layers 2 and 3 after SD and transient focal ischemia. The cortical induction was reduced by MK-801, an *N*-methyl-D-aspartic acid-receptor antagonist, and by dexamethasone and quinacrine, phospholipase A_2 (PLA_2) inhibiting compounds. MK-801 acted by blocking SD whereas treatment with PLA_2 inhibitors preserved the wave propagation. NBQX, an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate-receptor antagonist, did not affect the SD-induced COX-2 expression, whereas COX-inhibitors indomethacin and diclofenac, as well as a NO synthase-inhibitor, N^G-nitro-L-arginine methyl ester, tended to enhance the COX-2 mRNA expression. In addition, ischemia induced COX-2 expression in the hippocampal and perifocal striatal neurons and in endothelial cells. Thus, COX-2 is transiently induced after SD and focal ischemia by activation of *N*-methyl-D-aspartic acid-receptors and PLA_2, most prominently in cortical neurons that are at a high risk to die after focal brain ischemia.**

In focal brain ischemia a peri-infarct zone called ischemic penumbra is the tissue surrounding the core with reduced blood flow and at risk to die (1). Neurons are injured in all cortical layers in penumbra, especially in layers 2 and 3, in which glucose consumption is increased (2, 3). One major factor contributing to neuronal death in penumbra is thought to be spreading depression (SD) waves (1, 4, 5), which are accompanied by depolarization of both astrocytes and neurons (6), collapsed ionic gradients and impaired ATP generation (1, 7). Because penumbra constitutes potentially salvageable tissue, the molecular responses of the perifocal neurons to focal ischemia and SD are of interest (8).

Cyclooxygenase-2 (COX-2) is a key enzyme in conversion of arachidonic acid to prostaglandins and thromboxanes, and is a target enzyme for nonsteroidal anti-inflammatory drugs. It is normally expressed throughout the brain in discrete populations of neurons and is regulated by glutamate receptors and glucocorticoids (9–11). Recently, seizure (12) and brain ischemia (13) were reported to induce COX-2 mRNA or protein. Because COX enzyme-products modulate glutamate release and vasoconstriction, and free radicals are generated during the synthesis (14), COX-2 induction in neurons at risk to die could contribute to neuronal death in brain ischemia. This hypothesis is supported by reports that COX (15, 16) and phospholipase A_2 (PLA_2) inhibitors (17) reduce hippocampal damage in global ischemia. We studied the regulation of COX-2 mRNA and protein in the rat brain using a model of transient focal ischemia and a KCl model of SD. The results show that both focal ischemia and SD induce a strong COX-2 expression in cortical neurons in layers 2 and 3, the most vulnerable neurons in penumbra. The induction is reduced by *N*-methyl-D-aspartic acid (NMDA)-receptor antagonists that block SD waves, and by compounds that inhibit PLA_2. In addition, focal ischemia induced COX-2 mRNA and protein in neurons of other cortical layers, perifocal striatum, hippocampus, and in endothelial cells in the infarct core.

MATERIALS AND METHODS

Focal Ischemia Model. Male Wistar rats (250–300 g) were anesthetized with halothane in N_2O/O_2 (70:30). The rectal temperature was controlled with a heating pad. The right middle cerebral artery was occluded for 90 min by an intraluminal filament technique as previously described (18). For recording of physiological parameters, a polyethylene catheter was inserted into the femoral artery. Arterial blood pressure, PO_2, PCO_2, and pH were measured 15 min before and at 30 min intervals during and right after ischemia.

SD Model. After placing a rat in a stereotaxis frame under halothane anesthesia, a 2-mm craniotomy was made bilaterally 4 mm lateral to the sagittal suture and 4 mm posterior to bregma. Without disruption of the dura the brain was exposed for 60 min to 3 M KCl to induce SD in the right hemisphere. The left hemisphere was exposed to 0.9 M NaCl and served as a control. For recording of extracellular DC potentials a third craniotomy was made before SD induction 4 mm anterior to the site of KCl exposure. An extracellular low resistance needle electrode was inserted 1 mm into the cortex and the signals were led through a DC-amplifier to an instrumentation tape recorder. The data were assessed with Mann–Whitney *U*–Wilcoxon Rank Sum *W* test.

Drug Treatments. MK-801 hydrogen maleate (Research Biochemicals, Natick, MA; 3 mg/kg i.p.), N^G-nitro-L-arginine methyl ester (Research Biochemicals, 20 mg/kg, i.p.), diclofe-

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Abbreviations: COX, cyclooxygenase; NMDA, *N*-methyl-D-aspartic acid; PLA_2, phospholipase A_2; SD, spreading depression; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline.

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nac sodium salt (Research Biochemicals, 10 mg/kg, i.p.), indomethacin (Research Biochemicals, 3 mg/kg, i.p.), quinacrine dihydrochloride (Research Biochemicals, 5 mg/kg, i.p.), and dexamethasone sodium phosphate (Decadron, MSD Isotopes, 1.5 mg/kg i.p.) were given 30 min prior to ischemia or SD. 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX; Tocris Cookson, Bristol, U.K.) was administered as two 5-min i.v. infusions of a total dose of 60 mg/kg 5 min prior and 30 min after beginning of ischemia or KCl application. Control animals received an i.p. injection of saline.

***In Situ* Hybridization.** Two oligodeoxynucleotide probes were used: COX-1 (40-mer), complementary to the rat coding sequence 5′-CTGGAGGGAGGAGTTTTGTTCCGGAAGAAGACAGTCCTTG-3, and COX-2 (40-mer), complementary to rat coding sequence 5′-TCATCAACACTGCCTCAATTCAGCCTCTCATCTGCAATAA-3′. Oligonucleotides with same length and GC-ratio similar to the corresponding antisense oligonucleotides but without homology to any known gene sequences were used as controls. The slides were hybridized overnight with the oligonucleotides 3′end-labeled with ^{35}S-dATP (New England Nuclear) as described previously (18).

Image Analysis. A digital image analysis system (MCID 4, Imaging Research, St. Catherine's, ON, Canada) was used to obtain optical density measurements over the sections. The gray levels corresponding to the ^{14}C-plastic standards (Amersham) lying within the exposure range of the film were determined and used as a fourth degree polynomial approximation to construct a gray level to activity transfer. Densitometric measurements were done from 3–5 sections per each animal. The data were assessed with the two-tailed Mann–Whitney *U*–Wilcoxon Rank Sum *W* test.

Northern Blotting. Total RNA was isolated using the TRIzol reagent (Life Technologies, Gaithersburg, MD). Samples of 30 μg per lane were electrophoresed through a formaldehyde/1.2% agarose gel and transferred to a Hybond N (Amersham) nylon membrane by capillary blotting. The membrane was hybridized with ^{32}P-labeled oligonucleotide probes (the same as in *in situ* hybridization experiments and a cyclophilin probe complementary to nucleotides spanning amino acids 150–164) in 5× standard saline citrate (SSC)/5× Denhardt's/50% formamide/1% SDS at 42°C overnight, and washed twice for 5 min at room temperature with 2× SSC/0.1% SDS, and once for 5 min in 0.2× SSC/0.1% SDS also at room temperature. The blots were kept wet and exposed to Fuji x-ray film at −80°C until the signal was detected.

Immunoblotting. Tissue was homogenized and concentrated Laemmli sample buffer was added to a final concentration of 62.5 mM Tris·HCl, 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol prior to heating to 95°C for 5 min. Thirty micrograms of each sample were electrophoresed in 10% polyacrylamide gels. Proteins were electrophoretically transferred to nitrocellulose membranes (Hybond-C Extra, Amersham) that were blocked for 1 h at 25°C with 2% BSA diluted in 0.02 M PBS (pH 7.2) containing 0.2% Tween. Blots were incubated with anti-cox-2 mAb (Transduction Laboratories, Lexington, KY, 1:2,000) overnight at 4°C. After incubation with anti-mouse, peroxidase-conjugated antibody (1:1,000), blots were washed in PBS-Tween, incubated with enhanced chemiluminescence detection reagent (Amersham), and exposed to Kodak XAR-5 film.

Immunocytochemistry. Free-floating (50 μm) sections were reacted with the primary antibody (Transduction Laboratories, diluted 1:100). After incubating with biotinylated anti-mouse serum and avidin–biotin complex (Vectastain Elite kit, Vector Laboratories) for 3 h each, the avidin–biotin complex was visualized with 0.05% diaminobenzidine and 0.02% H_2O_2. After rinsing the slides were examined in a Leica 3000RB microscope.

RESULTS

The physiological variables of the saline-treated animals were within the normal range. The drug-pretreated animals did not significantly differ from the control animals, except dexamethasone-treated animals after reperfusion. This group showed shifted levels of PCO_2 [38.00 ± 1.0 → 52.20 ± 3.0 (mean ± SD) mmHg, $P < 0.01$].

In situ hybridization studies did not reveal detectable expression of COX-1 mRNA in the control or experimental brain samples, whereas COX-2 in the unstimulated brain was strongly expressed in the dentate gyrus and CA4-3 pyramidal cell layer in the hippocampus. COX-2 was expressed at relatively low but detectable levels in the piriform cortex, CA1 pyramidal cells in the hippocampus, and the superficial layers of the neocortex (Fig. 1*a*). COX-2-immunoreactive cells were detected in the regions that also expressed COX-2 mRNA (Fig. 2 *a, c,* and *e*; Fig. 3 *a, b,* and *e*). COX-2-positive cells were always neurons. However, the antibody also stained a population of small, microglia-like cells throughout the brain (Fig. 3 *g* and *h*). Since the immunoreactivity of this small cell population has not been reported previously, is not detectable with a specific polyclonal antibody (10) and did not change after experimental procedures, it may represent nonspecific immunostaining. In the neocortex the antibody stained a few neuronal somata and faintly some neurites (Fig. 2 *a* and *c*). COX-2-immunoreactive cells were most abundant in the piriform cortex (Fig. 2*e*), the dentate gyrus, and CA3 blade of the hippocampus (Fig. 2*a*). The low, hardly detectable expression of COX-2 mRNA and protein in the unstimulated brain was supported by Northern (Fig. 4*a*) and Western immunoblot analysis (Fig. 4*b*).

The topical application of 3 M KCl to the parietal cortex induced 4.36 ± 1.2 (mean ± SD) DC-potentials when recorded during the first hour. Saline did not induce any DC-potential. Figs. 1 *a–d* and 5*a* show the time course of COX-2 mRNA expression after KCl and saline exposure. The expression was about 3-fold 4 and 8 h after KCl treatment compared with the expression in control brains or on the contralateral side. At 24 h, no statistically significant difference was detected between KCl and saline-treated cortices, and at 48 h COX-2 mRNA expression was completely back to normal levels. The mRNA induction at 8 h was confirmed in Northern blots (data not shown). Immunocytochemistry (Fig. 2 *b, d,* and *f*) and immunoblotting (data not shown) revealed that the mRNA induction was followed by an increase in COX-2 protein. The immunoreactivity was strong in layers 2 and 3, but the staining was enhanced also in layer 5 when compared with the control side.

For studing the mechanisms mediating COX-2 induction by SD, the animals were pretreated with various pharmacological compounds and the brains processed for *in situ* hybridization or immunocytochemistry 8 h after SD induction (Figs. 1 *e–h* and 5*b*). MK801, an NMDA-receptor antagonist, and dexamethasone significantly blocked the SD-induced COX-2 expression, whereas indomethacin, a COX-1 inhibitor, and diclofenac, a COX-1/COX-2 inhibitor, were without effect. Quinacrine, a PLA_2 inhibitor, decreased COX-2 expression as well, but with $n = 4$ did not reach significant level. The mRNA inhibition was confirmed by Northern blotting, and immunocytochemistry showed a corresponding reduction in COX-immunoreactive cortical neurons in MK-801-treated SD-animals (not shown). Additional experiments were run after pretreatment with NBQX, an α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid/kainate receptor antagonist, and N^G-nitro-L-arginine methyl ester, a NO synthase inhibitor that mainly blocks endothelial NO synthase when given systemically. Neither one of these drugs blocked COX-2 expression (Fig. 5*b*). In fact, N^G-nitro-L-arginine methyl ester tended to increase COX-2 mRNA levels on both sides of the brain.

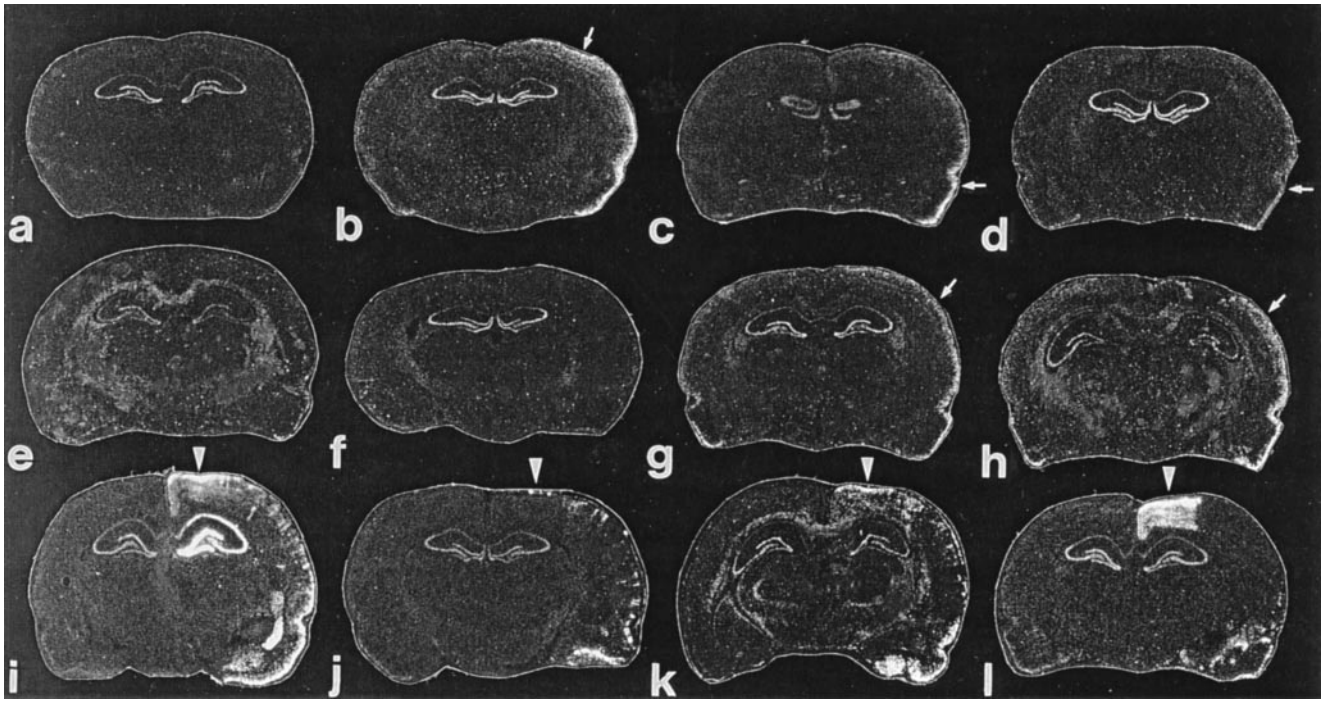

FIG. 1. *In situ* hybridization autoradiographs showing expression of COX-2 in the rat brain 0 h (*a*), 4 h (*b*), 8 h (*c* and *e–h*), and 24 h (*d*) after SD, and 12 h after focal brain ischemia (*i–l*). The pretreatments were as follows: *a–d* and *i*, 0.9% NaCl; *e*, 1.5 mg/kg dexamethasone; *f* and *j*, 3 mg/kg MK-801; *g*, 60 mg/kg NBQX; *h* and *l*, 3 mg/kg indomethacin; *k*, 5 mg/kg quinacrine. Arrows point to SD-induced COX-2 mRNA and arrowheads to the perifocal cortex that shows the maximal gene induction after focal brain ischemia.

Because MK-801, dexamethasone, and quinacrine inhibited SD-induced COX-2 expression, we studied whether the effect could be due to blockade of propagation of DC potentials.

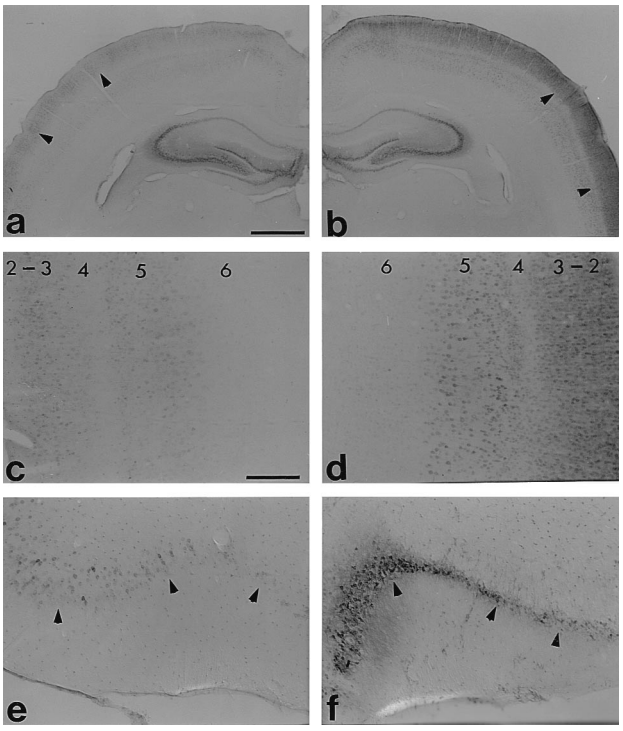

FIG. 2. COX-2 immunoreactivity on the contralateral side (*a*, *c*, and *e*) and SD-side (*b*, *d*, and *e*) 8 h after KCl application. The immunoreactivity is strongly induced in cortical layers 2 and 3 (arrowheads in *a* and *b*). High magnification of the neocortex shows a slightly increased immunoreactivity also in layer 5 (*c* and *d*) and in the piriformis cortex (arrowheads in *e* and *f*). Numbers refer to different cortical layers. Bar = 100 μm (*a* and *b*); 50 μm (*c–f*).

Practically no DC potentials were recorded after MK-801 pretreatment (0.2 ± 0.5, mean ± SD, significantly different from the controls, $P = 0.019$, Mann–Whitney *U*–Wilcoxon Rank Sum *W* test). Dexamethasone did not significantly affect the number of KCl-induced SD-potentials at the dose of 1.5 mg/kg (2.2 ± 1.6, $P > 0.05$, when compared with control) and 5.0 mg/kg (4.0 ± 1.0, $P > 0.05$). Thus, SD-induced-COX-2 expression in cortical neurons is indirectly diminished by NMDA-receptor antagonists, whereas corticosteroids act downstream to NMDA receptors.

SD is thought to be crucial in mechanisms of infarct enlargement after focal brain ischemia and to mediate most of the perifocal gene inductions in brain ischemia models (2, 4, 5, 8). Therefore, we next studied whether COX-2 is induced after transient middle cerebral artery occlusion in the same cell populations and with a mechanism similar to that after KCl-induced SD. Twelve hours following 90 min of ischemia COX-2 mRNA was induced in all layers in the neocortex, perifocal striatum, piriform cortex, dentate gyrus, and CA1–4 pyramidal cell layers in the hippocampus (Fig. 1*i*). In the cortex, the layers 2 and 3 had the highest expression. The induction was also seen at the protein level using immunocytochemistry (Fig. 3) and immunoblotting (Fig. 4*b*). The perifocal induction of COX-2 protein was only induced in neurons and was strongest in perinuclear area (Fig. 3*d*). In the hippocampus the dentate gyrus showed a strong COX-2 immunoreactivity and in the CA1 patches of immunoreactive pyramidal cells were seen (Fig. 3 *f* and *g*). In the infarcted core, immunoreactive endothelial cells were seen (Fig. 3*h*). Figs. 1 *i–l* and 5*c* show the effect of drug-pretreatments on ischemia-induced COX-2 expression. In the line with SD experiments, MK-801, dexamethasone, and quinacrine significantly inhibited COX-2 expression in the perifocal cortex, whereas their effect on hippocampal COX-2 expression was not consistent. Also NBQX-pretreatment decreased COX-2 expression when quantified from the whole perifocal cortex. This may be secondary to decreased ischemic damage because very high COX-2 expression was always found but was restricted to the

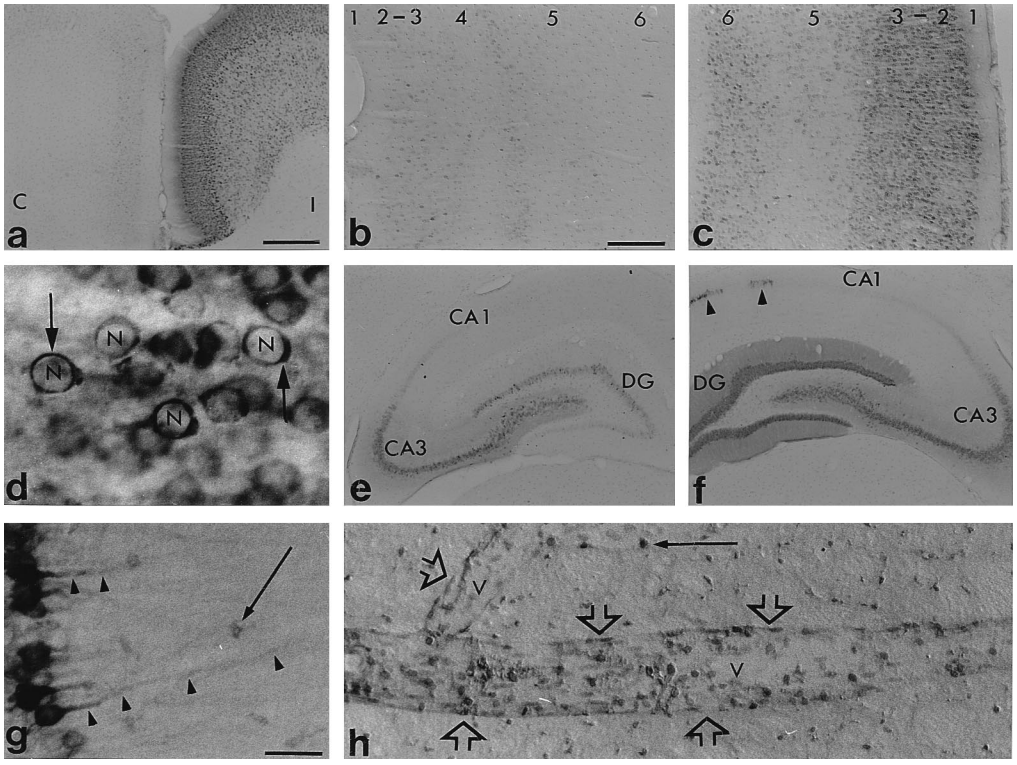

FIG. 3. Induction of COX-2 immunoreactivity 24 h after focal brain ischemia in the neocortex (*a–d*), hippocampus (*e–g*), and around blood vessels in the infarcted core (*h*). At low magnification (*a*) a clear induction is seen in the ischemic (I) neocortex compared with the contralateral (C) side. At high magnification a few positive cell is seen in the contralateral cortex (*b*), whereas a strong staining is seen on the ischemic side (*c*), especially in layers 2 and 3. The COX-2 positive material is mainly localized perinuclearly (*d*, arrows). In the hippocampus, the ischemia-induced COX-2 staining (*f*) is detected in the dentatus gyrus, CA3 and in patches in the CA1 pyramidal cell layer (arrowheads) when compared with the contralateral side (*e*). The staining in CA1 pyramidal cell layer (*g*) is localized in the somata and proximal neurites (arrowheads). Endothelial cells around the blood vessels in the infarcted core are strongly stained by COX-2 antibody (*h*, thick arrows). Long arrows in *g* and *h* point to microglia-like cells that were found to be immunoreactive throughout the brain in all samples. N, nucleus. [Bars = 100 μm (*a*); 50 μm (*b, c, e,* and *f*); 25 μm (*d* and *h*).]

infarct margin. Indomethacin, diclofenac, and N^G-nitro-L-arginine methyl ester had a tendency to increase the ischemia-induced COX-2 expression. Also in Northern blotting (Fig. 4*a*) and immunocytochemistry experiments (not shown) pretreatment with MK-801 showed decreased COX-2 expression.

DISCUSSION

The major finding of the present study is that COX-2, the inducible form of the enzyme converting arachidonic acid to prostaglandins, is induced within hours after SD and transient focal ischemia in perifocal cortical neurons by a mechanism dependent on NMDA-receptors and PLA_2. In addition, focal ischemia induces COX-2 expression in most of the hippocampal and perifocal striatal neurons, as well as in endothelial cells indicating that increased prostaglandin production after ischemia-reperfusion injury can take place not only in neurons at risk to die but also in neurons remote from the ischemic area and in non-neuronal cells.

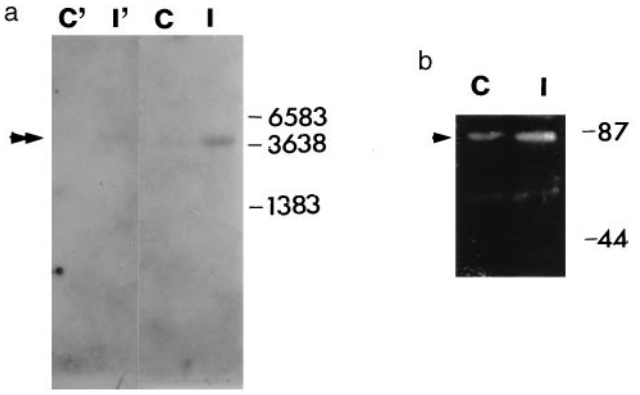

FIG. 4. Northern (*a*) and Western (*b*) blotting analysis of the ischemia-induced COX-2 expression in the ischemic (I) and contralateral (C) cortex. (*a*) The Northern blot shows a 4.1 kb-band (double arrowhead) that is barely detectable in the control sample but clearly seen in ischemic tissue. In MK-801-pretreated brain the signal is very low both in contralateral (C′) and ischemic (I′) tissue. In the Western blot (*b*) an arrowhead points to a 70-kDa COX-2-immunoreactive band.

The basal expression of COX-2 in the brain is regulated by NMDA-dependent synaptic activity and glucocorticoids (9, 11, 13). Kainate-induced seizures are associated with a sustained COX-2 expression in hippocampal regions that are damaged by the agonist (12). Seizures induced by kainate injection into the nucleus basalis increase COX-2 expression in the cortex by a mechanism mediated by NMDA receptors but blocked by glucocorticoids (11). Unlike seizures, SD does not cause irreversible neuronal injury under normoxic conditions (1). However, our results show that it does induce a strong neuronal expression of COX-2 mRNA and protein, which is regulated by the same mechanisms as in seizures. Because the propagation of SD waves itself is blocked by NMDA-receptor antagonists (1), the inhibiting effect of MK-801 on SD-induced COX-2 expression is not surprising. However, dexamethasone and quinacrine, PLA_2 inhibiting compounds, reduced SD-induced COX-2 expression without blocking SD waves indicating that these compounds may interfere in neurons downstream to NMDA receptor activation to block COX-2 transcription.

Quinacrine is known to block PLA_2 activity by forming complexes with phospholipids that then cannot serve as PLA_2 substrates (19), and inhibits NMDA-mediated arachidonic acid

release from neurons (20, 21). Because dexamethasone can block PLA_2 either by inducing synthesis of PLA_2-inhibiting compounds, annexins, or by direct inhibition of PLA_2 synthesis (22), our studies indicate that PLA_2 activation subsequently to NMDA-receptor stimulation increases COX-2 expression. The PLA_2-induced COX-2 expression may be mediated by platelet-activating factor that is produced in arachidonic acid synthesis and has been reported to induce COX-2 expression in the brain (12, 22). However, COX-2 inhibition by dexamethasone was more profound than by quinacrine, suggesting that dexamethasone has also direct interactions with COX-2 transcription. Glucocorticoids are known to inhibit activator protein-1 (AP-1) binding and especially NF-κB activity (23–25). Both AP-1 and NF-κB transcription factors bind to COX-2 promoter (23–26). Thus glucocorticoids may inhibit ischemia-induced COX-2 expression directly through affecting the binding activity of crucial transcription factors or indirectly through blocking PLA_2 activity and platelet-activating factor release.

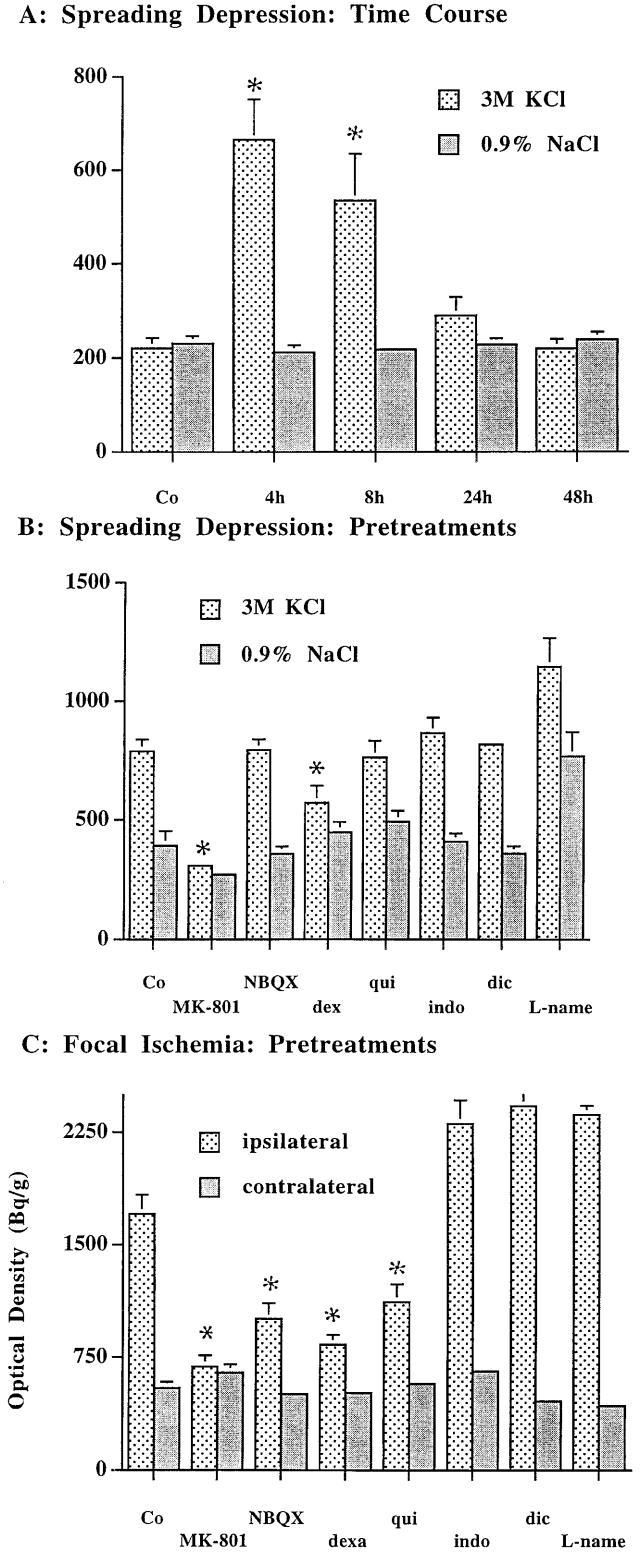

FIG. 5. Expression of COX-2 mRNA in the neocortex at dorsal hippocampal level measured on x-ray film *in situ* hybridization autoradiographs. Values represent the mean ± SEM from three to five animals. *, The difference between experimental (*A* and *B*, KCl-treated; *C*, ipsilateral cortex) and contralateral side statistically significant ($P < 0.05$, two-tailed Mann–Whitney *U*–Wilcoxon RankSum *W* test) when compared with the difference in control animals. Dex, dexamethasone; qui, quinacrine; indo, indomethacin; dic, diclofenac; L-name, N^G-nitro-L-arginine methyl ester.

The COX-2 expression induced by transient focal ischemia was more sustained than after SD, involving all the cortical layers, ipsilateral striatum, and hippocampal neurons. The perifocal neurons in the cortex and striatum, and pyramidal neurons in the CA1 region are especially at risk to die, whereas the cortical neurons outside of the area supplied by middle cerebral artery, and hippocampal neurons other than CA1 pyramidal neurons are not supposed to be damaged by middle cerebral artery occlusion. Similar to SD, ischemia-induced COX-2 expression in the cortex was inhibited by MK-801, dexamethasone, and quinacrine indicating that SD is responsible for cortical COX-2 expression following transient focal ischemia, whereas COX-2 expression in the hippocampus does not clearly correlate with its regulation in the cortex. The more sustained COX-2 expression following ischemia than after SD may be due to compromised energy sources, reperfusion-triggered production of oxygen free radicals or longer-lasting SD waves, all of which can contribute to increased presynaptic release of glutamate in ischemic brain (1, 3). Increased extracellular glutamate could then funnel its postsynaptic effect on COX-2 expression through NMDA-receptor activation as NBQX, an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainate -receptor antagonist did not block COX-2 expression.

Focal ischemia caused COX-2 induction in endothelial cells that have not been described before to express the gene in the brain. In culture, COX-2 expression is detected in several non-neuronal cells such as macrophages (27), astrocytes (28), and endothelial cells (29). The basal expression of COX-2 revealed by immunoblotting could be derived from these cells in addition to neurons. Even though all these non-neuronal cells are activated by ischemia, only endothelial cells in the infarct up-regulate COX-2. The induction could be caused by cytokines or calcium influx, both of which regulate COX-2 expression of these cells in culture. Overall, COX-2 induction in non-neuronal cells may be taken as an indication of a ruptured blood-brain barrier during focal ischemia (3, 8).

Induction of COX-2 has evidently profound effects on the brain and it is likely to affect the outcome of ischemic damage. First, increased COX-2 activity may control ischemia-induced increase of arachidonic acid levels that may have direct effects on neurotransmitter release and receptors (14). Second, COX, while converting arachidonic acid to eicosanoids, produces free radicals that may contribute to the ischemic neuronal damage (14). Third, prostaglandins may potentiate the post-ischemic inflammation by increasing edema and by delivery of proinflammatory cells into the brain. In addition, prostaglandins may facilitate sensory neuropeptide release and sensitize pain receptors (30, 31). COX-2 inducibility by focal brain ischemia and SD, together with the absence of COX-1 in the brain indicate, that specific COX-2 inhibitors may be beneficial for the therapy of brain ischemia and also of migraine, which is thought to be triggered through a SD-like phenomenon during the aura phase (32).

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