Role of Cyclooxygenase-2 in Neuronal Cell Cycle Activity and Glutamate-Mediated Excitotoxicity

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Received October 8, 2001; accepted January 9, 2002 This article is available online at http://jpet.aspetjournals.org

ABSTRACT

In previous studies we found that neuronal overexpression of human cyclooxygenase (COX)-2 in transgenic mice potentiated excitotoxicity in vivo and in vitro. To clarify the molecular mechanisms involved in COX-2-mediated potentiation of excitotoxicity, we used cDNA microarray to identify candidate genes the expression of which is altered in the cerebral cortex of homozygous human hCOX-2 transgenic mice. We found that the mRNA expression of the cell cycle kinase (CDK) inhibitor-inhibitor kinase (INK) p18INK4, a specific inhibitor of CDK 4,6, which controls the activation of the retinoblastoma (Rb) tumor suppressor protein phosphorylation, was decreased in the brain of adult hCOX-2 homozygous transgenics. Conversely, chronic treatment of the hCOX-2 transgensics with the preferential COX-2 inhibitor nimesulide reversed the hCOX-2-mediated decrease of cortical p18INK4 mRNA expression in the brain. Further in vitro studies revealed that in primary cortico-hippocampal neurons derived from homozygous hCOX-2 transgenic mice, COX-2 overexpression accelerates glutamate-mediated apoptotic damage that is prevented by the CDK inhibitor flavopiridol. Moreover, treatment of wild-type primary cortico-hippocampal neuron cultures with the COX-2 preferential inhibitor nimesulide significantly attenuated glutamate-mediated apoptotic damage, which coincided with inhibition of glutamate-mediated pRb phosphorylation. These data indicate that hCOX-2 overexpression causes neuronal cell cycle deregulation in the brain and provides further rationale for targeting neuronal COX-2 in neuroprotective therapeutic research.

Cyclooxygenase (COX) is the rate-limiting enzyme in the production of prostaglandins and, as such, is a key target for many anti-inflammatory drugs. There are two known isoforms, COX-1 and COX-2, which have quite distinct expression patterns and biological activities. COX-1 is a constitutively expressed protein found in most tissues, whereas COX-2 expression can be induced by a variety of mitogens, including cytokines, hormones, and phorbol esters (Kujubu et al., 1991; O'Banion et al., 1992). Inflammatory stimuli may influence excitotoxicity, we used cDNA microarray to identify candidate genes the expression of which is altered in the cerebral cortex of homozygous human hCOX-2 transgenic mice. We found that the mRNA expression of the cell cycle kinase (CDK) inhibitor-inhibitor kinase (INK) p18INK4, a specific inhibitor of CDK 4,6, which controls the activation of the retinoblastoma (Rb) tumor suppressor protein phosphorylation, was decreased in the brain of adult hCOX-2 homozygous transgenics. Conversely, chronic treatment of the hCOX-2 transgensics with the preferential COX-2 inhibitor nimesulide reversed the hCOX-2-mediated decrease of cortical p18INK4 mRNA expression in the brain. Further in vitro studies revealed that in primary cortico-hippocampal neurons derived from homozygous hCOX-2 transgenic mice, COX-2 overexpression accelerates glutamate-mediated apoptotic damage that is prevented by the CDK inhibitor flavopiridol. Moreover, treatment of wild-type primary cortico-hippocampal neuron cultures with the COX-2 preferential inhibitor nimesulide significantly attenuated glutamate-mediated apoptotic damage, which coincided with inhibition of glutamate-mediated pRb phosphorylation. These data indicate that hCOX-2 overexpression causes neuronal cell cycle deregulation in the brain and provides further rationale for targeting neuronal COX-2 in neuroprotective therapeutic research.

In vitro studies showing that the phosphorylation of the retinoblastoma (Rb) tumor suppressor protein, an index of CDK activity (Weinberg, 1995), is induced in neurons overexpressing hCOX-2 during response to glutamate-mediated excitotoxic apoptotic damage. Most importantly, we found that the CDK inhibitor, flavopiridol, attenuated the hCOX-2-mediated
ated potentiation of apoptotic neuron damage. The study suggests that a mechanism by which COX-2 may influence excitotoxicity is through promotion of cell cycle activity.

Materials and Methods

Transgenic Mice. Homozygous transgenic mice (C57BL/6J × C3H (B6C3) 9-month-old females) with overexpression of hCOX-2 in neurons and nontransgenic control littermates were previously described from our laboratory (Kelley et al., 1999). Transgenic mice were identified by dot blot hybridization of tail skin DNA samples with a random-primed, 930-bp EcoRI fragment that contains the entire simian virus 40 sequence present in the hCOX-2 transgene (Kelley et al., 1999). In this study, mice were sacrificed by cervical dislocation and the brains were stored at −70°C.

Drug Treatment. The preferential COX-2 inhibitor nimesulide was provided by Helsinn Healthcare (Lugano, Switzerland) (Warner et al., 1999). Nimesulide was mixed directly with powdered rodent feed in a mixing drum into a homogeneous preparation, which was then formulated into rodent half-inch feed pellets. All ingredients of the diet were from Zeigler Bros., Inc. (Garners, PA), and formulated diets were stored at 4°C. Mice (four per cage) had access to food and water ad libitum, and diets were replenished regularly every 3 days. Based on recent evidence indicating that 1500 mg celecoxib/kg in feed is the maximum tolerable dose in rodents for 50 weeks of treatment (Pentland et al., 1999), and that the relative potency of COX-2 inhibition by celecoxib and nimesulide is equivalent (Warner et al., 1999), we treated our mice with an equivalent dose of nimesulide (1500 mg/kg) for 3 months. At sacrifice, necropsied mice did not reveal any detectable gross alteration of renal or intestinal abnormalities in the nimesulide-treated group. In previous studies we found that treatment with 1500 mg/kg nimesulide in the diet for 3 months is well tolerated in B6C3 mice without detectable adverse effect based on weight loss criteria and overall lack of gastrointestinal pathology.

Complementary DNA Expression Arrays. Brain (cerebral cortex) poly(A⁺) RNA was purified from four mice per group using the Oligotex mRNA Mini Kit (Qiagen, Valencia, CA). Fluorescent-labeled antisense cDNA probes were generated by reverse transcription, following procedures recommended by Incyte Genomics, Inc. (Palo Alto, CA). The reference probe (control mRNA, pool of n = 4) was labeled with a green-fluorescent dye, Cy3 (P1), whereas the test probes (hCOX-2 mRNA, pool of n = 4) were labeled with the red-fluorescent dye Cy5 (P2). Individual mouse UniGem microarray chips (Incyte Genomics, Inc.) containing 8832 independent genes, including known and expressed sequence tag sequences, were simultaneously hybridized with both probes. Hybridization of Cy3- and Cy5-labeled probes for each of the elements on the microarray chip was simultaneously recorded and quantified using a two-head laser scanner. The linear fitting curves of the P1 and P2 fluorescence signal were then analyzed by linear regression scatter plot analysis that revealed a very tight distribution pattern clustered in an almost 45° diagonal line. This evidence suggested that probe preparation, hybridization condition, data collection, and microarray preparation had negligible influences in the microarray studies. In our studies, signals covering less than 40% of the surface area and/or signals having fluorescence intensity less than 600 units (range from 0 to 2200 units) were considered to be background hybridization and were excluded from analysis. The cDNA microarray study was conducted in duplicate, and genes that were consistently up- or down-regulated in hCOX-2 transgenics by ≥1.8-fold relative to wild-type (WT) were further analyzed. Genes, including p18INK4 mRNA were grouped into clusters defined by cellular and/or biochemical functions using the GEM Tool software package (Incyte Genomics, Inc.). The ≥1.8 cut-off level was derived from the analysis of independent UniGem complementary DNA microarrays (n = 4, cDNA microarray) designed to identify consistency and reproducibility in mouse cDNA chip hybridization. In this study the expression for each gene represented in the cDNA chip expressed in the mouse brain was normalized to a mean equal to 1. The standard deviations of each gene were then calculated. The 95th percentile of the empirical distribution of the standard deviations (0.236) was used to build the model for mouse gene expression variability. Our results suggested that for 95% of the genes, gene expression levels have to be outside the 1 ± 0.5-fold range for P < 0.05 significance level. Although the differentially regulated gene products that were considered for further investigation in this study by cDNA array had to be outside the 1 ± 0.5-fold range, we found that the altered expression of p18INK4 mRNA by RNase protection was in the range of a 30% change (see Results). Thus, the cDNA microarray evidence overestimated the differential expression of p18INK4 mRNA in the brain relative to the RNase protection assay evidence (Materials and Methods).

Western Analysis. Tissue culture lysates were homogenized and resolved by SDS-polyacrylamide gel electrophoresis (10% acrylamide). Proteins were transferred to a nylon membrane (Transblot Membrane; Bio-Rad, Hercules, CA), blocked overnight (4°C) (Superblock; Pierce Chemical, Rockford, IL) and immunoreacted for 3 h in a 1:1000 dilution of a rabbit polyclonal anti-phospho-Rb (Ser795) antibody (PhosphoPlus; New England Biolabs, Beverly, MA). A C-terminal control antibody, which detects phosphorylated-independen levels of Rb (1:1000; PhosphoPlus; New England Biolabs) was also used in parallel studies to control for specificity of phosphorylated Ser795 Rb, and gave negative results (not shown). Immunoreactivities were visualized autoradiographically using a chemiluminescence detection kit (SuperSignal; Pierce) for horseradish peroxidase-labeled goat antirabbit IgG (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instruction. B-Actin immunoreactivity (anti-β-actin, 1:1000; Sigma-Aldrich, St Louis, MO) controlled for selectivity of changes. Immunoreactivities were quantified by Western blot imaging analysis using the Bio-Rad Gel Doc 2000 gel documentation system.

RNase Protection Assay. Total RNA was assayed with the RibonQuant Multiprobe RNase Protection Assay System (BD PharMingen, San Diego, CA). A custom probe containing cDNA template for the p18INK4 was used. The probe set included the housekeeping genes L32 and glyceraldehyde phosphate dehydrogenase (GAPDH) for normalization of assay conditions. Details on the generation of 32P-labeled antisense RNA probes and conditions of the RNase protection assay were provided by the manufacturer’s instructions as previously described (Luterman et al., 2000). The radioactively labeled RNase protection fragments were quantified using a Storm 860 Phosphor Screen Scanner with the ImageQuant software package (Molecular Dynamics, Sunnyvale, CA). Each RNase protection assay analysis was conducted with 10 μg total RNA, according to A260 values. Data were expressed as a ratio of p18INK4 mRNA normalized to the constitutively expressed GAPDH mRNA. Normalization of p18INK4 mRNA signals to L32 did not change the outcome of the results (not shown).

The mouse p18INK4 cDNA clone (GenBank accession number AA 422352) was digested with EcoRI and PstI, and a 273-bp EcoRI/PstI cDNA fragment encoding part of the p18INK4 open-reading frame was subcloned into pBlueScript vector (Stratagene, La Jolla, CA), which is referred to as pBSm p18INK4. For generation of an RNA probe, the pBSm p18INK4 plasmid was linearized with EcoRI and the antisense p18INK4 cRNA probe was generated using T7 RNA polymerase (BD PharMingen). The sequence identity of the 333-bp probe (including 60 bp of vector) was verified by dyeoxy sequencing.

Neuronal Culture and Glutamate-Mediated Neurotoxicity. Embryonic (E14–E16) cortico-hippocampal primary neuronal cultures derived from hCOX-2 transgenic mice were prepared as previously described (Kelley et al., 1999). Briefly, after brain dissection, mechanical trituration, and centrifugation, neurons were seeded onto poly-D-lysine-coated 96-well plates at a density of 2 × 10⁴ cells per well. The absence of astrocytes (<1–2%) was confirmed by the lack of glial-fibrillary acidic protein immunostain-
ing (not shown). Glutamate and/or flavoperidol (L86-8725, \((-\text{cis}\)-5,7-dihydroxy-2-(2-chlorophenyl)-8-(4-(3-hydroxy-1-methyl)piperidinyl)-4H-benzopyran-4-one) (donated by David Park) were added to 7-day-old cultures for the appropriate time as discussed in the text. Detection of neuronal apoptotic nuclear morphology in vitro was assessed by incubation of cultures with 1 \(\mu\)g/ml of bisbenzimide (Hoechst 33258) (Sigma-Aldrich) for 15 min at room temperature and then coveredslipped as previously described (Didier et al., 1996). The number of neurons with apoptotic nuclei (condensed chromatin) were digitized, counted, and expressed as percentage of the total number of neurons in six to eight random fields of each chamber \((n = 4–6\) chambers per group, per culture).

For glutamate toxicity studies L-glutamate (Sigma-Aldrich) was dissolved in PBS (pH 7.4) and stored at 4°C in 500 mM aliquots. Disposable aliquots of nimesulide, flavoperidol, and DMSO were also stored at -20°C to mimic freeze-thaw conditions in vehicle-treated cultures. Cultures were treated with glutamate or vehicle (0.001% PBS or 0.01% DMSO, final concentration), as indicated. Glutamate exposure was performed in 7-day-old cultures by adding 50 \(\mu\)M glutamate and/or nimesulide or flavoperidol (1 \(\mu\)M) from concentrated stocks into the existing culture media for 24 h until neuron cultures were collected for viability assays. In our studies we found that cortico-hippocampal cultures treated with 0.01% DMSO did not differ from PBS-treated controls (not shown).

**Prostaglandin Assay.** PGE\(_2\) concentration was measured by an enzyme-linked immunoassay kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. Briefly, pulverized brain tissue stored in liquid \(N\_2\) was homogenized in 0.1 M phosphate-buffered saline (containing 1 mM EDTA and 10 \(\mu\)M indomethacin), mixed with an equal volume of ethanol, and centrifuged. The supernatant was diluted with 50 mM acetic buffer and purified through an affinity column (Cayman). After the column was equilibrated with column buffer (0.1 M phosphate-buffered saline, 7.7 mM \(\text{NaN}_3\), 0.5 M NaCl 2) followed by UltraPure water, the supernatant was eluted from the 4-ml column by adding the elution solution and allowing it to pass through the packing material. The eluate was then evaporated and redissolved in enzyme-linked immunoassay buffer, applied to a 96-well plate precoated with goat antimouse IgG, and incubated with PGE\(_2\) monoclonal antibody and recovery tracer buffer, applied to a 96-well plate precoated with goat antimouse IgG, then evaporated and redissolved in enzyme-linked immunoassay allowing it to pass through the packing material. The eluate was then eluted from the 4-ml column by adding the elution solution and allowing it to pass through the packing material. The eluate was then evaporated and redissolved in enzyme-linked immunoassay buffer, applied to a 96-well plate precoated with goat antimouse IgG, and incubated with PGE\(_2\) monoclonal antibody and recovery tracer buffer, applied to a 96-well plate precoated with goat antimouse IgG, then evaporated and redissolved in enzyme-linked immunoassay allowing it to pass through the packing material. The eluate was then eluted from the 4-ml column by adding the elution solution and allowing it to pass through the packing material. The eluate was then evaporated and redissolved in enzyme-linked immunoassay buffer, applied to a 96-well plate precoated with goat antimouse IgG, and incubated with PGE\(_2\) monoclonal antibody and recovery tracer buffer, applied to a 96-well plate precoated with goat antimouse IgG, then evaporated and redissolved in enzyme-linked immunoassay allowing it to pass through the packing material. The eluate was then eluted from the 4-ml column by adding the elution solution and allowing it to pass through the packing material.

**Statistical Analysis.** Differences between two groups were analyzed by a two-tailed \(t\) test. Analysis of variance was used to compare three or more groups, and Bonferroni's multiple comparisons test was used to detect differences across multiple groups.

**Results**

p18\(^{\text{INK4}}\) mRNA Expression Is Decreased in the Cerebral Cortex of hCOX-2 Transgenic Mice. Brains from 9-month-old female hCOX-2 transgenic mice overexpressing hCOX-2 under the regulation of the neuron-specific enolase promoter were collected and used for high-throughput mRNA screening with complementary DNA array microarray chips containing probes for 8832 mouse sequences; brain mRNA from age- and gender-matched WT mice were used as reference control. Genes showing consistent differential expression at the steady-state mRNA level or turnover in the brain of hCOX-2 transgenics were considered for further analysis by RNase protection assay. Among others, mRNA expression of CDK 4,6 p18\(^{\text{INK4}}\) was found to be consistently decreased in the cerebral cortex of hCOX-2 transgenics and further considered for its potential role in COX-2-mediated responses in the brain.

To perform an independent validation of the complementary DNA microarray data, we used the RNase protection assay (Fig. 1A). We observed an overall agreement (50% change by cDNA array versus 30% change by RNase protection assay) in the decreased expression of p18\(^{\text{INK4}}\) mRNA in the cerebral cortex of hCOX-2 transgenics \((P < 0.05, n = 4–6\) per group) between the two methods.

Next we tested the hypothesis that treatment of transgenic mice with COX-2 inhibitors could reverse the hCOX-2-mediated inhibition of p18\(^{\text{INK4}}\) mRNA expression in the brain. We found that treatment of the hCOX-2 transgenics with the preferential COX-2 inhibitor nimesulide for 3 months in the diet (1500 mg/kg of feeding) reversed the hCOX-2-mediated decrease of cortical p18\(^{\text{INK4}}\) mRNA expression in the brain of hCOX-2 transgenics (Fig. 1A). No detectable alteration of p18\(^{\text{INK4}}\) mRNA expression was found in WT mice treated with nimesulide compared with littermates fed a normal diet (Fig. 1A).
The decreased expression of the cortical p18\textsuperscript{INK4} mRNA expression in the hCOX-2 brain coincided with a 1.8-fold elevation of PGE\textsubscript{2} content assessed in the cerebral cortex of the same mice used for RNA studies, relative to wild-type control littermates (Fig. 1B).

Overexpression of Exogenous hCOX-2 Potentiates Glutamate-Mediated Apoptotic Neuron Damage in Vitro and Is Prevented by the CDK Inhibitor, Flavoperidol. Initial experiments were performed to characterize glutamate-mediated apoptotic damage using primary cortico-hippocampal neuron cultures derived from WT (B6C3) embryos. In these studies, neuronal death was evaluated by condensed pyknotic nuclear morphology using a bisbenzimide assay. We found that concentrations of glutamate ranging from 10 to 100 \( \mu M \) caused a dose-dependent increase of neurons with evident apoptotic damage (2- to 3-fold induction), 12 to 24 h after treatment (not shown). Based on this evidence, subsequent studies were performed using a dose of 50 \( \mu M \) glutamate for 24 h, which achieved a significant amount of apoptotic neuron damage within the linear range of increasing glutamate toxicity assessed by bisbenzimide in our culture conditions (not shown). In control studies we also found that the treatment of cortico-hippocampal neurons with the noncompetitive NMDA receptor antagonist MK801 (10 \( \mu M \)) blocked glutamate-mediated apoptotic damage (50 \( \mu M \) glutamate, 24 h treatment) (not show). Using primary cortico-hippocampal neuron cultures derived from transgenic embryos with neuronal overexpression of hCOX-2 or from WT littermate control embryos, we then tested the hypothesis that COX-2 in neurons could influence glutamate (50 \( \mu M \) glutamate, 24 h treatment)-mediated apoptotic damage.

We found that hCOX-2 overexpression in neuron potentiates by 2-fold the magnitude of the glutamate (50 \( \mu M \))-mediated neuronal apoptotic damage characterized by condensed pyknotic nuclear morphology, relative to control WT neuron cultures, 24 h post-treatment \((P < 0.05)\) (Figs. 2A and 3, d and e). Cotreatment of neuron cultures with the CDK inhibitor flavoperidol (1 \( \mu M \)) prevented the hCOX-2 potentiation of glutamate-mediated apoptotic damage, 24 h after treatment (Figs. 2A and 3f). Control hCOX-2 neuron cultures exposed to 1 \( \mu M \) flavoperidol alone for 24 h did not differ from vehicle (PBS) (Figs. 2A and 3, b and c) or 0.01% DMSO-treated hCOX-2 cultures (not shown). No detectable difference in number of neurons with apoptotic damage was found between control (untreated) cultures derived from WT or hCOX-2 embryos.

The hCOX-2-mediated induction of apoptotic neuron damage after treatment with glutamate coincided with a 50% potentiation of Ser\textsuperscript{795} pRb phosphorylation in a pool of parallel hCOX-2 cultures relative to the increase observed in a pool of WT cultures, 24 h after exposure to glutamate (Fig. 2B).

The COX-2 Preferential Inhibitor Nimesulide Neuroprotects While Preventing Glutamate-Mediated Induction of Ser\textsuperscript{795} pRb Phosphorylation in WT Cortico-hippocampal Neurons. Using primary cortico-hippocampal neuron cultures derived from WT control embryos (B6C3), we then tested the hypothesis that preferential COX-2 inhibitors may neuroprotect against glutamate-mediated apoptotic damage, possibly through the modulation of Ser\textsuperscript{795} pRb phosphorylation.

We found that treatment of cortico-hippocampal neuron cultures with the preferential COX-2 inhibitor nimesulide \((1 \mu M, 24 \text{ h})\) neuroprotected against glutamate-mediated apoptotic damage (Fig. 4A). Moreover, the nimesulide neuroprotection coincided with the inhibition of glutamate-mediated induction of Ser\textsuperscript{795} pRb phosphorylation, in parallel cultures (Fig. 4B). Neuron cultures exposed to 1 \( \mu M \) nimesulide alone for 24 h did not differ from untreated control cultures with respect to apoptotic damage (Fig. 4A) and the levels of Ser\textsuperscript{795}-phosphorylated pRb (Fig. 4B).

Discussion

In this study, using complementary DNA microarray technique, we found that the mRNA expression of the INK p18\textsuperscript{INK4}, an inhibitor of CDK 4,6 that controls the activation
clear morphology. Fluorescence-positive neurons characterized by condensed pyknotic nuclei were inhibited by flavoperidol alone. In panels a to f, arrows point toward bisbenzimide labeling which effectively inhibits multiple classes of cell cycle G1 phase-inhibitory proteins. Inhibition of cell cycle G1 phase by flavoperidol treatment coincides with elevation of Ser795 Rb phosphorylation. In A, cortico-hippocampal neuron cultures derived from wild-type (B6C3) embryos were treated with glutamate (GLU, 50 μM) and/or nimesulide (NMS, 1 μM) 24 h before apoptotic neuron damage was assessed. Cultures treated with 0.01% DMSO did not differ from treated controls (not shown). *P < 0.001 versus untreated group. In B, parallel cortico-hippocampal neuron cultures, derived from WT control embryos as shown in A, demonstrated that the elevation of Ser795 Rb phosphorylation by glutamate treatment (24 h, 50 μM) is prevented by cotreatment with NMS (1 μM). Values represent means ± S.E.M. of determinations made in five separate cultures for glutamate alone and three separate cultures for the other groups; *P < 0.01 versus untreated control cultures. In B inset, representative Western blot immunoreactivity of Ser795 Rb. In A and B, apoptotic neurons were counted from six to eight random fields within premarked reticules of 1 mm² (n = 4–6 culture chambers per group, per culture).

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Two classes of CDK inhibitors, the INK4 (p16, p18, p21) and the cycle-inhibitory protein (CIP) (p21, p27, p57) families, have been defined based on sequence similarity (Elledge et al., 1998) and found to differ in specificity and mechanisms of inhibition (Zindy et al., 1997). Unlike the CIP family, which effectively inhibits multiple classes of cell cycle G1 phase-inhibitory proteins including CDK2, CDK3, and CDK4 and CDK6, the INK4 family is specific for CDK4 and CDK6 (reviewed in Elledge et al., 1996). The link between CDK activity and cell cycle is provided by phosphorylation of the Rb, a phosphoprotein that regulates growth in the G1 phase of the cell cycle (Weinberg, 1995), in part by binding to and inhibiting critical regulatory proteins that include members of the E2F family of transcription factors (Connell-Crowley et al., 1997). We also note that, physiologically, Rb is differentially regulated by CDK complexes; for example, there is evidence that CDK 4,6 phosphorylation steps appear to activate pRb, with subsequent phosphorylation steps being inactivating (Ezhevsky et al., 2001). pRb is phosphorylated on a defined number of Serine and threonine residues by activation of CDK 4,6 (Wang et al., 1994). Chronic treatment of the transgenic mice with the preferential COX-2 inhibitor nimesulide (Warner et al., 1999) in the diet prevents the hCOX-2-mediated decrease of the CKI 4,6 mRNA expression. This evidence in vivo was extended to in vitro studies revealing that hCOX-2 overexpression in primary cortico-hippocampal neuron cultures potentiates excitotoxic-mediated apoptotic damage, which coincides with elevation of Ser795 pRb, an index of CDK 4,6 activation (Connell-Crowley et al., 1997). This finding, coupled with the observation that the CDK inhibitor flavoperidol and the preferential COX-2 inhibitor nimesulide may prevent apoptotic neuron damage, suggests that a mechanism by which neuronal COX-2 may influence excitotoxicity is through activation of cell cycle activity. Consistent with our evidence, recent studies also suggested that the loss of expression of the endogenous CDK inhibitor p16INK4a in neurons and the activation of cell cycle machinery may be responsible for delayed neuronal cell death in a model of neuronal ischemic damage (Katchanov et al., 2001).
Our in vitro studies show that hCOX-2 overexpression in primary cortico-hippocampal neurons derived from homozygous hCOX-2 transgenic mice promotes glutamate-mediated apoptotic damage. This is consistent with our previous finding, showing that neuronal hCOX-2 potentiates the intensity and lethality of KA excitotoxicity (Kelley et al., 1999). We have found that a potential mechanism by which hCOX-2 may promote excitotoxicity in vitro is by influencing Ser795 pRb phosphorylation. Conversely, this was found to be prevented by treatment of neurons with the COX-2 preferential inhibitor, nimesulide (Warner et al., 1999). This evidence is of high relevance in view of the finding that cell cycle abnormalities occur in several neurodegenerative diseases (reviewed in Raina et al., 2001, including Alzheimer’s disease (AD) (Raina et al., 2000) and models of AD (Giovanni et al., 1999, 2000; Xiang et al., 2001). Also interesting, and consistent with our findings, is the recent observation that the CDK inhibitor flavoperidol may neuroprotect against excitotoxicity (Park et al., 2000), thus further underscoring the implication of cell cycle activity involvement in excitotoxic neurodegeneration.

Ongoing studies on cancer continue to show that the loss of endogenous CDK inhibitors such as INKs and CIP-KIP is sufficient to precipitate abnormal cell proliferation, coincidental with pRb activation (Elleridge et al., 1996; Connell-Crowley et al., 1997; Schreiber et al., 1999). This is in agreement with other studies on brain tumors showing that the loss of endogenous CDK inhibitors is sufficient to precipitate uncontrolled proliferation (Nishikawa et al., 1995; Ueki et al., 1996). However, apoptotic neuron death appears to be closely tied together with abnormal cell cycle in neurodegenerative diseases. This has been also demonstrated in AD (Raina et al., 2001), where abnormal cell cycle activities in the brain were correlated with neuronal death (Herrup and Busser, 1995). The general interpretation of these findings is that if neurons committed to a permanent cessation of cell division, then, once they are forced to reenter the cell cycle, they die (Raina et al., 2000). Thus, the attempt to reenter the cell cycle may be a significant factor that accompanies neurodegeneration and AD. Our study brings a new perspective to this hypothesis by suggesting that neuronal COX-2, which is also elevated in the AD brain (Ho et al., 2001), may influence the excitotoxic neuronal cell in postmitotic neurons by promoting an unsuccessful attempt to reenter the mitotic cycle as schematized in Fig. 5.

The apparent protean effect of COX inhibitors in AD (reviewed in Pasinetti, 2001) suggests that COX may be involved in neurodegeneration. The mechanism of action for the beneficial role of nonsteroidal anti-inflammatory drugs in AD is unclear; it is generally assumed that their effects are mediated by competitive inhibition of COX catalytic activity, thus reducing the production of inflammatory prostaglandins from membrane-derived arachidonate (Warner et al., 1999). However, the role of COX-2 inhibitors in neurodegeneration may be far more complex. For example, although there is evidence that COX-2-specific inhibitors neuroprotect in vitro against glutamate toxicity (Hewett et al., 2000), recent evidence suggests that inhibition of COX-2 may also worsen responses to traumatic brain injury (Dash et al., 2000) and KA seizures (Baik et al., 1999). The demonstration in the present study that neuronal hCOX-2 overexpression in the brain may influence cell cycle activities and increase susceptibility to excitotoxicity suggests a novel, noninflammatory role for COX-2 in the brain, and has important implications for understanding its role in neurodegeneration and, possibly, the development of therapeutic strategies for neurodegenerative diseases.

Acknowledgments

We thank Dr. Paul Aisen and Patrick Pamplin for the discussion of the studies. We thank Isabela Diaconescu for the superb secretarial work.

References


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