

Cyclooxygenase-2 (COX-2) in Inflammatory and Degenerative Brain Diseases

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Abstract. Cyclooxygenase (COX) catalyses the first committed step in the synthesis of prostanoids, a large family of arachidonic acid metabolites comprising prostaglandins, prostacyclin, and thromboxanes, and is a major target of non-steroidal anti-inflammatory drugs (NSAIDs). COX exists as constitutive and inducible isoforms. COX-2 is the inducible isoform, rapidly expressed in several cell types in response to growth factors, cytokines, and pro-inflammatory molecules. Since its discovery in the early 1990s, COX-2 has emerged as a major player in inflammatory reactions in peripheral tissues. By extension, COX-2 expression in brain has been associated with pro-inflammatory activities, thought to be instrumental in neurodegenerative processes of several acute and chronic diseases. However, 2 major aspects should be borne in mind. First, in the central nervous system, COX-2 is expressed under normal conditions and contributes to fundamental brain functions, such as synaptic activity, memory consolidation, and functional hyperemia. Second, “neuroinflammation” is a much more controlled reaction than inflammation in peripheral tissues, and in many cases is triggered and sustained by activation of resident cells, particularly microglia. In spite of the intense research of the last decade, the evidence of a direct role of COX-2 in neurodegenerative events is still controversial. This article will review new data in this area, focusing on some major human neurological diseases, such as multiple sclerosis, amyotrophic lateral sclerosis, Parkinson disease, Creutzfeldt-Jakob disease, and Alzheimer disease. Furthermore, the emerging role of COX-2 in behavioral and cognitive functions will be discussed.

Key Words: Brain inflammation; Cyclooxygenase (COX); Neurodegeneration; Neuroprotection; Non-steroidal anti-inflammatory drugs (NSAIDs); Prostaglandin.

INTRODUCTION

Cyclooxygenase (COX), also known as prostaglandin (PG) H synthase, catalyses the first committed step in the synthesis of prostanoids, a large family of arachidonic acid metabolites comprising PGs, prostacyclin, and thromboxanes. COX has become a very popular enzyme since 1971, when it was demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory properties through the inhibition of COX enzymatic activity, thus preventing PG synthesis (1). We know now that this heterogeneous class of drugs affects many other important cellular targets, nonetheless COX remains central to the development of anti-inflammatory treatments of a variety of pathologies, including neuroinflammatory and neurodegenerative diseases.

COX is a unique enzyme. First, it exhibits 2 catalytic activities, a bis-oxygenase activity (cyclooxygenase), which catalyses PGG₂ formation from arachidonic acid, and a peroxidase activity, which reduces PGG₂ to PGH₂, the final substrate for the specific synthases. The peroxidase activity also results in the production of free radicals, which are in part utilized by COX itself (Fig. 1). The two enzymatic activities occur at distinct, interacting sites on the COX molecule and external factors can affect each of them independently (2). Second, during the cyclooxygenase activity, COX undergoes a conformational

rearrangement leading to an unstable intermediate, which eventually gives rise to an inactive enzymatic species. This process, known as “suicide” inactivation, occurs both in vitro and in vivo and represents a limiting factor in prostanoids synthesis (3).

COX is an integral membrane glycoprotein, consisting of a homodimer with an associated heme group involved in both enzymatic activities (2). Besides a constitutive isoform (COX-1), which is widely distributed in virtually all cell types and is thought to mediate physiological responses, a second and inducible isoform, termed COX-2, was identified in the early 1990s (4). COX-2 is rapidly expressed in several cell types in response to growth factors, cytokines, and pro-inflammatory molecules and has emerged as the isoform primarily responsible for prostanoid production in acute and chronic inflammatory conditions.

COX-1 and COX-2 are coded by 2 distinct genes located on human chromosome 9 and 1, respectively. The *COX-2* gene is characterized by the presence of a TATA box and a multitude of binding sites for transcription factors in its promoter region, which account for the complex regulation of COX-2 expression. In addition, a long 3'-untranslated region, which has been found in many immediate-early genes, acts as mRNA instability determinant or as translation inhibitory element, suggesting post-transcriptional control of COX-2 expression. On the contrary, the *COX-1* gene represents a classical “house-keeping” gene, lacking of a TATA box in its promoter.

At the protein level, the 2 isoforms show >60% homology in humans and rodents. While the functional sites are conserved, a few crucial substitutions cause important conformational variations in the active site pocket of the

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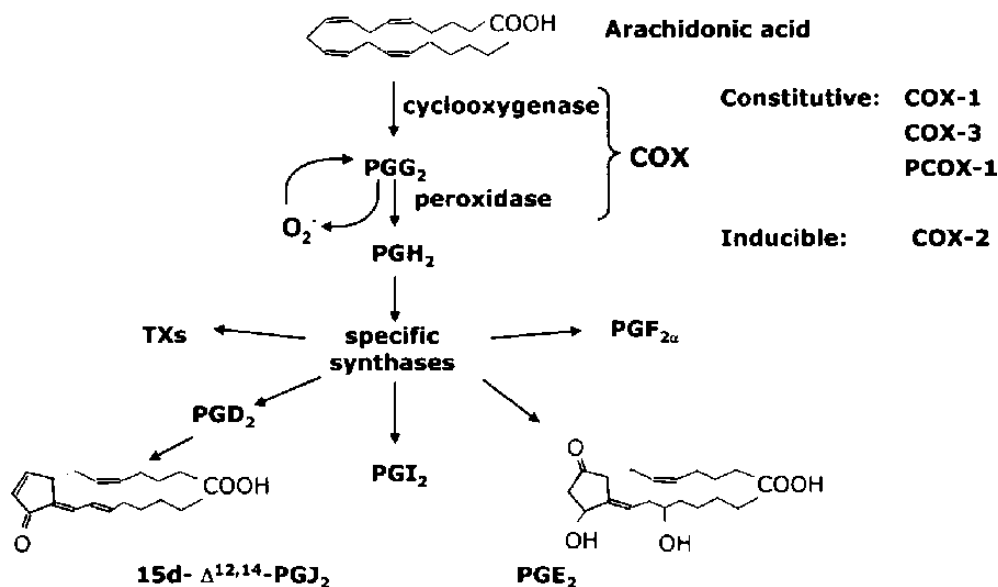


Fig. 1. Cyclooxygenase pathway. Prostaglandin (PG) biosynthesis starts after the release of arachidonic acid from membrane phospholipids by phospholipase A₂. Then, cyclooxygenase (COX) catalyses the first 2 steps (i.e. a cyclooxygenase reaction and a peroxidase reaction), converting arachidonic acid into the unstable intermediate PGH₂, the precursor of all PGs and thromboxanes (TXs). The final step in the biosynthesis of PGs involves specific synthases.

2 isoenzymes, which could account for the different sensitivities of COX-1 and COX-2 to specific inhibitors (5). One important difference between the 2 isoforms is the 18-amino acid insert near the COX-2 C-terminus, which is not present in COX-1 and has allowed the production of specific antibodies.

The distribution of the 2 COX isoforms has been extensively studied in rat and human tissues. In the majority of the tissues, COX-1 appears to be the only isoform constitutively expressed, confirming the involvement of this isoform in physiological functions, such as cytoprotection of stomach and platelet aggregation. However, in brain, testes, and kidney macula densa cells, both COX-1 and COX-2 are expressed under physiological conditions (2). In rat brain, COX-1 and COX-2 immunoreactivities are present in discrete neuronal populations distributed in distinct areas of cerebral cortex and hippocampus. In other regions, such as midbrain, pons, and medulla, COX-1 immunoreactivity prevails (6, 7). Similarly, mRNAs for both COX-1 and COX-2 are present in several regions of human brain, although COX-2 is the prominent isoform particularly in the hippocampus (8, 9).

The relative contribution of COX-1 and COX-2 activity to brain pathology and physiology has been recently questioned (10, 11). On one side, it has been argued that COX-1 activity in brain diseases has been overlooked; on the other side, mandatory evidence suggests that COX-2 plays a special role in normal neuronal function and in neurotoxicity. Although this debate will be solved only by further clinical and experimental studies, it is clear

that the popular paradigm by which COX-1 serves physiological functions and COX-2 is responsible for “pathological” PGs, cannot explain an increasing number of findings.

Recently, a third variant of COX, named COX-3, and 2 partial COX-1 proteins (PCOX-1 proteins) have been identified from canine and human cerebral cortex cDNAs (12). COX-3 and one of the PCOX-1 are products of the *COX-1* gene, but retain intron 1 in their mRNA. Both proteins are tissue-specific, with the highest expression in the brain followed by heart. Within the brain, the highest levels are in the cerebral cortex, where the expression of the *COX-1* variant gene accounts for ~5% of *COX-1*. As its counterpart COX-1, COX-3 is not induced by acute inflammatory stimulation (13). COX-3, but not PCOX-1, exhibits enzymatic activity that is glycosylation-dependent and especially sensitive to the inhibitory activity of paracetamol (acetaminophen). Thus, COX-3 could represent the brain-specific COX isoform, the existence of which was hypothesized a few decades ago to explain the potent analgesic and antipyretic actions of paracetamol in spite of its poor ability to inhibit COX from peripheral tissues (12). Nonetheless, the functional role of COX-3 is largely unknown and more intense research is required to elucidate the contribution of COX-3 to the overall PG production in brain.

The potential role of COX isoforms and PGs in brain diseases has been extensively reviewed in the past years (14–16). Over-expression of COX-2 has been associated with neurotoxicity in acute conditions, such as hypoxia/

ischemia and seizures. However, the beneficial or detrimental role played by COX-2 in inflammatory and neurodegenerative brain pathologies is still controversial.

The present article will review new data in this area, focusing on some major human neurological diseases, such as multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Parkinson disease (PD), Creutzfeldt-Jakob disease (CJD), and Alzheimer disease (AD). First, the emerging role of COX-2 in cognitive functions will be discussed since understanding the role of COX-2 in brain function is an important prerequisite to fully understanding how to exploit the potential benefits of COX-2 inhibition in disabling neurological diseases.

COX-2 in Brain Function

In mammalian brain, COX-2 is constitutively expressed in specific neuronal populations under normal physiological conditions. In rat brain, COX-2 mRNA and immunoreactivity were detected in dentate gyrus granule cells, pyramidal cell neurons in the hippocampus, the piriform cortex, superficial cell layers of neocortex, the amygdala, and at low levels in the striatum, thalamus, and hypothalamus (6, 7). This “constitutive” neuronal COX-2 expression should be more correctly regarded as “dynamically” regulated since it is dependent on normal synaptic activity, is rapidly increased during seizures or ischemia, and is downregulated by glucocorticoids (6). The dependence of COX-2 expression on natural excitatory synaptic activity is supported by the presence of COX-2 immunoreactivity in distal dendrites and dendritic spines, which are involved in synaptic signaling, and by its exclusive localization to excitatory glutamatergic neurons. Furthermore, the heterogeneous distribution within a neuronal population is compatible with induction of COX-2 in subsets of neurons in response to natural excitatory synaptic stimulation, as shown for other immediate early genes activated by excitatory stimulation (14).

The involvement of COX-2 in synaptic activity is further supported by the developmental profile of COX-2 expression. In rat brain, COX-2 expression follows developmental gradients and coincides with the critical period of activity-dependent cortical development (17). In Rett syndrome—a neurological disorder associated with mental retardation, defective development of cortical neurons, and abnormalities of dendritic branching—the laminar pattern of cortical COX-2 immunoreactivity is disrupted, in that COX-2-positive neurons are decreased in number and randomly distributed (18).

Rats subjected to selective destruction of basal forebrain cholinergic neurons during the first post-natal week showed decreased levels of COX-2, but not COX-1, in the hippocampus at adulthood. This effect was accompanied by impairment in social memory, suggesting that the early loss of hippocampal cholinergic input may impact on the expression of COX-2 in hippocampal neurons

and on the functional role of PGs in synaptic activity (19).

Indirect evidence of COX-2 involvement in synaptic plasticity has been obtained in the recent years by using COX inhibitors in *in vivo* and *in vitro* models of synaptic plasticity. COX-2 inhibitors, but not COX-1 selective inhibitors, administered systemically shortly after training in the Morris water maze (a hippocampal-dependent learning task) have been shown to impair spatial memory in rats (20). Similarly, intracerebral injection of COX inhibitors in chicks attenuated memory of a passive avoidance response (21). In addition, pre-training infusion of a COX-2-specific inhibitor (celecoxib) in the hippocampus of adult rats impaired acquisition of the Morris water maze, suggesting that in rats COX-2 activity in the hippocampus is necessary for both memory and learning of a spatial task (22). In keeping with these findings, systemic administration of ibuprofen, a non-selective COX inhibitor, caused deficits in spatial learning in the water maze and in the induction of long-term potentiation (LTP), a major model of synaptic plasticity (23). Ibuprofen administered 1 hour prior to behavioral task or electrophysiological procedures did not affect non-hippocampal tasks or baseline synaptic transmission. In addition, the drug abolished the increase in PGE₂ and brain-derived growth factor (BDNF) levels following LTP and spatial learning. A period of prior exercise in a running wheel reverted the inhibitory effect of ibuprofen on LTP and spatial learning, most likely through an increase in BDNF levels, supporting the hypothesis that COX activity plays a permissive role in synaptic plasticity and spatial learning via BDNF-associated mechanisms (23). In agreement with this hypothesis, PGE₂ but not PGD₂, reversed the suppression of LTP induced by COX-2-inhibitor in hippocampal dentate granule neurons *in vitro* (24). PGE₂, which is preferentially formed during the enzymatic activity of COX-2 rather than of COX-1, could participate to synaptic plasticity through several mechanisms, including modulation of adrenergic, noradrenergic, and glutamatergic neurotransmission, remodeling of actin in the cytoskeleton thus influencing the shape of spines and dendrites, and regulation of membrane excitability (25). Moreover, COX-2-derived PGs are involved in the coupling of synaptic plasticity with cerebral blood flow, as suggested by the attenuation of the increase in neocortical blood flow in response to vibrissal stimulation by the COX-2 inhibitor NS398. The hyperemic response was also impaired in mutant mice lacking of COX-2 (26).

In spite of the emerging evidence of a physiological role for COX-2 in brain development and function, COX-2 knockout mice show no gross abnormalities of brain anatomy. However, these mice exhibit early and progressive renal failure that prevents accurate behavioral analysis (27). In addition, significant compensatory effects of COX-1 and, possibly, COX-3 cannot be ruled out.

Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS, with a typical onset in the early adult life and characterized by perivascular infiltration of lymphocytes and macrophages into the brain parenchyma. The etiology of MS is not yet fully elucidated, but immunological mechanisms are crucial in the initiation and progression of the disease. Activation of autoreactive brain antigen-specific T-cells and inflammatory attack are associated with demyelination, oligodendrocyte death, axonal damage and, ultimately, neuronal loss.

Although there is evidence that inflammation may be beneficial in MS, several inflammatory mediators, including pro-inflammatory cytokines and free radicals, are thought to contribute to cell damage in this disease (28). In addition, glutamate-mediated excitotoxic death of oligodendrocytes has been reported to contribute to the pathogenesis of demyelinating diseases (29). In this light, the expression of COX-2 and its contribution to the pathogenic events in MS have been explored in several animal models and in MS patients.

COX-2 immunoreactivity has been found in experimental autoimmune encephalomyelitis (EAE), an extensively used animal model. Analysis of spinal cord of SJL mice immunized with a peptide of the myelin constituent proteolipid protein revealed that, during the acute phase of EAE, COX-2 expression is confined within infiltrating macrophages and ramified microglia close to the inflammatory infiltrates. Very rare reactive astrocytes expressed COX-2 in this phase, but their number significantly increased during relapse phase, suggesting that COX-2 induction in astrocytes could be due to soluble factors, i.e. cytokines, produced during the protracted inflammatory insults (30).

In a different EAE model, in which Lewis rats were immunized with a peptide of another myelin protein, the myelin basic protein, COX-2 immunoreactivity was exclusively found associated with neurons and endothelial cells (31). The number of COX-2-positive endothelial cells increased with the progression of the disease, most prominently in areas of cellular infiltration. Macrophage/microglia-like cells expressing COX-1 were disseminated throughout the brain parenchyma of control animals. In EAE, the number of COX-1-positive macrophages increased along with that of COX-2-positive cells.

In a rat model of delayed-type hypersensitivity response to heat-killed bacillus Calmette-Guérin, which results in T-cell and macrophage recruitment to the brain parenchyma, breakdown of BBB, primary demyelination, and axon damage, COX-2 expression was restricted to major infiltrating hematogenous cell populations such as neutrophils and mononuclear phagocytes, and to perivascular cells of the blood vessels in the vicinity of the lesion. These perivascular cells were identified as macrophages, but the possibility that some endothelial cells also

expressed COX-2 could not be ruled out. Neuronal COX-2 was not affected by the ongoing inflammation. In spite of the extensive astrocyte and microglial reaction occurring over a broad area surrounding the inflammatory lesions, there was no obvious COX-2 staining in these cells, indicating that the upregulation of COX-2 expression in this model of chronic, immune-mediated lesions is remarkably restricted to the lesion sites (32).

A similar restricted COX-2 expression has been described in brain tissues from 7 MS patients (33). In these specimens, characterized by the presence of chronic active lesions, COX-2 expression was studied by sophisticated confocal microscopy analysis. COX-2-positive cells were present in all chronic active lesions examined, and generally located on the border of myelinated regions. COX-2 immunoreactivity was largely, but not exclusively, associated with cells expressing the macrophage/microglial marker CD64, the FC receptor typically associated with activated macrophages. However, not all CD64-positive cells expressed COX-2. Expression of COX-2 was frequently associated with that of inducible NO synthase (iNOS), suggesting that both enzymes could contribute to the progression of MS, through their ability to produce free radicals such as superoxide anion and NO, which may combine into the more toxic free radical species peroxynitrite. The authors also propose that the colocalization of COX-2 and iNOS may be functionally linked to oligodendroglial excitotoxic death in MS. Indeed, both PGE₂ and peroxynitrite could increase the local concentration of glutamate to toxic levels by inducing Ca²⁺-dependent glutamate release and inactivating glutamate transporters, respectively (34, 35).

Nonetheless, a protective role of COX-2 in MS cannot be excluded. Despite its classical pro-inflammatory role on vascular permeability and leukocyte extravasation, PGE₂ regulates several immune functions and downregulates the process of macrophage and microglial activation, thus self-limiting the inflammatory process (15, 35). PGE₂ levels were elevated during the recovery phase in a murine model of MS, suggesting a protective effect of PGE₂ in this model (37). Conversely, Reder et al reported that the NSAID indomethacin suppressed active EAE (38). The suppressive effect was potentiated by co-administration of misoprostol, a PGE₂ analog, suggesting that distinct COX-derived products (i.e. ROS and PGs) may have protective or detrimental effects in EAE.

There is a controversial literature on PGs and, more generally, arachidonic acid metabolites in MS patients (39). Recently, cerebrospinal fluid (CSF) levels of PGE₂ and isoprostane 8-epi-PGF₂, a stable lipid peroxidation product currently used as an index of oxidative stress *in vivo*, were measured in a group of MS subjects (40). Both metabolites were increased in these subjects when compared to control subjects. The levels of 8-epi-PGF₂, but not PGE₂, correlated with the disability grade, supporting

the hypothesis that in MS lipid peroxidation, myelin damage, and disability are correlated. The amounts of PGE₂ and that of 8-epi-PGF₂ in the CSF of each single subject were not correlated, suggesting that PG synthesis and lipid peroxidation are unrelated process in this disease. The lack of correlation between PGE₂ levels and disability grade is consistent with some of the previous literature, which supports a dual role of inflammation and PGE₂ in the pathogenesis of MS, showing beneficial and detrimental effects (28).

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive loss of motor neurons, typically resulting in death within 5 years of onset. Although the sporadic form (sALS) is the most frequent, 5% to 10% of cases are familial, being associated with several genes. Missense mutations in the gene encoding for the Cu/Zn superoxide dismutase (SOD1) account for a familial form of ALS linked to chromosome 21q. This finding has led to the development of transgenic mice expressing mutant SOD1 with phenotype that mimics clinical and pathological characteristics of the human disease. Spinal cord tissue from patients who died of ALS shows several neuro-inflammatory changes that are found in other neurodegenerative diseases, such as increased levels of pro-inflammatory molecules, astrogliosis, and microglial activation. Thus, it has been suggested that inflammatory-related processes may promote motor neuron death. In addition, high CSF levels of glutamate and excitotoxicity have been reported in ALS (41).

The well-established role of COX-2 in inflammation and in glutamate-dependent neurotoxicity has set the basis for the hypothesis of COX-2 involvement in ALS pathogenesis.

COX-2 mRNA and protein were increased in post-mortem spinal cords of ALS patients (42) and transgenic mutated SOD1 mice (43). Elevation of PGE₂ tissue levels paralleled the increased expression of COX-2 (43). Consistently, 2 independent studies reported increased levels of PGE₂ in the CSF of ALS patients (44, 45). The cell types expressing COX-2 have been identified in both animal and human specimens. Under normal conditions, COX-2 is expressed in neurons in the spinal cord dorsal and ventral horns as well as in dorsal root ganglia. In postmortem spinal cord of ALS patients, COX-2 expression was markedly increased and localized to both neurons and glial cells. The number of COX-2-positive motor neurons and interneurons was significantly increased in spite of the expected overall reduction in the total number of neurons. In addition, COX-2 was associated with astrocytes and, and to a much lesser extent, with microglial cells. In contrast, COX-1 immunoreactivity was

confined to some microglial cells and there was no significant difference was detected between control and ALS specimens (45). A similar pattern of COX-2 expression was reported for the mutated SOD1 transgenic mice (43).

The role of COX-2 activity in ALS was examined by using selective COX-2 inhibitors. In the first study, the COX-2 inhibitor SC236 significantly protected motor neurons in an organotypic spinal cord culture model, in which neuronal death is induced by treo-hydroaspartate, an inhibitor of astrocytic glutamate re-uptake (46). These findings suggested that COX-2 could take part in the excitotoxic damage caused by elevated glutamate levels. Subsequently, the same group showed that treatment of SOD1 transgenic mice with COX-2 inhibitor celecoxib significantly delayed the onset of disease, prolonged the survival and reduced the spinal neurodegeneration and glial activation (47). These studies suggest that inhibition of COX-2 could have therapeutic benefits by altering the cascade of events leading to the progressive neuronal death in ALS patients. However, in these studies mice received the COX-2 inhibitor treatment beginning several weeks before the onset of disease, defined as a 30% decrease in motor performance. Thus, the efficacy of COX-2 inhibition in the presence of overt clinical signs of disease remains to be investigated.

Several mechanisms could be triggered by COX-2 overexpression. In addition to the enhancing effect of PGE₂ on glutamate release, COX-2 could contribute to oxidative stress-mediated damage by producing oxidizing reactive species during the peroxidase activity (Fig. 1). In transgenic mutated SOD1 mice, COX-2 and iNOS are induced with a similar temporal pattern and co-expression of the 2 enzymes, as discussed in the previous section, could lead to the formation of more reactive free radical species such as peroxynitrite.

COX-2 could also contribute to ALS by promoting inflammatory processes. However, as mentioned before, PGE₂ has a broad array of functions, including potential protective effect (36). In addition to PGE₂, other PGs can be generated and influence the course of disease. 15-deoxy- Δ^{12-14} -PGJ₂ (15d-PGJ₂) immunoreactivity was found in spinal cords of sporadic ALS patients (48). 15d-PGJ₂ derives from the non-enzymatic dehydration of PGD₂, a major brain PG, and activates the nuclear receptor peroxisome proliferator activated receptor- γ . This receptor has recently received great attention for the potential therapeutic benefits of its activation in several inflammatory neurological diseases (36).

Parkinson Disease

Parkinson disease (PD) is a major neurodegenerative disease, characterized by the progressive loss of dopamine-containing neurons in the substantia nigra, which

results in severe movement disorders. Idiopathic PD accounts for the majority (>90%) of the cases. The remaining cases are mostly familial PD forms that are correlated with mutations of few genes, including synuclein and parkin. Idiopathic PD, unlike the familial form occurring earlier, usually begins in the fifth decade of life and progress over long periods of time (10 to 20 years). The etiology of idiopathic PD is still not understood (49). Human and animal studies have consistently evidenced robust microglial activation, suggesting an important role for these immunocompetent cells in the pathogenesis of PD. On the contrary, astrogliosis has been sporadically observed in patients and in some animal models (50). Inflammation, oxidative stress, and mitochondrial impairment are thought to play a key role in the disappearance of dopaminergic neurons and increasing experimental observations in several PD models indicate that attenuation of inflammation may reduce neurodegeneration. At present, there are conflicting results on the ability of various NSAIDs to reduce neurodegeneration in cellular and animal PD models (49). In some cases, neuroprotection has been related to free radical scavenging effects rather than to COX inhibition or attenuation of inflammation (51, 52). Two studies have investigated the expression of COX isoforms in postmortem PD specimens or in PD experimental models. The first study reported an increased expression of COX-2 in amoeboid or activated microglial cells in the substantia nigra from 11 idiopathic PD patients, whereas neuronal and astroglial COX-2 expression was not different in the control and PD groups. Moderate COX-1 immunoreactivity was observed in some neuronal somata and processes and in few glial cells in both groups, suggesting that the greater potential for PG synthesis is associated to COX-2 and microglial cells (53). By contrast, the second and more recent study (52) showed that COX-2 is specifically induced in substantia nigra dopaminergic neurons in postmortem PD specimens and in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model. No obvious staining of astrocytes and activated microglia was detected. In addition, PGE₂ levels were increased in both human and mouse tissues. The involvement of COX-2 in PD neurodegeneration was further suggested by the observation that MPTP neurodegeneration was mitigated in COX-2, but not in COX-1, knock out mice. Several technical aspects can account for the apparent discrepancies between the 2 studies, including tissue preservation and fixation, specificity of primary antibodies, and sensitivity of detection systems. Nonetheless, the results of the 2 studies remain to be reconciled.

Creutzfeldt-Jakob Disease

Creutzfeldt-Jakob disease (CJD) is the best known human form of transmissible spongiform encephalopathies or prion diseases, a heterogeneous group of infectious,

sporadic, and genetic disorders characterized by rapidly progressive dementia and by more than 90% mortality within 1 year from the onset. The characteristic neuropathological signs of the disease are amyloid deposition of the proteinase-resistant prion protein (PrP^{res} or PrP^{Sc}), astrocytosis, and spongiform degeneration. A further disease hallmark is the extensive microglial activation observed in CJD patients as well as in experimental prion diseases, which supports the occurrence of a local non-immune mediated chronic inflammatory response (54).

In spite of the prominent microglial activation, classical pro-inflammatory mediators such as IL-1, IL-6, TNF- α , and IFN- γ were not detected in significant amount in a murine model of prion disease in which C57BL/6J mice are infected with scrapie, the prion form affecting sheep. By contrast, the immunoregulatory cytokine transforming growth factor- β and PGE₂ were increased (54, 55). The increase in hippocampal PGE₂ levels was associated with a strong induction of COX-2 expression, which increased with the progression of disease and was specifically localized to microglial cells (56). Few scattered COX-1-positive microglia-like cells were found in control and infected brains (Fig. 2). These findings were then confirmed in a second murine model in which CH3 mice were infected with homogenates from 2 cases of genetic CJD and 3 cases of sporadic CJD (57), suggesting that the selective upregulation of COX-2 in microglial cells is not characteristic of a specific prion agent or mouse strain.

Different results were reported in a recent study in which both COX-1 and COX-2 were increased in sporadic CJD cortex (58). COX-1 immunoreactivity was present in macrophages/microglial cells whereas COX-2 was predominantly in neurons. mRNAs and proteins of both isoforms were higher in tissue from temporal lobe of 1 CJD patient when compared to 1 neuropathologically unaltered control case.

Increased COX activity in prion diseases was confirmed by 2 studies (55, 59), reporting elevated CSF PGE₂ levels in a group of 62 subjects affected by sporadic and genetic CJD and in 18 cases of variant CJD, the novel human form associated with the consumption of contaminated bovine products. In sporadic CJD patients, higher CSF levels of PGE₂ were associated with shorter survival. PGE₂ levels were not dependent on the time of CSF sampling during the course of the disease, suggesting that PGE₂ may be an index of disease severity rather than progression (55).

The increased levels of PGE₂ in the CSF of CJD patients and the high expression of COX-2 in microglial cells in experimental prion diseases suggest that PGE₂ synthesis may be associated with the clearance of apoptotic neurons. In CJD, abundance of apoptotic neurons

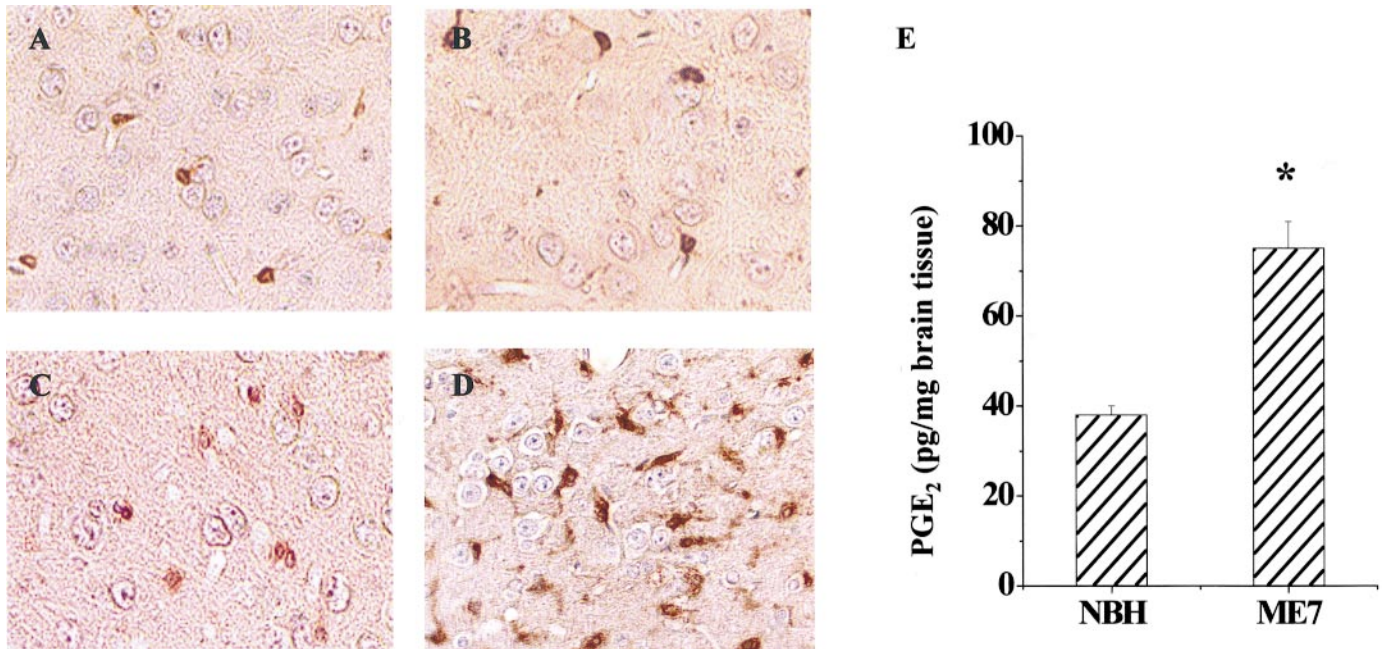


Fig. 2. COX-1 and COX-2 immunoreactivity and PGE₂ levels in murine prion disease brain. C57BL/6J mice were injected into the dorsal hippocampus with brain homogenate infected by the ME7 scrapie strain, and by 20 to 24 weeks post-injection develop the typical clinical signs of prion diseases, such as hunched posture, fur ruffling, and mobility reduction. At histopathological examination, the brains show an extensive astrocytosis and microglial activation in most cortical areas of both hemispheres, which are not found in age-matched mice injected by the same procedure with normal brain homogenate (NBH). COX-1 (A, C) and COX-2 (B, D) immunostaining of NBH- (A, B) and ME7-injected (C, D) mice. **E:** Hippocampal PGE₂ levels in NBH- and ME7-injected mice. Data are mean SEM (n = 5), p < 0.05 vs NBH-injected animals. Immunohistochemistry and PGE₂ measurements were performed as previously described (55, 56). Anti-COX-1 and anti-COX-2 antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); high sensitivity enzyme-immunoassay for PGE₂ was from Assay Design, Inc. (Ann Arbor, MI).

correlated well with microglial activation (60), and recently, interaction of microglial cells with apoptotic neurons has been reported to selectively promote COX-2 expression and PGE₂ synthesis (61).

Alternatively, PGE₂ could be associated with neuronal death, as suggested by the observation that in neuroblastoma cells, PrP peptides increase PGE₂ levels and COX-1 inhibitors protect against PrP toxicity (62). By contrast, the non-selective COX inhibitor indomethacin had no significant effect on onset of clinical signs and survival time in experimental prion disease (63). At present, whether PGE₂ contributes to neuronal death in CJD, is a consequence of neuronal apoptosis, or is just an index of the disease state remains to be established.

Alzheimer Disease

Alzheimer disease (AD) is the most common cause of dementia in the elderly, characterized by senile plaques, neurofibrillary tangles, and amyloid angiopathy. AD brains lack the classical hallmarks of inflammation such as neutrophil infiltration and perivascular mononuclear cuffing. However, as for other neurodegenerative diseases, a local inflammatory reaction is sustained by activated microglia and reactive astrocytes, as indicated by the

presence of antigens associated with microglia/macrophage activation and inflammatory mediators, such as elements of the complement system, cytokines, and free radicals (54).

The concept of a pathogenic role of COX in AD is deeply rooted in epidemiological studies reporting an association between long-term NSAID use and reduced risk of AD, although not every investigation has proved the same protective effect (64).

Over the last 10 years, several analyses of COX-1 and COX-2 expressions have been carried out in animal models and postmortem AD brain tissues, providing a substantial but still controversial body of evidence pointing to the involvement of COX-2 in the cascade of events leading to neurodegeneration in AD. COX-2 mRNA levels in AD brains were reported as either decreased or increased (9, 65, 66), possibly because of the short half-life of COX-2 transcripts or individual variability of inflammatory-related processes (67). Histological analyses of AD brains have also produced apparently conflicting results. Several studies reported increased neuronal COX-2 immunoreactivity compared to control brain tissues (9, 68). However, in other studies, in which COX-2 expression was related to specific hallmarks of the disease, such

as clinical dementia rating and Braak stage of disease, the number of COX-2-positive neurons decreased with the severity of dementia. In end stage AD, COX-2-positive neurons were significantly fewer than in non-demented controls (68, 69). In a more recent study (70), the number of neurons expressing COX-2 negatively correlated with the Braak score for amyloid deposits, although a moderate, albeit non-significant COX-2 increase was found at Braak stage A, corresponding to the mildest stage of disease when compared to non-demented control cases. COX-2 immunoreactivity did not correlate with Braak staging for neurofibrillary changes. These recent studies suggest that COX-2 expression varies with the disease stage and this may explain the controversial findings reported in the literature.

Hoozemans et al also reported a colocalization and a significant correlation of neuronal COX-2 expression with cell cycle regulators involved in controlling the G₀/G₁ phase, such as cyclin D1 and E and the retinoblastoma protein (69, 70). Disruption of cell cycle control due to altered expression of cell cycle proteins has been proposed as a primary mechanism by which post-mitotic neurons undergo apoptotic death in AD (71). Although there are some indications that COX-2 might regulate cell cycle progression (70), the functional link between COX-2 and cell cycle alteration remains elusive. Nonetheless, it can be suggested that COX-2 and cell cycle proteins are involved in early steps leading to neurodegeneration.

In contrast to COX-2, the levels of COX-1 mRNA and protein were not significantly altered in AD brains (9, 72). COX-1 appeared to be mainly expressed by microglial cells found in association with amyloid deposits, regardless their ramified or activated morphology (71).

CSF levels of PGE₂ were increased in probable AD patients (73). This finding was only partially confirmed by a longitudinal study in which PGE₂ was measured in CSF samples obtained on at least 3 annual visits in 35 controls and 33 AD patients (74). The study showed that CSF PGE₂ declines with the increasing dementia severity. Compared with controls, CSF PGE₂ was higher in patients with mild memory impairment, but lower in those with more advanced AD. This pattern is consistent with the slight increase in the number of COX-2-positive neurons at Braak stage A, as well as with the reduction in COX-2-positive neurons reported in patients with severe dementia and Braak end-stage disease, as previously reported (68–70).

The moderate increase in COX-2 expression and activity at very early stages of AD could explain the primary protective of NSAIDs, by preventing early steps leading to neurodegeneration. Upregulation of neuronal COX-2 is associated with ischemia and excitotoxicity, suggesting that COX-2 is involved in neurotoxic mechanisms. Increased susceptibility to excitotoxicity in COX-2 over-expressing neurons and neuroprotection by COX-2 inhibition has been shown in several experimental models

(64). Nonetheless, increased COX-2 expression could be an adaptive reaction to pathological events, such as cerebrovascular dysfunction, early inflammatory processes, or oxidative stress, in the attempt to restore lost physiological functions.

Taking into account the positive and negative effects of increased COX-2 activity and the emerging role of COX-2-derived PGs in brain function, it is difficult to predict the final outcome of long-term therapeutic COX-2 inhibition. At present, clinical trials of selective COX-2 inhibitors have not been as convincing as expected, but these failures may be related to drug selection and dose, duration of treatment, and state of disease of selected patients (64). Furthermore, it has to be noted that the protective effects of NSAIDs may be via non-COX-inhibitory mechanisms, such as lowering of A β peptide levels, reduction in the plaque pathology, and activation of the peroxisome proliferator-activated receptor- γ , suggesting that selective inhibition of COX-2 may not be the optimal therapeutic strategy (64).

Conclusions

Since its discovery in early 1990s, COX-2 has emerged as a major player in inflammatory reactions in peripheral tissues. Evidence from several laboratories indicates that COX-2 is induced in various inflammatory settings, is the main source of PGs responsible for clinical signs of inflammation, and its inhibition leads to anti-inflammatory effects. By extension, COX-2 expression in brain has been associated with pro-inflammatory activities, thought to be instrumental in neurodegenerative processes of several acute and chronic diseases.

However, 2 major aspects should be borne in mind when considering the significance of COX-2 activity in brain diseases. First, COX-2 is expressed under normal conditions and contributes to fundamental brain functions such as synaptic activity, memory consolidation, and functional hyperemia. Second, “neuroinflammation” is a much more controlled reaction than inflammation in peripheral tissues. In degenerative diseases, it mainly occurs in the absence of blood-borne infiltrating cells and is sustained by activated glial cells, particularly microglia. In more typical brain inflammatory diseases such as MS, inflammation is tightly regulated and the final outcome of this complex process is not exclusively detrimental.

In spite of the intense research of the last decade, the evidence of a direct role of COX-2 in neurodegenerative events is still controversial and further experimental and clinical studies are required to improve our knowledge of how and when COX-2 inhibition may have beneficial effects for patients suffering from inflammatory and degenerative neuropathologies.

Numerous features contribute to the complexity of the problem. Several cell types, including resident cells (i.e. neurons, glia, endothelial cells) and infiltrating blood cells, can express COX-2 in brain. Over-expression of COX-2 in each of these cells may have different functional consequences and its final outcome is likely to depend on the prevailing product of COX-2 activity, including PGs with different functions and free radicals. Neurons are particularly susceptible to damage caused by free radicals generated through COX-2 peroxidase activity,

whereas glial cells are more resistant. Specific signals seem responsible for COX-2 induction and/or over-expression in particular cell types, such as glutamate in neurons, cytokines in astrocytes, and apoptotic neurons in microglia. Such signals can be specifically related to a disease or to a stage of disease, thus explaining some of the discordances in the reported results.

The beneficial effects of specific and non-specific COX-2 inhibitors in several experimental models and epidemiological studies are an indirect proof of the causative role of COX-2 in neurodegeneration, as COX-independent mechanisms cannot be excluded. Furthermore, whether these drugs act in the periphery, thereby interfering with systemic inflammatory processes and their consequences on central inflammation, remains to be investigated.

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