# Cyclopentenone Eicosanoids as Mediators of Neurodegeneration: A Pathogenic Mechanism of Oxidative Stress-Mediated and Cyclooxygenase-Mediated Neurotoxicity

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**The activation of cyclooxygenase enzymes in the brain has been implicated in the pathogenesis of numerous neurodegenerative conditions. Similarly, oxidative stress is believed to be a major contributor to many forms of neurodegeneration. These 2 distinct processes are united by a common characteristic: the generation of electrophilic cyclopentenone eicosanoids. These cyclopentenone compounds are defined structurally by the presence of an unsaturated carbonyl moiety in their prostane ring, and readily form Michael adducts with cellular thiols, including those found in glutathione and proteins. The cyclopentenone prostaglandins (PGs) $PGA_2$, $PGJ_2$, and 15-deoxy-$\Delta^{12,14}$ $PGJ_2$, enzymatic products of cyclooxygenase-mediated arachidonic acid metabolism, exert a complex array of potent neurodegenerative, neuroprotective, and anti-inflammatory effects. Cyclopentenone isoprostanes ($A_2$/$J_2$-IsoPs), products of non-enzymatic, free radical-mediated arachidonate oxidation, are also highly bioactive, and can exert direct neurodegenerative effects. In addition, cyclopentenone products of docosahexaenoic acid oxidation (cyclopentenone neuroprostanes) are also formed abundantly in the brain. For the first time, the formation and biological actions of these various classes of reactive cyclopentenone eicosanoids are reviewed, with emphasis on their potential roles in neurodegeneration. The accumulating evidence suggests that the formation of cyclopentenone eicosanoids in the brain may represent a novel pathogenic mechanism, which contributes to many neurodegenerative conditions.**

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## INTRODUCTION

Activation of cyclooxygenase (COX) enzymes and oxidative stress are 2 separate pathogenic mechanisms, which have been implicated as major contributors to central nervous system (CNS) diseases. A common link between these seemingly disparate processes is the oxidation of arachidonic acid (AA) to yield bioactive oxidized lipids. Interestingly, both COX-mediated and oxidative stress-mediated oxidation of AA can lead to the generation of electrophilic lipid species containing unsaturated cyclopentenone ring structures. Due to this reactive carbonyl moiety, these cyclopentenone eicosanoids rapidly form Michael adducts with cellular thiols, including those found in glutathione (GSH) and proteins. Two major classes of cyclopentenone eicosanoids have been described: cyclopentenone prostaglandins ($PGA_2$, $PGJ_2$, and their metabolites such as 15-deoxy-$\Delta^{12,14}$ $PGJ_2$), which arise from the enzymatic oxidation of arachidonic acid by COX enzymes, and cyclopentenone isoprostanes ($A_2$/$J_2$-IsoPs), which are formed as a result of non-enzymatic, free-radical mediated peroxidation of AA. While cyclopentenone molecules derived from AA have been most thoroughly studied, similar compounds can also arise from the oxidation of other polyunsaturated fatty acids (PUFAs), such as and docosahexaenoic acid (DHA). Significant evidence has accumulated demonstrating that cyclopentenone eicosanoids exert potent biological actions in the CNS, and may mediate some of the pathogenic consequences of both COX-2 activation and oxidative stress in the brain.

## CYCLOOXYGENASE EXPRESSION IN NEURODEGENERATION

Enzymatic oxidation of free AA by cyclooxygenase (COX) enzymes plays important roles in many physiological processes, and is reviewed extensively elsewhere (94). Two COX isoforms exist: a constitutive form (COX-1), and an inducible form (COX-2), both of which are expressed in brain and are the targets of non-steroidal anti-inflammatory drugs. The sequential actions of COX and the prostaglandin (PG) synthase enzymes convert AA to the potent eicosanoids $PGE_2$, $PGD_2$, $PGI_2$, $PGF_{2a}$, and thromboxane, the proportions of each being dictated by the relative abundance of various PG synthases in a given tissue. These "classic" PGs then interact with cognate G-protein coupled receptors and mediate many vital actions in the body. Considerable evidence now suggests that COX enzymes, particularly COX-2, play a role in neurodegeneration. Overexpression of COX-2 in neurons has been documented in several neurodegenerative conditions, including stroke (123), Alzheimer disease (AD) (104, 106), amyotrophic lateral sclerosis (ALS) (137), and Parkinson disease (PD) (132). In animal models of cerebral ischemic injury (45, 102), ALS (25), or PD (28, 131, 132), neuronal COX-2 expression is correlated with cell death, and genetic deletion or pharmacologic inhibition of COX-2 provides neuroprotection. Thus, it appears that overexpression of COX-2 in neurons is toxic to these cells. COX-2 expression also occurs in activated microglia and promotes neuroinflammation, which can contribute to neuronal death (71, 78). Furthermore, increased intake of non-steroidal anti-inflammatory drugs, which inhibit COX activity in all cells, is correlated with a decreased relative risk of developing AD (46, 127, 139) and PD (18). However, the mechanism by which COX-2 contributes to neuronal death is unknown. Attempts

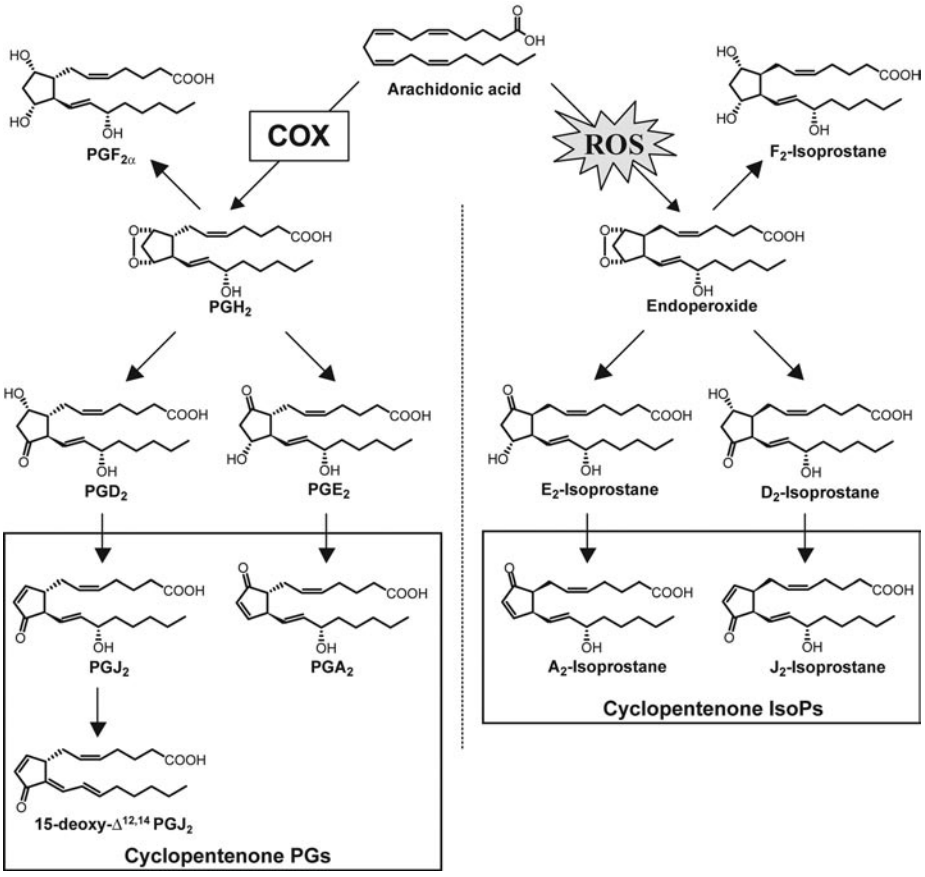


**Figure 1.** *Diagram of the formation of cyclopentenone eicosanoids.* COX-dependent metabolism of arachidonic acid yields $PGH_2$, which can be converted to several PGs. $PGE_2$ and $PGD_2$ spontaneously dehydrate to formed $PGA_2$ and $PGJ_2$, respectively. $PGJ_2$ can be further metabolized to 15-deoxy-$\Delta^{12,14}$ $PGJ_2$. Free-radical mediated oxidation of arachidonic acid leads to the formation of an unstable endoperoxide intermediate, which can be reduced to form stable, non-reactive $F_2$-IsoPs (top right), which are commonly measured as an index of oxidative stress, or can isomerize to $E_2/D_2$-IsoPs, which spontaneously dehydrate to form the reactive $A_2/J_2$-IsoPs, also known as cyclopentenone IsoPs. Note that prostaglandins have *trans* stereochemistry of their side chains with respect to the prostane ring, while IsoPs have a predominantly *cis* orientation. While IsoPs are formed as a mixture of 4 regioisomers, only the 15-series regioisomers are depicted for simplicity. The $A_2$-IsoP isomer depicted is 15-$A_{2t}$-IsoP.

to identify which "classic" prostaglandins mediate COX-2 neurotoxicity have been inconclusive, and have lead some investigators to suggest that cyclopentenone PGs, highly reactive dehydration products of $PGE_2$ and $PGD_2$, may in fact be the toxic COX products (34, 56).

## CYCLOPENTENONE PROSTAGLANDINS: AN OVERVIEW

$PGE_2$ and $PGD_2$ are abundant COX products in brain tissue (1, 103). However, both of these PGs are intrinsically unstable, and can spontaneously dehydrate to yield $PGA_2$ and $PGJ_2$, respectively (Figure 1). Unlike other PGs, both $PGA_2$ and $PGJ_2$ contain an unsaturated carbonyl moiety in their cyclopentenone ring structure, which is highly reactive and can readily form Michael adducts with nucleophilic substrates, such as thiol groups. Thus, $PGA_2$ and $PGJ_2$, also known as cyclopentenone PGs, are unique electrophilic products of COX-mediated AA metabolism, and therefore, have been thoroughly studied. $PGA_2$, which is formed by spontaneous dehydration of $PGE_2$, was initially identified in human seminal fluid (36) and in rabbit adrenal medulla (60), and was subsequently shown to be present in human plasma (31, 142). $PGJ_2$, the product of the spontaneous dehydration of $PGD_2$, was identified in 1982 (32), and its metabolite $\Delta^{12,14}PGJ_2$ was subsequently quantified in human urine (39). In the presence of serum albumin, $PGD_2$ rapidly converts not only to $PGJ_2$, but also to the highly bioactive metabolites $\Delta^{12}$-$PGJ_2$ and 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ (29, 52). Unlike other PGs, which interact with membrane-bound receptors, cyclopentenone PGs are taken up by cells via an active transport process and accumulate intracellularly (99) with nearly 50% of the compound transported to the nucleus (100). Cyclopentenone PGs are rapidly metabolized in cells via glutathione transferase (GST)-mediated conjugation to glutathione (GSH) (7, 11), then removed from the cell by the action of ATP-dependent efflux pumps (107). Variability between cell types in GSH and GST levels and efflux pump activity may explain the differential susceptibility of various cell lines to the effects of cyclopentenone PGs (24, 40). Accordingly, depletion of intracellular GSH levels potentiates the effects of cyclopentenone PGs, while augmentation of cellular GSH content protects cells from these compounds (6, 63).

The biological actions of cyclopentenone PGs appear to depend on the reactive cyclopentenone ring structure, as GSH conjugates of cyclopentenone PGs are biologically inactive, as are non-reactive cyclopentenone PGs analogs (41). Cyclopentenone PGs form reversible adducts with a specific population of vulnerable cysteine thiol groups on numerous intracellular proteins (16, 109, 110). Cyclopentenone PGs can inhibit the transcriptional activity of several important transcription factors, including p53 (87), AP-1 (109), and NF-κB (16) via covalent modification of specific cysteine residues in the DNA binding sites of these proteins.

While direct thiol adduction is the primary mechanism of cyclopentenone PG action, it should be noted that 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ is a ligand for the peroxisome proliferator activated receptor-gamma (PPARγ) nuclear receptor, which is thought to contribute to some of the biological effects of this compound (17, 30, 53, 118). PPARγ regulates numerous cellular processes including apidogenesis and inflammation, all of which can be modulated in PPARγ-expressing cells by addition of 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ (30, 53, 118). However, significant controversy exists over whether 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ is formed in vivo at levels required for PPARγ ligation (10).

Another pronounced effect of cyclopentenone PGs is their ability to inhibit the inflammatory response (128). Cyclopentenone PGs prevent the expression of pro-inflammatory molecules such as cytokines and inducible nitric oxide synthase (iNOS) in lipopolysaccharide-stimulated macrophages (17, 69, 118) or in tumor necrosis factor (TNF)-α stimulated microglia (57,

111). Indeed, cyclopentenone PGs suppress inflammatory protein expression in a variety of cell types in response to multiple stimuli (48, 122, 128, 136). The anti-inflammatory effects of cyclopentenone PGs are largely due to the ability of these compounds to inhibit the NF-κB pathway at several steps (16, 109, 118, 121, 129), as NF-κB is a central mediator of inflammatory protein transcription.

Cyclopentenone PGs can also potently induce cell stress responsive proteins, including the cytoprotective chaperone heat shock protein 70 (hsp70), and the anti-inflammatory, anti-oxidant protein heme oxygenase-1 (54). Finally, cyclopentenone PGs can activate the cytoprotective Antioxidant Response Element pathway via interaction with the redox-sensitive protein Keap1 (64). By this mechanism, cyclopentenone PGs can increase GSH levels and precondition certain cells against future insults (50, 63).

However, the cytoprotective stress responses activated by cyclopentenone PGs are balanced by their many cytotoxic effects. Cyclopentenone PGs potently induce apoptosis in several cancer cell lines (22, 51, 68, 138), as well as in non-cancerous cells, including neurons (120, 125), endothelial cells (63), macrophages (15, 42), hepatic myofibroblasts (65), and dendritic cells (101). Cyclopentenone PGs can induce intracellular oxidative stress in a variety of cell types (4, 55, 62, 65). This increased reactive oxygen species (ROS) production appears to originate from the mitochondria (55), and contributes to some of the biological effects of cyclopentenone PGs (4, 62, 65), including cytotoxicity (55). Furthermore, cyclopentenone PGs can impair the cellular glutathione system by direct scavenging and depletion of GSH (55), impairment of GST enzymatic activity, and inhibition of efflux pump function (133). Thus, a tenuous balance exists between the toxic and protective effects of cyclopentenone PGs, which is cell type, concentration, and context dependent (63).

## NEUROTOXIC EFFECTS OF CYCLOPENTENONE PGs

Several lines of evidence suggest that cyclopentenone PGs may be neurotoxic mediators in the CNS. While cyclopentenone PGs have never been shown to be formed in brain, $PGE_2$ and $PGD_2$, the precursors to cyclopentenone PGs, are produced abundantly in the CNS and are elevated in several neurodegenerative diseases (47, 78, 86). Furthermore, Kondo et al demonstrated increased 15-deoxy-$\Delta^{12,14}$ $PGJ_2$-like immunoreactivity in spinal cord sections from ALS patients (56). 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ potently induces apoptosis in primary cortical neurons (120) and cerebellar granule cells (125) in culture, as well as in SH-SY5Y neuroblastoma cells. In SH-SY5Y cells, 15-deoxy-$\Delta^{12,14}$ $PGJ_2$-induced apoptosis is mediated by increased p53 expression and activation of the Fas-Fas ligand pathway (56). A second study with SH-SY5Y cells demonstrated that 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ caused a loss of mitochondrial membrane potential and increased mitochondrial ROS production, depletion of GSH, accumulation of ubiquitinated proteins, and increased lipid peroxidation (55). 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ toxicity was prevented by antioxidants, and was closely correlated with the degree of oxidation damage. This finding is intriguing, as mitochondrial dysfunction and oxidative stress are hallmarks of many neurodegenerative diseases (9), and are often associated with COX-2 expression (108). Furthermore, basal NF-κB activity in neurons is required for survival, while NF-κB inhibition can precipitate neuronal death and enhance neurodegeneration caused by various insults (21, 23, 49, 70). As cyclopentenone PGs are potent inhibitors of NF-κB, this mechanism could contribute to their neurotoxicity. These studies thus suggest that cyclopentenone PGs can contribute to neurodegeneration by several potential mechanisms.

## POTENTIAL NEUROPROTECTIVE EFFECTS OF CYCLOPENTENONE PGs

The role of cyclopentenone PGs in the CNS is far from clear, however, because in addition to toxic effects, neuroprotective actions of these molecules have been described. While inhibition of basal NF-κB activity can be fatal to neurons, excessive activation of NF-κB has also been associated with neuronal death (73). The mitochondrial complex I inhibitor rotenone induces pronounced NF-κB activation and cell death in SH-SY5Y cells, both of which can be prevented by $PGA_1$, a $PGA_2$ analog (134). Similarly, DNA fragmentation and NF-κB overactivation caused by striatal quinolinic acid injection in rats is blocked by co-injection of $PGA_1$ (113). In both cases, $PGA_1$ neuroprotection was also correlated with increased expression of hsp70, suggesting that stress response elicited by cyclopentenone PGs, rather than NF-κB inhibition, may mediate their protective effects. Similarly, 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ protects HT22 hippocampal cells, but not SK-N-SH neuroblastoma cells, from glutamate and hydrogen peroxide toxicity, but only when cells were preincubated with low concentrations of the PG for several hours prior to insult (5). Similarly, 24-hour pre-incubation with low (0.5 and 2 μM) concentrations of 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ protects PC12 cells from subsequent nitrosative stress-induced cell death (67). These 2 studies further suggest that exposure to sublethal concentrations of cyclopentenone PGs precondition cells against future insults, as has been reported for other cellular insults (72). Accordingly, cyclopentenone PGs can activate the Nrf2-antioxidant response element pathway (64), a signaling system shown to protect neurons from various stressors (58, 61). These studies suggest that cyclopentenone PGs can exert direct neuroprotection by modulating NF-κB activity and neuronal stress response pathways, particularly when cells are exposed to sublethal concentrations prior to a second insult.

Neuroinflammation is a key contributor to several neurodegenerative conditions, including AD and PD (44, 71). Activation of NF-κB in microglia and macrophages by cytokines, bacterial endotoxin or other stimuli facilitates the expression of numerous pro-inflammatory proteins, including TNFα, COX-2, and iNOS, which can enhance neurodegeneration (33, 71). Indeed, inhibition of microglial activation is protective in several mouse models of neurodegenerative diseases (14, 26, 74). Cyclopentenone PGs have been shown to inhibit neuroinflammation both in vivo and in vitro by blocking glial NF-κB activation and thereby suppressing the expression of cytokines and inflammatory proteins such as iNOS in both microglia and macrophages (38, 48, 57, 111). Accordingly, 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ can exert neuroprotective effects in models of inflammatory neurodegeneration (37, 38). These studies also suggested that PPARγ activation is crucial for 15-deoxy-$\Delta^{12,14}$ $PGJ_2$-mediated protection, as other PPARγ agonists were also protective.

Thus, while cyclopentenone PGs on their own appear to be neurotoxic, they can potentially act as neuroprotective mediators in the brain through activation of stress response or inhibition of inflammation.

## CYCLOPENTENONE ISOPROSTANES: REACTIVE PRODUCTS OF FREE-RADICAL MEDIATED LIPID PEROXIDATION

Enzymatic metabolism is not the only possible oxidative fate of AA. IsoPs, a family of PG-like molecules, are formed non-enzymatically as a result of free radical-mediated peroxidation of AA. IsoPs containing various prostane ring structures are formed in vivo, including $F_2$-IsoPs, which are isomeric to $PGF_{2a}$ (92), and $D_2$/$E_2$-IsoPs, which are isomers of $PGD_2$ and $PGE_2$, respectively (Figure 1) (117). Unlike PGs, which have a set stereochemistry due to their enzymatic generation, each type of IsoP is formed as a racemic mixture of 64 possible regio- and stereoisomers. A second important distinction between IsoPs and PGs is that the former contain side chains that are predominantly oriented *cis* to the prostane ring while the latter possess exclusively *trans* side chains, a stereochemical disparity which can greatly affect bioactivity (92). Furthermore, while PGs can only be generated from free arachidonic acid, IsoPs are initially formed in situ esterified to phospholipids, and are subsequently released by unidentified phospholipase(s) (90). Because of their stability, the measurement of $F_2$-IsoPs by mass spectrometry has been extensively employed as a marker of oxidant stress, and is widely considered to be the "gold-standard" index of lipid peroxidation in vivo (91, 95).

As oxidative stress has been implicated in the pathogenesis of numerous neurodegenerative conditions (9), IsoP formation in the brain has been extensively studied. Increased $F_2$-IsoPs have been observed in human AD brain samples (115), and in post-mortem cerebrospinal fluid (CSF) from AD patients (82, 83), as well as in CSF from living patients with probable AD (80, 112). Significantly elevated CSF $F_2$-IsoPs have also been reported in patients with Huntington disease (81), Creutzfeldt-Jakob disease (79), traumatic brain injury (8), and multiple sclerosis (35), suggesting that oxidative stress and IsoP formation are conserved characteristics of the neurodegenerative process.

As described previously, the cyclopentenone PGs, $PGA_2$ and $PGJ_2$, arise from the spontaneous dehydration of $PGE_2$ and $PGD_2$, respectively. The finding that $E_2$/$D_2$-Isoprostanes, which are isomeric to $PGE_2$ and $D_2$, respectively, are formed in vivo under conditions of oxidative stress suggested that cyclopentenone IsoPs ($A_2$/$J_2$-IsoPs) would likely be present in oxidized tissue (Figure 1) (93). The potent and diverse biological actions of cyclopentenone PGs spurred interest in the existence of these analogous cyclopentenone IsoPs, as these non-enzymatic products might also possess bioactivity. Indeed, $A_2$/$J_2$-IsoPs were found to be formed in vivo in rat liver (19). Cyclopentenone IsoPs were significantly more abundant that $F_2$-IsoPs in the same tissue, and increased 22-fold following exposure to $CCl_4$, which causes severe hepatic oxidant injury (19). Previous experiments had shown that 15-$E_{2t}$-IsoP, an E-ring isoprostane, was formed abundantly in $CCl_4$-treated rat liver tissue (93, 96). In keeping with our hypothesis that $A_2$-IsoPs arise from the dehydration of $E_2$-IsoPs, 15-$A_{2t}$-IsoP, the dehydration product of 15-$E_{2t}$-IsoPs and a stereoisomer of $PGA_2$, was formed in vivo and was found to be a relatively abundant cyclopentenone IsoP isomer (20).

Interestingly, cyclopentenone IsoPs in free acid form were undetectable in the aforementioned experiments (19), suggesting that following hydrolysis from membrane phospholipids, these IsoPs are either rapidly metabolized or quickly form protein adducts. Accordingly, cyclopentenone IsoPs are highly reactive and readily form thiol adducts with proteins (19). Furthermore, 15-$A_{2t}$-IsoP is efficiently conjugated to GSH in cells, and is metabolized more quickly and extensively than $PGA_2$, suggesting that the IsoP has equal or greater reactivity than its analogous PG (77). 15-$A_{2t}$-IsoP metabolism is largely mediated by human or rat GST A4-4 (43). Studies in HepG2 cells revealed that 15-$A_2$-IsoP isomers are rapidly converted to 4 distinct GSH conjugates, with over 60% of total IsoPs metabolized within 6 hours (77). The primary urinary metabolite of 15-$A_{2t}$-IsoP was also identified in rat as a modified mercapturic acid conjugate, and was found to increase dramatically in rats following treatment with $CCl_4$ (G. Milne, unpublished data). Thus, cyclopentenone IsoPs differ from other IsoPs (particularly $F_2$-IsoPs) in that they are unstable, reactive, and rapidly metabolized via GST-mediated conjugation to GSH.

## FORMATION OF CYCLOPENTENONE IsoPs IN THE BRAIN

IsoPs are products of lipid peroxidation that are formed abundantly in affected brain tissue from patients with numerous neurodegenerative diseases. However, until recently, the formation and biological actions of reactive cyclopentenone IsoPs in the brain were completely unexplored. Several pieces of evidence suggested that cyclopentenone IsoPs should be formed abundantly in the brain. The loss of reducing environment in the brain, manifested by depletion of GSH and vitamin E, shifts the IsoP pathway toward the formation of E/D-ring IsoPs and away from reduced F-ring IsoPs (85). In peroxidizing brain synaptosomes, $E_2$/$D_2$-IsoP are the favored products of the IsoP pathway, and their levels far exceed those of F-ring IsoPs (85). Moreover, $E_2$/$D_2$-IsoP levels are significantly elevated in the brains of human AD patients, and the ratio of E/D-ring to F-ring IsoPs is increased in this disease (115). As $E_2$/$D_2$-IsoPs are the direct precursors of $A_2$/$J_2$-IsoPs, these findings strongly suggest that $A_2$/$J_2$-IsoPs are formed in brain tissue. To address this question, we developed a novel liquid chromatography electrospray ionization tandem mass spectrometric method employing a [$^4H_2$]-$PGA_2$ internal standard to quantify cyclopentenone IsoPs in human tissue. This method has proven to be highly specific, sensitive, and accurate. Using this assay, we found that cyclopentenone IsoPs are indeed formed abundantly in rat brain tissue and are nearly 7-fold more abundant than $F_2$-IsoPs (Musiek et al, manuscript submitted). Oxidative injury caused a marked elevation in brain cyclopentenone IsoPs, as levels increased 12-fold following 24-hour exposure of rat brain tissue to the oxidant AAPH, while $F_2$-IsoPs increased only 2-fold. In post-mortem samples of human cerebral cortex, cyclopentenone IsoPs were again present at levels considerably higher than previously reported concentrations of $F_2$-IsoPs in human cerebral tissue. Thus, cyclopentenone IsoPs are formed abundantly in brain tissue, and are elevated under conditions of oxidative stress, suggesting that these molecules could mediate some of the

neurodegenerative effects of cerebral oxidant injury.

## NEURODEGENERATIVE EFFECTS OF CYCLOPENTENONE IsoPs

Due to their chemical similarity to the cytotoxic cyclopentenone PGs, we have recently explored the potential neurotoxicity of cyclopentenone IsoPs. We have observed that 15-$A_{2t}$-IsoP causes cell death in primary cortical neuronal cultures with an $LD_{50}$ of 950 nM (Musiek et al, manuscript submitted). In HT22 hippocampal cells, both 15-$A_{2t}$-IsoP and 15-$J_2$-IsoP, a J-ring cyclopentenone IsoP, induce cell death with $LD_{50s}$ ~4 μM. 15-$A_{2t}$-IsoP-induced neuronal death is apoptotic, as cells exposed to this IsoP exhibited condensed nuclei and asymmetric chromatin formations, as well as increased caspase-3 cleavage, and were completely protected by the pan-caspase inhibitor zVAD-FMK. Similar to the effects of cyclopentenone PGs in SH-SY5Y cells, 15-$A_2$-IsoP caused rapid GSH depletion in neurons, and induced membrane lipid peroxidation via promotion of mitochondrial ROS production. Furthermore, 15-$A_{2t}$-IsoP toxicity was mitigated by the free radical scavengers, suggesting that redox alterations caused by 15-$A_{2t}$-IsoP contribute to its toxicity.

Neurotoxicity caused by depletion of GSH can be modeled in embryonic neurons or HT22 hippocampal cells, neither of which express functional NMDA receptors, via application of millimolar concentrations of extracellular glutamate. This insult, known as oxidative glutamate toxicity, blocks cellular uptake of cystine, which is required for GSH synthesis, and leads to severe GSH depletion within several hours (98, 114). Fortunately, the signaling pathways mediating oxidative glutamate toxicity are well described. As 15-$A_{2t}$-IsoP also depletes GSH, we sought to compare the pathogenic signaling pathways activated by these 2 insults. We found great overlap between the cell death cascades activated by both insults, as both required increased ROS production, translocation and activation of the enzyme 12/15-lipoxygenase, and phosphorylation of ERK1/2 (66, 126, 130). These findings suggest that this pathway is a conserved response to neuronal oxidation, and that cyclopentenone IsoPs might be one of the pathogenic products formed secondary to GSH depletion that mediate oxidative glutamate toxicity. Furthermore, each of these signaling events activated by 15-$A_{2t}$-IsoP have been previously implicated in neurodegeneration.

As cyclopentenone IsoPs are products of oxidative injury and can induce ROS production and further lipid peroxidation, we have suggested that cyclopentenone IsoPs can set in motion a feed-forward cycle of increasing intracellular oxidation which ultimately pushes a neuron toward cell death. The activation of conserved cell death pathways in neurons by 15-$A_{2t}$-IsoP also suggests that cyclopentenone IsoPs might exacerbate neuronal injury caused by other insults. Indeed, subtoxic concentrations of 15-$A_{2t}$-IsoP as low as 100 nM significantly potentiate neuronal death caused by sublethal oxidative glutamate toxicity. 15-$A_{2t}$-IsoP also greatly enhances death of neurons induced by oxygen-glucose deprivation, an in vitro model of cerebral ischemic injury (Musiek et al, manuscript submitted). As esterified cyclopentenone IsoPs are present in oxidized rat brain tissue at levels, which roughly convert to ~550 nM, these findings demonstrate that these molecules, at biologically relevant concentrations, can contribute to the neurodegenerative process. Previously, the study of the role of lipid peroxidation in neurodegeneration has been largely focused on the actions of 4-hydroxynonenal (HNE) (12, 59, 105). We have found that cyclopentenone IsoPs are more biologically potent than HNE (unpublished data), and can be more accurately quantified in vivo. Thus, the actions of cyclopentenone eicosanoids in the brain should no longer be neglected, and merit further exploration.

## POTENTIAL NEUROPROTECTIVE EFFECTS OF CYCLOPENTENONE IsoPs

Unlike cyclopentenone PGs, no data exists to suggest that cyclopentenone IsoPs can exert direct neuroprotective effects. While 15-deoxy-$\Delta^{12,14}$ $PGJ_2$ can protect neurons from oxidative glutamate toxicity in some instances (5), 15-$A_{2t}$-IsoP potentiates this insult at all concentrations tested. This is perhaps partly explained by our findings that 15-$A_2$-IsoPs are not ligands for PPARγ, and are not potent inducers of hsp70 or heme oxygenase-1 (unpublished data). However, it remains to be seen if other cyclopentenone IsoP isomers, such as 15-$J_2$-IsoPs, have neuroprotective properties.

Like cyclopentenone PGs, however, cyclopentenone IsoPs can inhibit the inflammatory response. We have found that both 15-$A_2$- and 15-$J_2$-IsoPs are potent inhibitors of LPS-induced NF-κB activation in RAW264.7 macrophages, preventing expression of iNOS and COX-2, as well as elaboration of nitric oxide, PGs (Musiek et al, manuscript submitted), and various cytokines (76). 15-$A_2$-IsoPs also inhibit LPS-induced nitric oxide production in BV-2 microglial cells at sub-micromolar concentrations, suggesting that this process is relevant to the CNS (unpublished data). As NF-κB inhibition can also be toxic to neurons (21, 23), ongoing studies are examining the effects of NF-κB inhibition by cyclopentenone IsoPs in the CNS.

## OTHER CYCLOPENTENONE PRODUCTS OF LIPID PEROXIDATION

Previous studies in our lab have shown that oxidation of docosahexaenoic acid (DHA), an omega-3 PUFA, leads to the formation of IsoP-like molecules termed neuroprostanes (NPs) (119). NPs are so named because DHA is highly enriched in neuronal membranes (124). The mechanism of NP formation is similar to that of IsoP formation, and F-, D-, and E-ring NPs have been described (119). Due to the high concentration of DHA in the brain, cerebral $F_4$-NPs are highly abundant, and are significantly increased in human AD brain samples (115), as well as in animal models involving CNS oxidative stress (75, 84, 116). We have recently described the formation of cyclopentenone NPs ($A_4$/$J_4$-NPs) in vivo in rat brain (27). Like other cyclopentenone compounds, cyclopentenone NPs are reactive and form adducts with GSH and proteins. Interestingly, cyclopentenone NPs are extremely abundant in the CNS, exceeding the levels of any other IsoP or PG measured in brain to date. While the biological effects of cyclopentenone NPs have not yet been explored, several interesting possibilities exist. DHA is a primary constituent of fish oil, dietary consumption of which has been associated with numerous neuroprotective effects (2, 13, 135), including decreased risk of AD (89). DHA and fish oil also have potent anti-inflammatory effects, and can protect tissue from inflammatory damage (88). Thus, one might expect that cyclopentenone NPs will preferentially activate cytoprotective responses in

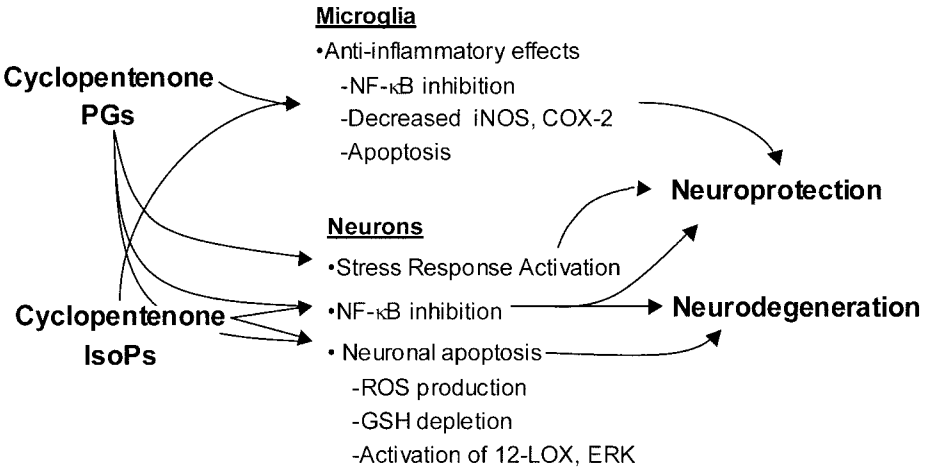


**Figure 2.** *Summary of the effects of cyclopentenone eicosanoids on neurons and microglia.* Cyclopentenone eicosanoids exert a mixture of potentially neuroprotective and neurodegenerative effects in the CNS, the final consequence of which is highly context dependent.

neurons and suppress the microglial inflammatory response, with minimal activation of cell death pathways, thereby exerting primarily neuroprotective effects. However, the actual impact of cyclopentenone NPs in the brain remains to be determined experimentally.

## SUMMARY

Cyclopentenone eicosanoids exert numerous potent and sometimes conflicting effects in the brain (Figure 2). While these compounds are generally neurotoxic, some cyclopentenone species (particularly cyclopentenone PGs) are able to protect neurons from other insults, largely through activation of cytoprotective stress response pathways. It is common for neurotoxic compounds to elicit neuroprotection when administered at low concentrations; however, many sublethal insults can precondition cells against future damage (72). Our preliminary studies with 15-$A_{2t}$-IsoP demonstrate that co-application of this compound during an insult is not protective, but rather potentiates neuronal death. As cyclopentenone eicosanoids are produced during an inflammatory or oxidative insult in the brain, not hours before, this result is likely more indicative of actual pathophysiology.

The ability of all tested cyclopentenone eicosanoids to inhibit neuroinflammation, particularly through microglial NF-κB inhibition, could offer neuroprotection from inflammatory insults. However, inhibition of microglial/macrophage function is not always a protective effect in the brain, as these cells actively remove debris and prevent the accumulation of certain neurotoxic compounds, such β-amyloid (3). Thus, inhibition of glial function and induction of macrophage/microglial apoptosis, an effect seen with cyclopentenone eicosanoids (42), could exacerbate certain aspects of neurodegenerative disease, such as amyloid plaque development (3). Furthermore, the findings that cyclopentenone eicosanoids perturb mitochondrial function and promote oxidative stress in both neurons and glia suggest that these compounds are more likely toxic than protective.

While numerous cyclopentenone IsoP isomers are formed, the biology of only a few has been examined. Thus, further studies must be conducted to explore the formation of these lipids in the brain under neurodegenerative conditions, as well the diversity of effects of these compounds in the CNS. The recent development of novel mass spectrometric methods to quantify these compounds in brain tissue in our laboratory, as well as the chemical synthesis of several cyclopentenone IsoP isomers (140, 141), should expedite these investigations. The existing data support the speculation that cyclopentenone eicosanoids likely play a role in neurodegenerative disease, and suggest that the further study of these molecules might provide insight for novel neuroprotective therapeutic strategies.

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