Published in final edited form as:

Neurotoxicology. 2011 June ; 32(3): 312–319. doi:10.1016/j.neuro.2011.02.004.

Prostanoid signaling: dual role for prostaglandin E₂ in neurotoxicity

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Abstract

The prostanoids, a naturally occurring subclass of eicosanoids, are lipid mediators generated through oxidative pathways from arachidonic acid. These cyclooxygenase metabolites, consisting of the prostaglandins (PG), prostacyclin and thromboxane, are released in response to a variety of physiological and pathological stimuli in almost all organs, including the brain. They are produced by various cell types and act upon targeted cells via specific G protein-coupled receptors. The existence of multiple receptors, cross-reactivity and coupling to different signal transduction pathways for each prostanoid, collectively establish their diverse effects. Notably, these effects can occur in functionally opposing directions within the same cell or organ. Prostaglandin E₂ (PGE₂) is the most versatile prostanoid because of its receptors, E Prostanoid (EP) receptor subtypes 1 through 4, its biological heterogeneity and its differential expression on neuronal and glial cells throughout the central nervous system. Since PGE₂ plays an important role in processes associated with various neurological diseases, this review focuses on its dual neuroprotective and neurotoxic role in EP receptor subtype signaling pathways in different models of brain injury.

Introduction

The prostanoids, a naturally occurring subclass of eicosanoids, are lipid mediators generated through the oxidative metabolism of 20-carbon fatty acids (eicosa is Greek for 20), primarily arachidonic acid (AA), that modulate various physiological responses and pathophysiological processes, including inflammation and tumorigenesis, as well as autoimmune and neurological disorders. The biosynthesis of eicosanoids begins with the hydrolysis of arachidonic acid (AA) from membrane phospholipids by members of the phospholipase A₂ (PLA₂) family (Simmons et al., 2004; Hao and Breyer, 2007). Free AA is a major substrate for three major groups of enzymes, cyclooxygenases (COX), lipoxygenases (LOXs) and epoxygenases, that catalyze the formation of the prostanoids, prostaglandins (PGs), prostacyclin and thromboxane A₂ (TxA₂), the leukotrienes or the epoxyeicosatrienoic acids, respectively. An analogous family of free radical catalyzed isomers, the isoeicosanoids (e.g., isoprostanes) and 4-hydroxynonenal, are formed by the

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nonezymatic peroxidation of AA that can still be esterified to phospholipid (Smyth et al., 2009). The COX pathway of AA metabolism is depicted in Figure 1. COX enzymes first catalyze the cyclization of AA to PGG₂ via its bis-oxygenase activity, followed by the catalytic conversion of unstable PGG₂ to PGH₂ by peroxidase activity (Hao and Breyer, 2008). Tissue-specific terminal prostaglandin synthases or isomerases then convert PGH₂ into biologically active PGs, namely, PGD₂, PGE₂, PGF_{2α}, and PGI₂ (also known as prostacyclin), as well as thromboxane A₂ (TxA₂) (Figure 1). These prostanoids undergo rapid metabolic degradation and typically act within the local microenvironment on the parent cell and/or neighboring cells. PGs and TxA₂ activate specific G protein-coupled receptor(s) (Breyer and Breyer, 2000; Narumiya and FitzGerald, 2001; Hao and Breyer, 2008), which differ in their agonist selectivity, tissue distribution and signal transduction pathways. Autocrine and paracrine stimulation and the differentially restricted expression of the receptors allow these prostanoids to achieve a wide variety of biological effects in different cell types and tissues.

The levels of PG production are mediated by the expression and activity of COX. COX exists in two distinct isoforms, COX-1 and COX-2. Both isoforms catalyze the same reactions and share about 60% homology at the amino acid level (Smith et al., 2000), but have different patterns of expression and are encoded by two different genes, which are located in different chromosomes. COX-1, constitutively expressed in most cells, is responsible for maintaining basic physiological functions and is thought to be responsible for the baseline production of prostanoids (Smith, 1992; Smith and Langenbach, 2001; Hao and Breyer, 2007). In contrast, COX-2 is induced by inflammatory mediators and mitogens and is thought to play a key role in numerous pathophysiological processes (Herschman, 1996; Smith and Langenbach, 2001). COX-2 is responsible for the increased production of prostanoids during inflammation and stress (Feldman and McMahon, 2000; Rocha et al., 2003). Further, COX-2 is markedly up-regulated both in neurons and glial cells in a variety of neurological disorders (Adreasson 2010). Its reaction products play critical roles in a wide variety of diseases associated with glutamate excitotoxicity, cerebral ischemia, traumatic brain injury and inflammatory injury in neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), HIV associated dementia (HAD) and amyotrophic lateral sclerosis (ALS) (Montine et al., 1999; Minghetti, 2004; McGeer and McGeer, 2004; Cimino et al., 2008; Liang et al., 2008; Adreasson, 2010). Although COX-2 is a major source of proinflammatory mediators, data suggest that COX-1 is involved in the production of prostanoids and also plays a role in the initial phase of an acute inflammation, with COX-2 up-regulation occurring several hours later (Smith et al., 2009).

The importance of these products of COX isoforms in pathophysiological processes is exemplified by the well-known clinical effects of pharmacological inhibitors, the non-steroidal anti-inflammatory drugs (NSAIDs). Because NSAIDs, such as ibuprofen, indomethacin and naproxen also exert multiple deleterious side effects predominately characterized by gastrointestinal bleeding due to the suppression of COX-1 and COX-2, the development of COX-2 selective blockers, called Coxibs, seemed to have resolved this issue (FitzGerald and Patrono, 2001). However, while COX-2 selective inhibitors have been widely used for their efficacy in reducing inflammation and sparing the constitutive actions of prostanoids, clinical trials have revealed that these agents increase the risk of adverse cardiovascular events, such as heart attacks and strokes (Couzin, 2005). These events have lead to an increased emphasis on researching alternative targets in the prostanoid pathways. Specifically, targeting individual prostaglandin receptors, rather than suppressing the entire PG pathway through the use of NSAIDs, represents a more focused approach to retaining the therapeutic effects of PGs while limiting their toxicity.

Additional limiting steps in the generation of prostanoids are the activation of phospholipases and protein expression of specific down-stream PG synthases. Cellular levels of free AA available for eicosanoid production are primarily controlled by PLA₂ (Narumiya and FitzGerald, 2001). PLA₂ activity is regulated by hormones and cytokines (Roberts, 1996; Sapirstein et al., 2000). During inflammation, pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- α), increase the activity of phospholipases and contribute to the increased production of eicosanoids (Rocha et al., 2003). The PLA₂ family is classified into four groups: secretory PLA₂ (sPLA₂), cytosolic PLA₂ (cPLA₂), calcium-independent PLA₂ (iPLA₂) and PAF acetylhydrolases (PAF-AH) (Bonventre, 1999; Kudo and Murakami, 2002). Among these, calcium-dependent cPLA_{2 α} has been best characterized and has been shown to play a dominant role in AA release and eicosanoid biosynthesis (Narumiya and FitzGerald, 2001; Hao and Breyer, 2007).

Prostanoid synthesis is completed by the cell- and tissue-specific prostanoid synthases' (PGS) catalyzed conversion of PGH₂ to specific PGs and TxA₂. Such synthases include PGE₂ synthase (PGES), prostacyclin synthase (PGIS), PGD₂ synthase (PGDS), PGF₂ synthase (PGFS), and tromboxane synthase (TxAS), which are responsible for PGE₂, PGI₂, PGD₂, PGF_{2 α} and tromboxane A₂ biosyntheses, respectively (Smith and Langenbach, 2001; FitzGerald, 2003). The existence of different isoforms, the intracellular localization and the functional coupling of these enzymes, as well as different biological demand modulates the AA pathway and prostanoid production. In addition, the existence of multiple receptors coupling to different signal transduction pathways for a given prostaglandin allows for diverse PG-mediated effects, which at times, can occur in functionally opposing directions within the same cell or organ.

Prostanoids and their receptors

The prostanoids exert a variety of actions by binding to membrane receptors on the surface of the targeted cells. There are nine G protein-coupled prostanoid receptors (GPCR) with several additional splice variants and divergent carboxyl terminal regions (Narumiya and FitzGerald, 2001; Hata and Breyer, 2004). They are named based upon the ligand that binds to the receptor with the greatest affinity: I prostanoid (IP) receptor binds PGI₂, DP binds PGD₂, EP binds PGE₂, FP binds PGF_{2 α} and TP binds TxA₂. Among the prostanoids, PGE₂ has the most known receptors with EP subtypes characterized as EP1–4. All types and subtypes of the prostanoid receptors have been experimentally knocked out in mice. Given their structural similarities, prostanoids may activate more than one subtype of the receptor and have ligand-binding cross-reactivity.

Prostaglandin D₂

(PGD₂) is the most abundant prostanoid in the rat brain and in other mammals, including humans (Narumiya et al., 1982; Huang et al., 2007). PGD₂ is relatively unstable (Golovko and Murphy, 2008) and it has a wide variety of roles *in vivo* (Giles and Leff, 1988), including opposing effects on the immune system. Generated as the major prostanoid in activated mast cells (Lewis et al., 1982), PGD₂ induces inflammation in a variety of settings. However, its metabolite, 15-deoxy-delta-(12,14)-prostaglandin J₂ (15-d-PGJ₂), a ligand for peroxisome proliferator-activated receptor γ (PPAR γ), has been implicated as mediator of many of the anti-inflammatory effects of PGD₂ (Gilroy et al., 1999; Trivedi et al., 2006). 15-d-PGJ₂ is also able to inhibit nuclear factor-kB (NF-kB) signaling and to form adducts with numerous cellular proteins (Straus et al., 2000; Rossi et al., 2000). In addition to its role in inflammatory processes, PGD₂ mediates a number of additional effects, including smooth muscle relaxation (Narumiya and Tod, 1985), vasodilation and vasoconstriction (Giles and Leff, 1988), mucous secretion (Wright et al., 2000) and sleep induction (Mizoguchi et al., 2001; Huang et al., 2007). The latter is mediated by the activation of DP receptors (Onoe et

al., 1988; Huang et al., 2007). There are two distinct PGD₂ GPCRs, the DP (DP1) and the chemoattractant-receptor homologous molecule expressed on Th2 cells (CRTH2, also called DP2). While DP receptor activation leads to stimulatory G protein-mediated increases in intracellular cAMP with calcium-flux (Hirata et al., 1994; Boie et al., 1995; Hata and Breyer, 2004), the CRTH2 receptor couples to an inhibitory type G protein (Gi) leading to the inhibition of cAMP and the increase of intracellular calcium in a variety of cell types (Hirai et al., 2001; Sawyer et al., 2002). Stimulation of the DP receptor enhances the barrier function of endothelial cells (Murata et al., 2008) and confers protection to neurons (Liang et al., 2005a; Schuligoi et al., 2010). In contrast, PGD₂ and DP receptors contribute to astrogliosis and demyelination in the twitcher mouse model (Mohri et al., 2006). Importantly, PGD₂ and its metabolites play a complex role in inflammation. *In vivo* and *in vitro* studies have shown that PGD₂ has potent a pro-inflammatory function (Emery et al., 1989; Fujitani et al., 2002), particularly in activating leukocytes. However, PGE₂ also inhibits inflammation in other settings (Trivedi et al., 2006; Sandig et al., 2007).

Prostaglandin F_{2α}

(PGF_{2α}) plays a major role in reproduction (Sugimoto et al., 1997; Hata and Breyer, 2004), renal function (Breyer and Breyer, 2000, 2001), cardiac hypertrophy (Lai et al., 1996) and the regulation of intraocular pressure. A high concentration of PGF_{2α} was also noted in the hippocampus following systemic administration of kainic acid (KA). A previous study showed that PGF_{2α} alone did not affect KA-induced seizures, but the PGF_{2α} receptor antagonist, AL 8810, potentiated KA-induced seizure activity dose-dependently (Baran et al., 1987; Yoshikawa et al., 2006; Kim et al., 2008). PGF_{2α} binds a single prostanoid receptor, namely FP, with two splice variants, FP_A and FP_B. This receptor has low selectivity and, in nanomolar concentrations, binds other PGs, such as PGD₂ and PGE₂ (Hata and Breyer, 2004). Its activation leads to an increase in the level of intracellular calcium (Abramovitz, 1994; Watanabe et al., 1994) as well as the activation of other signal transduction pathways, including phospholipase C (PLC), protein kinase C (PKC) and mitogen-activated protein (MAP) kinase (Bos et al., 2004). PGF_{2α} as PGD₂ also induces the bronchoconstriction of airways *in vitro* and in asthmatic patients (Coleman and Sheldrick, 1989; Fish et al., 1984). Further, PGF_{2α} is associated with acute and chronic inflammatory diseases (Basu et al., 2001a,b), oxygen-deprived brain injury (Basu et al., 2003) and leukocyte migration in the experimental model of lipopolysaccharide (LPS)-induced inflammation in rats (de Mendezes et al., 2008).

Prostaglandin I₂

(PGI₂), or prostacyclin, is a potent vasodilator and possesses anti-thrombotic, anti-proliferative and anti-inflammatory properties. Produced primarily by endothelial cells, PGI₂ inhibits platelet aggregation and induces vasodilation (Vane and Botling, 1995), thus acting as a physiological antagonist of TxA₂ (Gomberg-Maitland and Olschewski, 2008). Moreover, prostacyclin is a powerful cytoprotective agent that exerts its action through the activation of adenylate cyclase, followed by the intracellular accumulation of cyclic-AMP. Accordingly, PGI₂ cooperates with the system consisting of NO synthase (eNOS)/nitric oxide free radical (NO)/guanylate cyclase/cyclic-GMP. A beneficial role for prostacyclin in brain trauma is evidenced by a reduction in contusion volume and an increased flow of cortical blood in the rat after prostacyclin infusion (Bentzer et al., 2001, 2003; Lunblad et al., 2008). A recent study has showed that brain-derived neurotrophic factor (BDNF) favors production of PGI₂ and thus rejuvenates the cerebral arterial wall by enhancing vasodilator capacity and protecting against vasoconstrictor stimuli (Santhanam et al., 2010). Increased local concentration of PGI₂ in the arterial wall is also known to activate prosurvival signaling by activation of peroxisome proliferator-activated receptor delta (He et al., 2008). This, in turn, may increase resistance of cerebral circulation to injury. A microdialysis study

in men has also indicated that prostacyclin infusion in doses approximating physiological endogenous production improves oxygenation and reduces cell damage in the traumatized brain (Grände et al., 2000). PGI₂ activates the IP receptor, which leads to an increase in cAMP followed by PKA activation (Tang et al., 1995; Bos et al., 2004). Recent studies have suggested that IP receptor activation by PGI₂ may be beneficial after brain injury in mice by promoting the enhancement of cortical perfusion and the reduction of neuronal cell loss (Lunblad et al., 2008). In the brain trauma model, PGI₂ plays a role in edema formation following inflammation in peripheral tissue, thus exacerbating injury (Murata et al., 1997). The prostacyclin sodium salt and its synthetic stable analogues (iloprost, beraprost, treprostinil, epoprostenol, cicaprost) are efficacious for the treatment of advanced critical limb ischemia (e.g., in the course of Buerger's disease) and pulmonary artery hypertension (PAH).

Tromboxane A₂

(TxA₂) is a strong activator of platelets, a vasoconstrictor and a smooth-muscle mitogen that is produced in blood platelets, monocytes and vascular smooth muscle cells (VSMC) (Dogne et al., 2004, 2005). Because TxA₂ and PGI₂ possess opposing biological functions, the balance between these two mediators is crucial in both healthy and diseased vasculature (Dogne et al., 2005). TxA₂ is also synthesized in the central nervous system (Kong et al., 1991; Yalcin et al., 2005a), where it plays an important neuromediating role, including in neuroendocrine activities (Murakami et al., 1998; Yalcin et al., 2005b). The endogenous production and release of cerebral TxA₂ increases during ischemia and hypotensive conditions, such as cardiogenic and hemorrhagic shock (Kong et al., 1991). TxA₂ binds to TP, a 7-membrane-spanning G protein-coupled receptor that, upon activation, initiates pathophysiological processes, including the production of interleukin-1 β and the activation of monocytes (Miggin et al., 1998; Giannarell et al., 2010). TP-receptors are expressed in platelets and in the endothelium, smooth muscle cells, macrophages, monocytes (Meja et al., 1997; Giannarell et al., 2010), brain stem and astroglial cells (Gao et al., 1997). TP stimulation also increases the production of reactive oxygen species (ROS) and peroxynitrites by up-regulating PKC-dependent NAD(P)H oxidase activation. Persistent TP stimulation causes endothelial dysfunction by the uncoupling of eNOS activity (Zhang et al., 2010). The TP receptor is also activated by isoprostanes (F₂-IsoPs). As free-radical-catalyzed oxidation products of AA, isoprostanes, in addition to providing specific biomarkers of lipid peroxidation, are potent vasoconstrictors that cause bronchoconstriction, promoting platelet activation and the proliferation of fibroblast and endothelial cells (Pratico et al., 2001; Dogne et al., 2005). TP-receptor signaling transduction involves calcium signaling (Shenker et al., 1991; Berridge and Irvine, 1984), alterations in adenylyl cyclase, the activation of phospholipase C and changes in cAMP levels. TP-receptor antagonists inhibit the prostanoid-mediated vasoconstriction associated with aging, diabetes and hypertension, which are related to increased oxidative stress and the consequent up-regulation of COX-1 and/or induction of COX-2 (Vanhoutte et al., 2005).

Prostaglandin E₂

(PGE₂) is the most widely produced prostaglandin in the body, and it exhibits a variety of actions, including reproductive, neuronal, metabolic and immune functions. PGE₂ is generally considered to be a proinflammatory molecule associated with redness, swelling and pain (Harris et al., 2002; Legler 2010) and has significant effects on proliferation, the apoptosis of lymphocytes and the regulation of cytokine production in T cells (Harris et al., 2002). Endogenous PGE₂ might contribute to either neurotoxicity or neuroprotection in the injured brain via the induction of BDNF release from microglial cells and astrocytes (Hutchinson et al., 2010). In addition, PGE₂ may also reduce microglial activation and secondary neuronal toxicity by decreasing the levels of pro-inflammatory cytokines and

inducible nitric oxide synthase (iNOS) during brain inflammation (Minghetti et al., 1997; Caggiano et al., 1998; Zhang and Rivest, 2001). Thus, PGE₂ may exert functionally opposing effects; it induces pro- and anti-inflammatory effects, stimulating both toxic and protective effects in a variety of neuronal tissues (Akaike et al., 1994; Takadera et al., 2004; Andreade da Costa et al., 2009), and it elicits both smooth muscle relaxation and constriction (Walch et al., 2001; Davis et al., 2004). These different physiological effects of PGE₂ are mediated by the four PGE₂ receptor subtypes, EP1–4, which are widely expressed and linked to functionally antagonistic second messenger systems. In addition, the EP3 receptor has three transcriptional splice variants, EP3 α , EP3 β and EP3 γ . The EP3 β receptor is unique because it does not desensitize and thus displays persistent signaling when opposed to its ligand (Breyer et al., 2001; Nakamura et al., 2001). EP receptors exhibit structural, pharmacological and functional differences, which together determine the biological effects of PGE₂. EP1 receptors mediate signaling events by inducing the activation of phospholipase C and the elevation of cytoplasmic signaling intermediates including inositol triphosphate, diacylglycerol and calcium. While EP2 and EP4 receptors are linked to the stimulation of adenylyl cyclase and cAMP/protein kinase A (PKA) (Honda et al., 1993), signaling via EP3 receptors is unique, as cAMP levels are decreased (Narumiya et al., 1999; Dey et al., 2006). EP receptors are differentially expressed on almost all organs, including within the central nervous system. Their expressions are also located in specific regional and cellular areas in the rodent brain (Cimino et al., 2008). EP receptors are found in endothelial cells, microglia, astrocytes and neurons (Cimino et al., 2008; Carlson et al., 2009). EP4 expression in neurons is restricted to hypothalamic nuclei, while neuronal EP1, EP2 and EP3 are expressed in multiple brain regions including the hippocampus, striatum and cortex (Kawano et al., 2006; Bhattacharya et al., 1998; Nakamura et al., 2000; Oka et al., 2000; Sugimoto et al., 1994; Adreasson, 2010).

PGE₂ binds to EP3 and EP4 with higher affinity than EP1 and EP2. In mice, PGE₂ binding affinities are as follows: EP3 (k_d =0.9 nM) > EP4 (k_d =1.9 nM) > EP2 (k_d =12 nM) > EP1 (k_d = 20 nM). Relatively selective ligands for all four EP receptors have been used to investigate the differential effects of PGE₂. Commonly used selective EP receptor agonists include ONO-DI-004, ONO-AE1-259, ONO-AE-248 and ONO-AE1-329 for EP1, EP2, EP3 and EP4, respectively. Additional agonists for EP1 are Iloprost and 17-trinor PGE₂. The agonist for EP4 is PGE1-alcohol. Butaprost and 11-deoxy PGE1 are used as EP2 agonists. EP3 agonists include Sulprostone and Misoprostol. To date, no selective antagonist for EP2 exists. However, the antagonists, SC 51322, SC 51089 and ONO-8713, are used for EP1; ONO-AE3-240 and L-826266 are used for EP3 and AH 23848; and ONO-AE3-208 and CJ-023,423 are used as selective antagonists for EP4.

PGE₂ and neurotoxicity

PGE₂ plays an important role in variety of neurotoxic processes. Multiple PGE₂ receptor subtypes with different ligand affinities, cellular and tissue receptor expression profiles and coupling to opposing second messenger systems mediate PGE₂'s signaling versatility, and often, its opposing biological actions. Levels of PGE₂ in the central nervous system (CNS) are up-regulated in various neurological disorders including Alzheimer's disease, Parkinson's disease, multiple sclerosis, HAD and amyotrophic lateral sclerosis (Montine et al., 2002; Minghetti 2004; McGeer and McGeer, 2005; Cimino et al., 2008; Liang et al., 2008; Adreasson, 2010) (Table 1). The mechanisms associated with neuronal death in these diseases include direct or indirect neurotoxicity, glutamate receptor-mediated excitotoxicity and innate immune activation (Minghetti, 2004).

The relevance of PGs, particularly PGE₂, in the process of innate immune activation is highlighted by the effectiveness of NSAIDs as COX-inhibitors. However, studies in mice

deficient in individual EP receptors have revealed that PGE₂ acts not only as a pro-inflammatory mediator, but also exerts anti-inflammatory responses (Shi et al., 2010; Legler et al., 2010). We have shown that the EP2 receptor plays a critical role in the generation of ROS as well as in increased NOS activity in response to innate immune activation (Montine et al., 2002; Milatovic et al., 2005). Using an intracerebroventricular (ICV) model, we have identified the molecular and pharmacological determinants of lipopolysaccharide (LPS)-initiated innate immune response and cerebral neuronal damage *in vivo* (Montine et al., 2002; Milatovic et al., 2003, 2004). The bacterial endotoxin, LPS, specifically activates innate immunity through a Toll-like receptor (TLR)-dependent signaling pathway (Imler and Hoffmann, 2001; Akira, 2003). Responses to LPS that require the CD14 protein and the adaptor protein, MyD88, initiate a signal transduction cascade that culminates in altered gene transcription, primarily via the activation of the NF- κ B pathway, but also through c-Fos/c-Jun-dependent pathways. These signaling events result in the generation of effector molecules, including bacteriocidal molecules, which are primarily free radicals generated by NADPH oxidase and myeloperoxidase (MPO), as well as cytokines and chemokines that can mediate an adaptive immune response (Milatovic et al., 2004).

For the evaluation of oxidative damage, we used a stable isotope dilution method with gas chromatography and negative ion chemical ionization mass spectrometry (GC-MC/NICI) (Morrow and Roberts, 1997; Milatovic et al., 2009). Results from our studies showed that single ICV LPS injections in mice induced delayed, transient elevation in both F₂-IsoPs and neuroprostanes (F₄-NeuroPs, peroxidation product of docosahexaenoic acid) at 24 hr post exposure, with a return to baseline levels by 72 hr post exposure (Table 2) (Milatovic et al., 2003). While others have shown that altered gene transcription and increased cytokine secretion occur rapidly and peak within a few hours of LPS exposure, it is likely that the delay in neuronal oxidative damage as observed in our experiments is related, at least in part, to the time required to deplete anti-oxidant defenses.

We also examined the dendritic compartment of neurons using Golgi impregnation and NeuroLucida-assisted morphometry in hippocampal CA1 pyramidal neurons (Leuner et al., 2003; Milatovic et al., 2010). Initially, we determined the time course of dendritic structural changes following ICV LPS in mice. Our results showed a time course similar to neuronal oxidative damage with maximal reduction in both dendrite length and dendritic spine density at 24 hr post LPS and, remarkably, a return to baseline levels by 72 hr post treatment (Table 2). These data strongly suggest that neuronal oxidative damage is closely associated with dendritic degeneration following ICV LPS exposure. We and others have shown that primary neurons enriched in cell culture do not respond to LPS (Minghetti and Levi, 1995; Fiebich et al., 2001; Xie et al., 2002). Therefore, our results have conclusively established that LPS-activated microglia mediated paracrine oxidative damage to neurons. Importantly, our experiments with knock-out animals demonstrated that EP2 deficient (EP2 $-/-$) mice failed to mount the inflammatory oxidative response and synaptic damage seen in wild-type mice (Table 2). Additional *in vitro* experiments with microglia obtained from EP2 $-/-$ mice showed that microglial EP2 was critical to LPS-activated microglia-mediated neurotoxicity (Shie et al., 2005). The pharmacological suppression of microglia with COX inhibitors (with ibuprofen the most potent likely due its well-described activities in addition to COX suppression, Insel, 1996) also completely suppressed microglia-mediated neurotoxicity (Shie et al., 2005). Given the concordance between these *in vivo* and *in vitro* findings as well as between our genetic and pharmacological manipulations with NSAIDs, these data strongly implicate the microglial EP2-dependent release of paracrine effectors of neuronal damage following activation by LPS and establish that PGE₂ signaling through microglial EP2 plays a central role in the inflammatory oxidative response and secondary neurotoxicity.

An important role for EP2 in the regulation of microglia-mediated neurotoxicity is also supported by an *in vitro* study showing that the ablation of microglial EP2 enhanced A β phagocytosis while, at same time, suppressed damage to neurons (Shie et al., 2005). Our additional data indicate a reinforcing cycle between the production of oxidative damage and the formation of neurotoxic A β deposition (Woltjer et al., 2007). Moreover, studies using knock-out mice demonstrated that EP2 depletion in aged APPSwe-PS1 Δ E9 (APPS) transgenic mice resulted in lower levels of A β peptide, fewer amyloid depositions and a significant decrease in neuronal oxidative damage (Liang et al., 2005).

Analogous to AD, microglia and PG signaling play a significant role in disease progression in PD. EP2 $^{-/-}$ microglia displayed enhanced *ex vivo* clearance of aggregated α -synuclein in the mesocortex of the Lewy body disease patient while attenuating neurotoxicity and α -synuclein aggregation in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD (Jin et al., 2007). An additional *in vitro* study examining PGE $_2$ signaling in rat dopaminergic neurons showed that EP1 receptor inhibition protected against 6-hydroxy dopamine (6-OHDA)-mediated selective neurotoxicity (Carrasco et al., 2007).

Recent studies also point to a significant role for the EP2 receptor in glial-mediated secondary neurotoxicity in motor neuron degeneration in the G93ASOD transgenic model of familial amyotrophic lateral sclerosis (Boillee et al., 2006; Di Giorgio et al., 2007; Nagai et al., 2007). Genetic deletion of the EP2 receptor in this model significantly reduced the expression of oxidative enzymes, improved motor strength and extended animal survival (Liang et al., 2008).

However, in contrast to microglial EP2-mediated secondary neurotoxicity due to increases of ROS and pro-inflammatory cytokines, EP2 signaling by a PKA-dependent mechanism mediates significant neuroprotection in acute models of cerebral ischemia and excitotoxicity. Studies examining glutamate receptor-mediated toxicity (Ahmad et al., 2006) and middle cerebral artery occlusion/reperfusion (Liu et al., 2005) have demonstrated the protective effects of the EP2 receptor and have indicated that this mechanism of protection is dependant upon cAMP signaling for its functionality (McCullough et al., 2004). These findings have been corroborated by studies using organotypic slice cultures from the hippocampus (Liu et al., 2005) and the spinal cord (Bilak et al., 2004). Therefore, the neurotoxic effects in the models of innate immunity, AD and amyotrophic lateral sclerosis opposed to a cerebroprotective role in models of excitotoxicity and cerebral ischemia reflect the dichotomy of EP2's action with contradictory biological effects for PGE $_2$.

In contrast to the protective effect of EP2 observed in models of cerebral ischemia and excitotoxicity, the pharmacological inhibition or gene inactivation of the EP1 receptor ameliorates brain injury induced by middle cerebral artery (MCA) occlusion, oxygen glucose deprivation and excitotoxicity (Kawano et al. 2006; Carlson et al. 2009; Ahmad et al., 2006; Adreasson 2010). Studies examining the effect of pretreatment with the EP1 receptor selective agonist, ONO-DI-004, have demonstrated the exacerbation of the intrastriatal excitotoxic lesion induced by NMDA, whereas treatment with the selective EP1 antagonist, ONO-8731, was shown to produce a significant protective effect against NMDA-induced neurotoxicity. EP1 receptor involvement in the neurotoxicity has also been supported by experiments in EP1-deficient mice, which have demonstrated a significant decrease in NMDA-induced lesions compared to wild-type mice subjected to the same excitotoxic paradigm (Ahmad et al., 2006). Since NMDA-induced neurotoxicity is associated with increased calcium flux through the NMDA receptor as well as disrupted intracellular calcium homeostasis, the strategic blockade of EP1 or specific genetic deletions revealed the normalization of intracellular Ca $^{2+}$ and improved function of the Na $^{+}$ /Ca $^{2+}$ exchange (Kawano et al., 2006). Furthermore, EP1 receptor involvement in ischemic brain

damage has been demonstrated in a study wherein significant cerebroprotection and the rescue of behavioral effects were observed upon administration of an EP1 antagonist (Ahmad et al., 2006; Abe et al., 2009). EP1 receptor activation is also associated with vasoconstriction in the peripheral vasculature; therefore, the inhibition of EP1 signaling may lead to cerebroprotection not only by exerting effects on Ca^{2+} homeostasis, but also by attenuating cerebral blood flow.

Stimulating EP3 pharmacologically has also been shown to increase infarct size in the middle cerebral artery occlusion model of ischemia (Ahmad et al., 2007). However, glutamate excitotoxicity studies using hippocampal neurons and organotypic slices showed a protective effect for EP3 neuronal signaling (Bilak et al., 2004; Burks et al., 2007). These contradictory data may, in part, be explained by the existence of three distinct isoforms with different signaling pathways, desensitization and constitutive activity (Breyer et al., 2001; Bilson et al., 2004; Hasegawa et al., 1996; Adreasson, 2010).

Similar to the EP2 receptor, the EP4 receptor is also positively coupled to cAMP with strong expression induced by systemic LPS administration (Zhang et al., 1999). However, recent evidence suggests that the EP4 receptor exerts significant anti-inflammatory effects *in vitro* and *in vivo* by suppressing the proinflammatory gene response in the LPS model of innate immunity. Beneficial EP4 receptor signaling and its selective targeting also indicate therapeutic potential in brain injury and disease (Shi et al., 2010).

In summary, pharmacological inhibition or receptor gene inactivation in mice has revealed diverse and contrasting biological effects for PGE_2 . Depending upon cell-specific signaling cascades and injury context, EP receptors can mediate either toxic or protective effects in processes known to mediate various neurological diseases. Therefore, the development of highly selective agonists and antagonists which readily cross the blood-brain barrier to each EP subtype, clinical trials as well as studies in mice deficient in each EP receptor subtype should be broadened in order to provide new insights into the neurotoxic mechanisms associated with neurodegenerative disease. Importantly, targeting the neurotoxic sequelae of PGs, while maintaining their neuroprotective actions, may lead to the development of efficacious therapeutic strategies.

Acknowledgments

Supported by grants from the National Institute of Health NS057223 (DM), NIEHS ES07331, NIEHS ES16754 (TJM) and NIEHS ES10563 (MA).

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Figure 1.

Prostanoid biosynthesis and response pathway. Arachidonic acid is metabolized by cyclooxygenases to the unstable endoperoxide PGH_2 , the common precursor for the prostaglandins and thromboxane. Prostaglandins $\text{F}_{2\alpha}$, D_2 , I_2 , E_2 and thromboxane A_2 , generated by individual prostaglandin synthase enzymes, elicit their biological effects by activating G protein-coupled receptors. EP receptors mediate both neurodegenerative and neuroprotective effects in models of brain injury.

Table 1

PGE₂ levels in cerebrospinal fluid from patients with neurological disease.

Disease	Cerebrospinal fluid PGE ₂ levels
AD	Increased 5-fold in early AD (Montine et al., 1999); Highest increase in early AD, but decline with progressive cognitive impairment (Combrinck et al., 2006)
ALS	Increased 6-fold; Increased 2 to 10 fold (Ilzecka, 2003; Almer et al., 2002); no increase (Cudkowicz et al., 2006)
Ischemic stroke	Increased 2-fold during initial 72 hr (Aktan et al., 1991)
HAD	Increased 40% in all HIV-seropositive patient (Griffin et al., 1994)

(adopted from Cimino et al., 2008)

Table 2

Cerebral oxidative damage and dendritic degeneration in wild-type (w.t) and EP₂ deficient (EP₂ ^{-/-}) mice. Effects of ICV saline (5 µl, control) and ICV LPS (5 µg/5 µl) treatment determined at 24 hr and 72 hr post exposure.

	24 hr w.t.	24 hr w.t.	24 hr EP ₂ ^{-/-}	72 hr w.t.
	ICV saline	ICV LPS	ICV LPS	ICV LPS
F ₂ -IsoPs (ng/g tissue)	3.26 ± 0.19	4.77 ± 0.26	3.33 ± 0.21	2.98 ± 0.17
F ₄ -NeuroPs (ng/g tissue)	13.91 ± 1.17	58.50±5.98	10.95 ± 0.98	16.80 ± 0.96
Dendritic length (µm)	1018 ± 113	324 ± 37	1038 ± 124	1030 ± 61
Spine density (spine no./100 µm dendrite)	16.89 ± 1.67	5.86 ± 0.57	18.41 ± 0.92	16.77 ± 0.87

Adult (6 to 8 week old, n > 5 in each group) mice homozygous deficient (knockout) for EP₂ receptor and wild type (wt) mice received ICV saline or ICV LPS 24 hr or 72 hr prior to sacrifice. Ipsilateral cerebral hemispheres were processed for F₂-IsoPs and F₄-NeuroPs evaluation and tissue sections of hippocampus and surrounding structures were processed for Golgi stain and then evaluated by Neurolucida. Morphometric data are dendrite length and spine density for CA1 hippocampal pyramidal neurons (n > 15 neurons for each group). *Significant difference for one-way ANOVA with Bonferroni-corrected repeated pair comparisons p<0.001