Published in final edited form as:

Mol Cell Neurosci. 2008 April ; 37(4): 682–695. doi:10.1016/j.mcn.2007.12.019.

COX-2 oxidative metabolism of endocannabinoids augments hippocampal synaptic plasticity

Hongwei Yang¹, Jian Zhang¹, Katrin Andreasson², and Chu Chen^{1,*}

¹Neuroscience Center of Excellence, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112

²Department of Neurology & Neurological Sciences, Stanford University, Stanford, CA 94305, USA

Abstract

Endocannabinoids (eCBs) are important endogenous lipid mediators in synaptic transmission and plasticity and are oxygenated by cyclooxygenase-2 (COX-2) to form new types of prostaglandins. However, little is known about whether COX-2 oxidative metabolism of eCBs and their metabolites alter synaptic signaling. Here we demonstrate that increased COX-2 expression significantly enhances basal synaptic transmission and augments long-term potentiation (LTP) in the mouse hippocampus. This augmentation was inhibited in the presence of a selective COX-2 inhibitor or with deletion of the COX-2 gene. The CB₁ receptor-mediated depolarization-induced suppression of inhibition (DSI) was diminished when COX-2 expression was increased either with lipopolysaccharide (LPS) stimulation or transgenic neuronal over-expression of COX-2. Conversely, DSI was potentiated when COX-2 activity was pharmacologically or genetically inhibited. Interestingly, COX-2 oxidative metabolites of eCBs elevated LTP, an effect opposite to that of their parent molecules 2-arachidonoylglycerol (2-AG) and arachidonoyl ethanolamide (AEA). In addition, the ERK/MAPK and IP₃ pathways were found to mediate PGE₂-G-induced enhancement of LTP. Our results indicate that COX-2 oxidative metabolism of eCBs is an important signaling pathway in modulation of synaptic transmission and plasticity.

Keywords

Prostaglandin; Endogenous cannabinoids; Long-term potentiation

Introduction

Endocannabinoids (eCBs) are endogenous lipid mediators that play an important role in modulating cannabinoid CB₁ receptor-mediated inhibitory and excitatory synaptic transmission and plasticity in brain (Alger, 2005; Chevalleyre et al., 2006; Freund et al., 2003; Mackie, 2006; Piomelli, 2003; Sugiura et al., 2006). The most studied eCBs are 2-arachidonoylglycerol (2-AG) and arachidonoyl ethanolamide (AEA). The production and degradation of 2-AG and AEA occur through different pathways (Piomelli, 2003; Freund et al., 2003; Sugiura et al., 2004; Stella, 2004; Mackie, 2006). 2-AG is mainly produced from diacylglycerol (DAG) by diacylglycerol lipase (DGL) and hydrolyzed to arachidonic acid (AA) by monoacylglycerol lipase (MGL), whereas AEA is largely synthesized from N-arachidonoylphosphatidylethanolamine (NAPE) by phospholipase D (PLD) and degraded to

*Correspondence should be addressed to: Chu Chen, Ph.D., Neuroscience Center of Excellence, Louisiana State University Health Sciences Center, 2020 Gravier Street, Suite D, New Orleans, LA 70112, Tel. (504) 599-0918, Fax: (504) 599-0488, Email: cchen@lsuhsc.edu

AA by fatty acid amide hydrolase (FAAH, Freund et al., 2003; Piomelli, 2003; Sugiura et al., 2004). Recent studies show that 2-AG and AEA are substrates for cyclooxygenase-2 (COX-2), an inducible enzyme that converts AA to classic prostaglandins. 2-AG and AEA can also be oxygenated by COX-2 to novel prostaglandin isoforms: prostaglandin glyceryl esters (PG-Gs) and prostaglandin ethanolamides (PG-EAs, Kozak et al., 2000; 2002; 2004; Sang and Chen, 2006; Yu et al., 1997). Interestingly, 2-AG and AEA are poor substrates for COX-1, suggesting that the COX-2 oxidative metabolism is an important pathway in degrading eCBs (Kozak et al., 2004; Sang and Chen, 2006). This means that up- or down-regulation of COX-2 expression and activity will significantly influence eCB signaling in synaptic activity. This prediction has been confirmed by recent studies where the COX-2 inhibition augments hippocampal DSI (Kim and Alger, 2004; Sang et al., 2006) and elicits a CB₁-mediated decrease of excitatory transmission and LTP in rat CA1 hippocampus (Slanina and Schweitzer, 2005; Slanina et al., 2005).

COX-2 has been demonstrated to participate in synaptic transmission and plasticity (Chen et al., 2002; Murray and O'Connor 2003; Chen and Bazan, 2005; Sang et al., 2005; Akaneya and Tsumoto et al., 2006). COX-2 effects on synaptic transmission are dependent on increased production of AA-derived prostaglandins, mainly PGE₂. Since eCBs are important mediators involved in synaptic transmission and plasticity, COX-2 oxidative metabolism of eCBs likely alters eCB signaling. In this study, we determined effects of the COX-2 elevation and inhibition, and COX-2 oxidative metabolites of eCBs on hippocampal synaptic plasticity. We observed that increased COX-2 expression increases hippocampal basal synaptic transmission, augments LTP, and abolishes depolarization-induced suppression of inhibition (DSI), while inhibition of COX-2 reduces LTP and potentiates DSI. Moreover, we demonstrate that COX-2 oxidative metabolites of eCBs enhance LTP, an effect that is opposite to that of their parent molecules 2-AG and AEA. In addition, we provide evidence that ERK, p38 MAPK and IP₃ pathways mediate PGE₂-G-, a major COX-2 oxidative metabolite of 2-AG, induced elevation of LTP. Our results suggest that COX-2 oxidative metabolism of eCBs is an important mechanism in regulation of eCB signaling in synaptic plasticity.

Results

Elevation of COX-2 expression enhances basal synaptic transmission and augments LTP

Pharmacologic or genetic inhibition of COX-2 decreases LTP and LTD (Akaneya and Tsumoto et al., 2006; Chen et al., 2002; Murray and O'Connor 2003; Slanina et al., 2005). However, it is not known whether elevated COX-2 activity influences synaptic efficacy. To determine whether increased COX-2 expression produces an effect on long-term synaptic plasticity, we first examined the time course of COX-2 expression following the injection of lipopolysaccharide (LPS), a commonly used inducer of COX-2 expression. Injection of LPS (3 mg/kg, i.p.) had little effect on hippocampal COX-1 expression by real time PCR (Fig. 1A). However, COX-2 was robustly elevated 4 hrs after injection of LPS, with a gradual return to control level by 24 hrs following the LPS injection ($P<0.01$ and $P<0.05$, $n=3$; one way ANOVA, Fisher's PLSD). In the meantime, we monitored the levels of FAAH and MGL, which hydrolyze AEA and 2-AG, respectively. While the expression of MGL was not affected by LPS injection, FAAH was elevated 12 hrs following LPS injection ($P<0.05$ and 0.01 , one way ANOVA, Fisher's PLSD, Fig. 1A). This elevated FAAH may facilitate hydrolysis of AEA, which in turn affects synaptic activity. We confirmed that COX-2 protein levels were also elevated (Figure 1B) 4 hrs after LPS injection, remaining stable to 12 hrs and returning back to baseline by 24 hrs following LPS injection ($P<0.01$, $n=3$, one way ANOVA, Fisher's PLSD).

Having established the regulation of COX-2 expression in response to LPS, we recorded LTP at hippocampal perforant path-dentate granule synapses in animals that received LPS (3 mg/kg). LTP was induced by high-frequency stimulation (HFS) consisting of 8 trains, each of 8

pulses at 200 Hz with an inter-train interval of 2 sec as described previously (Chen et al., 2002; Chen, 2004). Field excitatory postsynaptic potentials (fEPSPs) were recorded at the molecular layer in response to perforant path stimulation at a frequency of 0.05 Hz. As shown in Figure 1C, LTP was significantly enhanced 4 and 12 hrs following LPS injection ($p < 0.01$) and returned to the control level 24 hrs after the injection. The LPS-enhanced LTP was inhibited by NS398 (10 mg/kg, i.p.), a selective COX-2 inhibitor. These results clearly show that the elevation of COX-2 augments hippocampal LTP.

Previously we demonstrated that an elevation of COX-2 expression induced by LPS or interleukin-1 β enhances mEPSCs in hippocampal neurons in culture (Sang et al., 2005). To determine whether LPS *in vivo* injection also alters basal excitatory synaptic transmission, we measured input-output function, paired-pulse ratio and miniature EPSCs (mEPSCs) in the animals injected with vehicle, LPS (3 mg/kg) and LPS plus NS398 (10 mg/kg). While LPS did not induce significant changes in the paired-pulse ratio (vehicle: 1.26 ± 0.02 , $n=6$, versus 12 hrs after LPS injection: 1.22 ± 0.05 , $n=8$, $P > 0.05$), it significantly enhances the input-output function and the frequency of mEPSCs (Figure 2). The LPS-induced increases in input-output function and mEPSCs were inhibited by NS398, indicating that the enhanced basal synaptic activity is associated with the elevation of COX-2. We noticed that the amplitude of mEPSCs was not significantly changed in animals that received LPS. This is consistent with our previous observations (Sang et al., 2005).

To confirm that increased COX-2 enhanced hippocampal long-term synaptic plasticity, we recorded LTP in COX-2^{-/-} (KO) and Thy-1-COX-2 transgenic (Tg) mice. As seen in Figure 3A, LTP was not elevated in COX-2 KO mice that received LPS (3 mg/kg), while LTP was significantly elevated in age-matched wild type littermates injected with LPS, indicating that the LPS-enhanced LTP resulted from expression of COX-2. Furthermore, the LPS-induced increase in LTP was inhibited by NS398 in wild type control animals (Fig. 3A). To confirm that increased expression of COX-2 potentiated LTP, LTP was examined in neuronal COX-2 Tg mice (Andreasson et al., 2001) and was found to be enhanced in COX-2 Tg animals as compared to wild type controls (Fig. 3C). Taken together, these data indicate that increased COX-2 expression increases LTP. These data are consistent with previous studies demonstrating that acute inhibition of COX-2 activity by selective COX-2 inhibitors or transient suppression of COX-2 expression by silencing the COX-2 gene reduces LTP in the hippocampus and visual cortex (Akaneya & Tsumoto, 2006; Chen et al., 2002; Murray & O'Connor, 2003). However, we observed that HFS-induced LTP is normal in COX-2 KO animals when compared to that of the wild type littermates, possibly from developmental compensatory effects.

Effects of alterations in COX-2 on DSI

Endocannabinoids have been proposed to act as retrograde messengers (Alger, 2002; Wilson and Nicoll, 2001; 2002). The reduction of synaptic transmission and plasticity by eCBs released from postsynaptic neurons may result from the activation of the G-protein-coupled CB₁ receptor on presynaptic sites. This hypothesis derives from results of depolarization-induced suppression of inhibition (DSI), which refers to eCB-induced suppression of GABAergic synaptic transmission, and depolarization-induced suppression of excitation (DSE), which refers to eCB-induced suppression of glutamatergic synaptic transmission (Alger, 2002; Diana and Marty, 2004; Freund et al., 2003; Piomelli, 2003;). Several lines of evidence indicate that manipulations that increase or decrease the synthesis/degradation of eCBs alter DSI or DSE (Freund et al., 2003; Wilson & Nicoll, 2001; 2002). Recent evidence demonstrates that eCBs are oxidized by COX-2 (Kazok et al., 2004; Sang and Chen, 2006). Inhibition of COX-2 augments DSI, indicating that COX-2 inhibition prevents depolarization-produced eCBs from being degraded (Kim & Alger, 2004; Sang et al., 2006). Therefore, DSI or DSE represent robust

assays to functionally determine whether COX-2 inhibition or elevation induces alterations in eCB metabolism and associated synaptic signaling.

We used the whole-cell patch clamp recording technique to characterize how eCBs modulate synaptic activity when the expression of COX-2 is elevated or inhibited in dentate granule neurons in hippocampal slices from COX-2 KO and wild type control mice. As indicated in Figure 4A, a 5-sec depolarizing step from a holding potential of -70 mV to 0 mV induced a reduction of inhibitory postsynaptic currents (IPSCs) in wild type slices. This reduction was more pronounced in slices pretreated with NS398 ($P < 0.05$). SR141716 (SR, 1 μ M), a selective CB₁ receptor antagonist (provided by Chemical Synthesis and Drug Supply Program, the National Institute of Mental Health), abolished DSI both in control and NS398 treated slices from wild type slices, indicating that DSI is mediated by the CB₁ receptor. Interestingly, the magnitude of DSI recorded in slices from COX-2 KO slices was similar to that recorded in slices treated with the COX-2 inhibitor in wild type slices, and DSI was also blocked by SR (Fig. 4B). These results indicate that pharmacologic or genetic inhibition of COX-2 abolishes the COX-2 oxidative metabolism of eCBs and augments eCB-mediated retrograde signaling in synaptic transmission (Kim and Alger, 2004; Sang et al., 2006). We then determined how COX-2 elevation regulates DSI. As shown in Figure 4C, LPS injection eliminated DSI in wild type animals, but failed to inhibit DSI in COX-2 KO mice. This indicates that the elevation of COX-2 expression, which facilitates oxidative degradation of eCBs, abolishes eCB-mediated retrograde signaling in synaptic transmission. To further confirm this finding, we recorded DSI in neuronal COX-2 Tg mice. As expected, DSI was eliminated in COX-2 Tg mice (Fig. 4C). These observations provide functional evidence that COX-2 metabolizes eCBs, resulting in alterations in eCBs signaling in synaptic efficacy.

Alternative reasons why LPS treatment eliminated DSI might be that CB₁ receptors are not functional, that they are saturated by endocannabinoids, or that they are constitutively maximally active. To test these possibilities, we applied Win 55,212-2 (Win), a potent synthetic cannabinoid, in slices from animals injected with vehicle and LPS. As shown in Figure 5B, application of Win (5 μ M) resulted in a significant decrease in IPSCs by $47.5 \pm 5.1\%$ (vehicle, $n=6$) and $48.6 \pm 3.5\%$, (LPS, $n=6$), respectively. The Win 55,212-2-induced decrease in IPSCs was blocked by SR141716 both in vehicle and LPS-treated animals ($2.4 \pm 3.2\%$, $n=7$ versus $1.4 \pm 3.5\%$, $n=6$). Taken together, these data indicate that the CB₁ receptor is functionally active in animals that received LPS treatment.

COX-2 oxidative metabolites of eCBs enhance LTP

Recent evidence demonstrates that COX-2 regulates the formation of eCBs that decrease LTP, and that inhibition of COX-2 decreases excitatory synaptic response in a CB₁R-dependent manner (Slanina & Schweitzer, 2005; Slanina et al., 2005). To determine whether COX-2 inhibition would lead to a decrease in LTP, animals were injected with NS398 (10 mg/kg) and NS398 plus SR (5 mg/kg) for 12 hrs. As shown in Figure 5A, injection of NS398 significantly reduced LTP when compared to vehicle control and this reduction was attenuated in the presence of SR (NS398: $122.3 \pm 5.3\%$ of base, $n=9$ versus NS398+SR: $153.7 \pm 4.8\%$ of base, $n=10$, $P < 0.01$), indicating that endocannabinoids are involved in COX-2 inhibition-mediated depression of LTP. To determine whether inhibition of CB₁ receptors affects LPS-induced enhancement of LTP, animals were injected with LPS with SR (5 mg/kg). The magnitude of LTP in animals that received LPS was similar to that of animals injected with LPS plus SR (LPS-12hr: $202.9 \pm 12.5\%$ of base versus LPS+SR-12hr: $208.8 \pm 7.8\%$ of base; Figure 5A). This suggests that the blockade of the CB₁ receptor does not affect the LPS-enhanced LTP. Presumably the levels of endocannabinoids are reduced by COX-2 oxidative metabolism in animals injected with LPS.

Previous studies indicate that COX-2 functions in long-term synaptic plasticity via its major reaction product PGE₂, acting through the EP2 receptor subtype (Akaneya & Tsumoto, 2006; Chen et al., 2002; Sang et al., 2005). To further examine the role of COX-2-mediated potentiation of LTP, the animals were co-injected with LPS and AH 6809, an EP1/EP2 antagonist. LTP was attenuated in animals that were administered AH 6809 and LPS as compared to LPS injection alone, but was still significantly elevated compared to that of vehicle controls ($149.2 \pm 5.3\%$ baseline, $n=6$ versus $171.2 \pm 5.8\%$ baseline, $n=9$, $P=0.02$, one way ANOVA). This suggests that in addition to PGE₂, there are other components involved in COX-2-mediated effects on LTP. One potential mechanism is COX-2-mediated oxidative metabolites generated from eCBs that negatively regulate synaptic transmission and plasticity. We hypothesized that novel prostaglandins, derived from COX-2 oxidative metabolism of eCBs, likely contribute to COX-2-mediated potentiation of LTP. To test this hypothesis, we first determined effects of 2-AG and AEA on hippocampal perforant path LTP. As indicated in Figure 6A, bath application of 2-AG (5 μ M) or AEA (10 μ M) significantly reduced LTP, and the reduction was blocked by SR (1 μ M), suggesting that 2-AG- and AEA-induced reduction of LTP is mediated via a CB1R. We noticed that application of 2-AG (5 μ M) or AEA (10 μ M) slightly reduced the baseline slope of fEPSPs (2-AG: $4 \pm 2\%$, $n=9$; AEA: $11 \pm 2\%$, $n=10$) with 10 min application. This reduction of baseline fEPSPs may shift the threshold for LTP, thereby limiting LTP induction. Apparently, however, this may not be the case. For instance, Stella et al (1997) observed that bath application of 2-AG (20 μ M) abolishes hippocampal CA₁ LTP without affecting baseline fEPSPs (Stella et al., 1997). To determine whether 2-AG or AEA induced a reduction of IPSCs, we recorded IPSCs in perforant path in the presence of 2-AG or AEA. 2-AG and AEA also slightly reduced IPSCs (2-AG: $7 \pm 3\%$, $n=5$; AEA: $5 \pm 2\%$, $n=6$). We also noticed that application of SR alone increased LTP, suggesting that there is a basal release of eCBs in regulation of synaptic transmission and plasticity (Robbe et al., 2002; Slanina et al., 2005).

We then individually applied PGD₂-EA, PGE₂-EA and PGF_{2 α} -EA which are COX-2 oxidative metabolites of AEA; we also applied PGD₂-G, PGE₂-G and PGF_{2 α} -G which are COX-2 oxidative metabolites of 2-AG to hippocampal slices for 20 min. As shown in Figure 6B & 6C, most of these novel prostaglandins, with the exception of PGD₂-EA, PGD₂-G, and PGF_{2 α} -G elevated LTP, suggesting that COX-2 oxidative metabolites of eCBs contribute to the COX-2-mediated enhancement of LTP. Taken together, these and previous results (Akaneya & Tsumoto, 2006; Chen et al., 2002; Murray and O'Connor 2003; Sang et al., 2005) suggest that the enhanced long-term synaptic plasticity associated with increased COX-2 expression arises from an increased production of AA-derived PGE₂, eCB-derived PG-Gs, PG-EAs, and reduced levels of eCBs.

PGE₂-G-induced enhancement of LTP is mediated via ERK/MAPK and IP3 pathways

As mentioned above, 2-AG and AEA are substrates for COX-2 and are oxygenated by COX-2 to generate the novel prostaglandins PG-Gs and PG-EAs (Kozak et al., 2002;2004; Yu et al., 1997; Sang and Chen, 2006). However, COX-2 efficiently oxidizes 2-AG to PG-Gs as it converts AA to classic prostaglandins (Kozak et al., 2000;2004), while the reaction of COX-2 metabolism of AEA to form PG-EAs is relatively slower (Kozak et al., J. 2000;2004). This suggests that 2-AG will be efficiently oxygenated and more PG-Gs will be produced when COX-2 activity is elevated, whereas AEA will not be as highly metabolized and less PG-EAs will be synthesized. To elucidate signal transduction mechanisms, we focused on the PGE₂-G-induced response. We demonstrated previously that MAPK and IP3, but not PKA and PKC, mediate the PGE₂-G-induced modulation of miniature IPSCs/EPSCs in hippocampal neurons in culture (Sang et al., 2006;2007). Recently we also observed that PKA and PKC were not involved in the PGE₂-G-induced modulation of miniature EPSCs in cultured hippocampal neurons (Sang et al., 2007). Thus, we tested the effects of the ERK, p38 MAPK and IP₃

inhibitors, PD 98059, SB 203580, SB 239063 and 2-APB, to determine whether these signaling pathways are involved in the PGE₂-G-mediated response. Figure 7 shows that the PGE₂-G-induced increase in LTP was blocked by bath application of PD 98059, SB 239063 and 2-APB, while these inhibitors did not significantly affect LTP. These results are consistent with our previous findings where the inhibition of ERK/MAPK or IP₃ blocks the PGE₂-G-mediated effect on inhibitory synaptic transmission (Sang et al., 2006; 2007), as well as other observations demonstrating that PGE₂-G triggers an IP₃-mediated intracellular Ca²⁺ mobilization in RAW264.7 macrophage cells (Nirodi et al., 2004).

To confirm the involvement of these signal transduction pathways mediating the PGE₂-G-induced effect, we assayed for phosphorylation of ERK and p38 MAPK in the presence of PGE₂-G. As seen in Figure 7D, PGE₂-G induced a time dependent phosphorylation of ERK and p38 MAPK in hippocampal slices. ERK and p38 MAPK inhibitors were then applied to determine whether the phosphorylation of ERK/MAPK would then be inhibited. We observed that PD 98059 and SB 203580 blocked the PGE₂-G-induced phosphorylation of ERK and p38 MAPK, respectively (data not shown). These results indicate that ERK and p38 MAPK are involved in the PGE₂-G-mediated response.

Discussion

We demonstrate here that hippocampal synaptic transmission is strengthened when COX-2 activity is elevated. The COX-2 mediated enhancement of synaptic plasticity is likely to be a result of increased production of AA-derived PGE₂ and eCB-derived PG-Gs and PG-EAs, and reduced eCBs. This is evidenced by (1) enhanced LTP and diminished DSI when COX-2 expression is induced by LPS or over-expressed in a transgenic COX-2 model, (2) by enhanced DSI when COX-2 is pharmacologically or genetically inhibited, and (3) by augmented LTP produced by eCB oxidative metabolites. Our findings indicate that COX-2 oxidative metabolism of eCBs is an important and novel signaling pathway in the modulation of synaptic plasticity.

COX-2 participates in synaptic transmission and plasticity (Chen et al., 2002; Murray and O'Connor 2003; Chen and Bazan, 2005; Sang et al., 2005; Akaneya and Tsumoto et al., 2006) via its downstream metabolite PGE₂. However, eCBs also are substrates for COX-2 and are oxygenated to new types of prostaglandins (Kozak et al., 2004; Sang and Chen, 2006). In particular, 2-AG is an endogenous substrate for COX-2 and its oxygenation by COX-2 is more efficient than that of AEA (Kozak et al., 2000; 2004). In addition, 2-AG is the most abundant endogenous cannabinoid and a full agonist for cannabinoid receptors (Alger, 2005; Chevalyere et al., 2006; Freund et al., 2003; Mackie, 2006; Sugiura, 2006). This suggests that the role of AEA in COX-2-oxidative events may not be as significant as that of 2-AG, although we observed in the present study that COX-2 oxidative metabolites of AEA increase LTP. Recent studies show that 2-AG is likely a retrograde signaling molecule responsible for DSI (Alger, 2005; Chevalyere et al., 2006; Mackie, 2006; Sugiura, 2006). Enzymes that synthesize 2-AG are present in postsynaptic dendritic spines, providing direct evidence that 2-AG is synthesized in postsynaptic sites and acts on presynaptic CB₁ receptors (Katona et al., 2006; Yoshida et al., 2006). We observed in the present study that the expression of MGL is not altered during LPS injection, suggesting that COX-2 oxidative metabolism is the major 2-AG degradation pathway when COX-2 activity is elevated. COX-2 is expressed in postsynaptic dendritic spines (Kaufmann et al., 1996; Sang et al., 2005). COX-2 and enzymes synthesizing 2-AG are colocalized in the same subcellular compartment, suggesting that 2-AG can be easily oxygenated by COX-2, particularly when COX-2 is elevated. This may explain the enhanced LTP and diminished DSI in LPS-treated and COX-2 transgenic mice we observed in the present study. Of note however are studies indicating that LPS inhibits LTP in the rat dentate gyrus (Vereker et al., 2000). The discrepancy between our observations and these is not clear, but

may be due to differences in doses used (3 mg/kg versus 0.2 mg/kg) and in the methods used to record (ex-vivo slices versus in vivo). We observed that the time courses of enhanced LTP were similar to that of COX-2 elevation following LPS injection. In addition, the data generated from the experiments where the LPS-induced enhancement of LTP is blocked by a selective COX-2 inhibitor and LPS failed to enhance LTP in COX-2 KO mice support the notion that COX-2 elevation promotes LTP.

We reported previously that COX-2 oxidative metabolites of endocannabinoids induce an increase in inhibitory GABAergic synaptic transmission, while classic prostaglandins, derived from AA, induce a decrease in mIPSCs. This means that the effect of novel prostaglandins derived from eCBs in modulation of synaptic transmission is different from that of classic prostaglandins derived from AA, and suggests that effects of these novel prostaglandins are not mediated via known prostaglandin receptors (Sang and Chen, 2006; Sang et al., 2006; 2007). Since COX-2 mainly targets 2-AG and efficiently oxidizes it to PG-Gs, we examined the signaling pathways of PGE₂-G (a major COX-2 metabolite of 2-AG). While the receptor mediating the PGE₂-G-induced enhancement of LTP is not known, our study provides evidence that ERK and p38 MAPK pathways are involved in the PGE₂-G-induced increase in synaptic plasticity. These results are consistent with our previous findings where inhibition of ERK blocks PGE₂-G-induced enhancement of synaptic transmission in cultured hippocampal neurons (Sang et al., 2006; 2007). Recent evidence shows that PGE₂-G mobilizes intracellular Ca²⁺ via IP₃ and PKC pathways in RAW264.7 macrophage cells (Nirodi et al., 2004). Although we did not detect intracellular Ca²⁺ in the presence of PGE₂-G, the PGE₂-G-induced increase in LTP is attenuated by an IP₃ inhibitor, indicating the involvement of the IP₃-mediated mobilization of intracellular Ca²⁺ in PGE₂-G-induced increase in LTP. The identification of these novel signaling pathways not only provides a mechanism by which PGE₂-G modulates long-term synaptic plasticity, but also establishes the existence of novel receptor signaling pathways.

Accumulated information indicates that eCBs play a variety of roles as endogenous signaling mediators in physiological, pharmacological, and pathological functions (Alger, 2002; Chevalere et al., 2006; Freund et al., 2003; Mackie, 2006; Piomelli, 2003; Stella, 2004; Sugiura et al., 2004; 2006). COX-2 is an inducible enzyme, but it is also constitutively expressed in the brain (Yamagata et al., 1993). Many factors such as neuroinflammation, brain ischemia, traumatic brain injury, and epileptic activity significantly induce COX-2 expression. It has been also demonstrated that COX-2 expression is regulated by NMDA receptor-dependent synaptic activity (Yamagata et al., 1993), meaning that changes in NMDA receptor-dependent synaptic activity will regulate COX-2 expression. The results obtained in the present study indicate that increases in COX-2 alter synaptic signaling not only through increased production of PGE₂ from AA, but also through the oxidative metabolism of eCBs to form new types of prostaglandins. Thus, COX-2 plays a central role in regulation of prostaglandin and eCB signaling in synaptic transmission and plasticity. Our results demonstrate that COX-2 oxidative metabolism of eCBs constitutes an important mechanism contributing to COX-2-mediated synaptic modification. Our observations provide evidence that prostaglandins derived from eCBs comprise a new class of signaling mediators involved in hippocampal synaptic transmission and plasticity. The demonstration of the augmented hippocampal synaptic plasticity by COX-2 oxidative metabolism of eCBs furthers our understanding of how eCB signaling is regulated by COX-2 in synaptic transmission and plasticity.

Experimental Methods

Animals

C57BL/6 mice (Charles River), COX-2^{-/-} (Ptgs2^{tm1Jed} Jackson Laboratory) and human Thy-1-COX-2 transgenic mice (Andreasson et al., 2001) weighing 20-25 g were used according to

the guidelines approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center. Mice were intraperitoneally (i.p.) injected with vehicle, LPS (3 mg/kg), NS398 (10 mg/kg), AH 6809 (10 mg/kg) or SR141716 (3 mg/kg), and killed 4, 12 and 24 hrs after injection.

Hippocampal slice preparation

Hippocampal slices were prepared from mice as described previously (Chen et al., 2002; Chen, 2004). Briefly, after decapitation, brains were rapidly removed and placed in cold oxygenated (95% O₂, 5% CO₂) low-Ca²⁺/high-Mg²⁺ slice medium composed of (in mM) 2.5 KCl, 7.0 MgCl₂, 28.0 NaHCO₃, 1.25 NaH₂PO₄, 0.5 CaCl₂, 7.0 glucose, 3.0 pyruvic acid, 1.0 ascorbic acid, and 234 sucrose. Slices were cut at a thickness of 400 μm and transferred to a holding chamber in an incubator containing oxygenated artificial cerebrospinal fluid (ACSF) composed of (in mM) 125.0 NaCl, 2.5 KCl, 1.0 MgCl₂, 25.0 NaHCO₃, 1.25 NaH₂PO₄, 2.0 CaCl₂, 25.0 glucose, 3 pyruvic acid, and 1 ascorbic acid at 36 °C for 0.5 to 1 hour, and maintained in an incubator containing oxygenated ACSF at room temperature (~22-24 °C) for >1.5 h before recordings. Slices were then transferred to a recording chamber where they were continuously perfused with 95% O₂, 5% CO₂-saturated standard ACSF at ~32-34 °C. Individual dentate granule neurons were viewed with an upright microscope (Olympus BX51WI) fitted with a 60× water-immersion objective and differential interference contrast (DIC) optics.

Electrophysiological recordings

Field EPSP (fEPSP) recordings in response to stimulation of the perforant path at a frequency of 0.05 Hz were made using an Axoclamp-2B patch-clamp amplifier (Molecular Devices, CA) in bridge mode. The external solution contained (in mM): 130.0 NaCl, 2.5 KCl, 1.0 MgCl₂, 10.0 HEPES, 1.25 NaH₂PO₄, 2.0 CaCl₂, 25.0 glucose (pH 7.4 with NaOH). Recording pipettes were pulled from borosilicate glass with a micropipette puller (Sutter Instrument), filled with artificial CSF (2-4 MΩ) and placed at the middle one third of the molecular layer of the dentate gyrus. As described previously (Chen et al., 2002; Chen, 2004), LTP was induced by a high-frequency stimulation (HFS), consisting of 8 trains, each of 8 pulses at 200 Hz with an inter-train interval of 2 seconds at 1.5× baseline stimulus intensity. The baseline stimulation strength was set to provide fEPSP with an amplitude of ~30% from the subthreshold maximum. Paired-pulse stimulation was induced by delivering two pulses with an inter-pulse interval of 40 ms (Zucker, 1989; Chen & bazan, 2005; Sang et al., 2005). Paired-pulse ratio was calculated as P2/P1 (P1, the amplitude of the first EPSP; P2, the amplitude of the second EPSP). The bath-perfused solutions contained 10 μM bicuculline to block ionotropic GABA receptors.

Inhibitory postsynaptic currents (IPSCs) were recorded under voltage clamp using an Axopatch-200B amplifier in dentate granule neurons in response to stimulation of the perforant path. The membrane potential was held at -70 mV. Stimuli were elicited via a bipolar tungsten electrode placed in the molecular layer of the dentate gyrus. Recording pipettes (4-5 MΩ) were filled with the internal solution containing (in mM) 140.0 CsCl, 5.0 NaCl, 1.0 CaCl₂, 10.0 HEPES, 10.0 EGTA, 4.0 Mg₂ATP, 0.2 Na₂GTP and 3.0 QX-314 (pH 7.25 with CsOH). DNQX (10 μM) and APV (50 μM) were applied via the bath perfusion to block glutamate AMPA and NMDA receptors, respectively. Depolarization-induced suppression of inhibition (DSI) was induced by a 5 sec depolarizing step from a holding potential of -70 mV to 0.

Miniature Excitatory postsynaptic currents (mEPSCs) were recorded in hippocampal neurons in hippocampal slices under voltage clamp as described previously (Chen & Bazan, 2005; Sang et al, 2005; 2007). The membrane potential was held at -70 mV. To isolate mEPSCs, tetrodotoxin (TTX, 0.5 to 1 μM), a voltage-gated Na⁺ channel blocker, bicuculline (10 μM) was included in the external solution. The frequency, amplitude and kinetics of mEPSCs were analyzed using the MiniAnalysis program. All experiments were performed at 32~34°C.

Reverse transcription and real-time PCR

Total RNA was prepared from harvested hippocampal tissue or slices with the RNeasy Mini Kit (Qiagen) and treated with RNase-free DNase (Qiagen) according to the manufacturer's instructions. The RNA concentration was measured by spectrophotometer (DU 640; BECKMAN). RNA integrity was verified by electrophoresis in a 1% agarose gel. The Iscript cDNA synthesis kit (BioRad) was used for the reverse transcription reaction. We used 1 µg total RNA, with 4 µl 5× Iscript reaction mix and 1 µl Iscript reverse transcriptase. The total volume was 20 µl. Samples were incubated for 5 min at 25 °C. All samples were then heated to 42 °C for 30 min, and reactions were stopped by heating to 85 °C for 5 min.

Specific primers for COX-1, COX-2, FAAH, MGL and GAPDH were selected using Beacon Designer Software (BioRad) and synthesized by IDT (Coralville, IA). Primer sequences are as follows: COX-1: 5'-agaaggagatggctgctgag-3', 5'-cacacggaaggaaacatagg-3' (296 bp, Genebank BC005573); COX-2: 5-aagcgaggacgtgggttcac-3, 5-acacctctccaccaatgacctg-3 (142 bp, BC052900); FAAH: 5'-tgtatcgccagtcgctcattg-3', 5'-gtcatcagccgttcacctc-3' (361bp, NM_010173); MGL: 5'-agggtgaaggtgtgcttg-3', 5'-gctgctgctatcctgtgagac-3' (282 bp, NM_011844); GAPDH: 5-accacagtcctatgccatcac-3, 5-acctgcccacagccttg-3 (134 bp, M32599). PCR products were confirmed with sequence analysis. The reactions were set up in duplicate in total volumes of 25 µl containing 12.5 µl 2× iQSYBR green Supermix (BioRad) and 5 µl template (1:10 dilution from RT product) with a final concentration of 400 nM of the primer. The PCR cycle was as follows: 95 °C/3 min, 45 cycles of 95 °C/30 sec, 58 °C/45 sec and 95 °C/1 min. Melt-curve analysis was performed at the end of each experiment to verify that a single product per primer pair was amplified. The sizes of the amplified DNA fragments were verified by gel electrophoresis on a 3% agarose gel. The amplification and analysis were performed using an iCycler iQ Multicolor Real-Time PCR Detection System (BioRad). Samples were compared using the relative CT method. The fold increase or decrease was determined relative to a vehicle-treated control after normalizing to a housekeeping gene using $2^{-\Delta\Delta CT}$, where ΔCT is (gene of interest CT) - (GAPDH CT), and $\Delta\Delta CT$ is (ΔCT treated) - (ΔCT control) as previously described (Sang et al., 2005).

Immunoblot

Hippocampal tissue or slices were extracted and immediately homogenized in a 1:1 weight: volume of RIPA lysis buffer and protease inhibitors. Supernatants were fractionated on 10% SDS-PAGE gels (Bio-Rad) and transferred onto PVDF membranes (Bio-Rad). The membrane was incubated with anti-COX-2 rabbit polyclonal antibody (1:1,000, Cayman, Ann Arbor, MI), anti-p38 and anti-phospho-p38 MAPK, anti-p44/42 and anti-phospho-p44/42 MAPK antibodies (1:1,000, Cell Signaling, Danvers, MA) at 4°C overnight. Blots were washed and incubated with secondary antibody (goat anti-rabbit 1:2000, Cell Signaling) at room temperature for 1 hour. Proteins were visualized by enhanced chemiluminescence (ECL, Amersham Biosciences, UK). Bands were quantified by densitometry using FUJIFILM Multi Gauge software (version 3.0) with normalization to β -actin (1:4000 Sigma, St. Louis).

Chemicals and drugs

2-AG, AEA, PG-Gs (PGE₂-G, PGD₂-G, and PGF_{2 α} -G) and PG-EAs (PGE₂-EA, PGD₂-EA, and PGF_{2 α} -EA) were purchased from Cayman Chemical (Ann Arbor, MI) and dissolved in ethanol as 20 mM stock solution and frozen at -80°C until use. Stock solutions were diluted with slice medium to the desired concentrations just before recordings. 2-APB, PD98059 (PD), SB 203580 and SB 239063 (Tocris, Ellisville, MO), NS398 (Cayman Chemical, Ann Arbor, MI), and SR141716 (Provided by Chemical Synthesis and Drug Supply Program, the National Institute of Mental Health) were dissolved in DMSO as stock solutions of 50 100 mM. All other drugs and chemicals were obtained from Sigma (St. Louis, MO). Vehicle consisted of either ethanol or DMSO.

Data analysis

Data are presented as mean $\pm$ S.E.M. Unless stated otherwise, Student's t-test and analysis of variance (ANOVA) with Fisher's PLSD test or Student-Newman-Keuls test were used for statistical comparison when appropriate. Differences were considered significant when $P < 0.05$.

Acknowledgement

This work was supported by National Institutes of Health grant R01NS054886 and the Alzheimer's Association grant IIRG-05-13580 (to CC).

References

- Akaike A, Kaneko S, Tamura Y, Nakata N, Shimoi H, Ushikubi F, Narumiya S. Prostaglandin E2 protects cultured cortical neurons against N-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. *Brain Res* 1994;663:237–244. [PubMed: 7874506]
- Akaneya Y, Tsumoto T. Bidirectional trafficking of prostaglandin E2 receptors involved in long-term potentiation in visual cortex. *J Neurosci* 2006;26:10209–10221. [PubMed: 17021176]
- Alger BE. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog Neurobiol* 2002;68:247–286. [PubMed: 12498988]
- Alger BE. Endocannabinoid identification in the brain: Studies of breakdown lead to breakthrough, and there may be NO hope. *Sci STKE* 2005;51:1–4.pe
- Andreasson KI, Savoneko A, Videnky S, Goellner JJ, Yang Y, Shaffer A, Kaufmann WE, Worley PF, Isakson P, Markowska AL. Age-dependent cognitive deficits and neuronal apoptosis in cyclooxygenase-2 transgenic mice. *J Neurosci* 2001;21:8198–8209. [PubMed: 11588192]
- Chen C, Magee JC, Bazan NG. Cyclooxygenase-2 regulates prostaglandin E2 signaling in hippocampal long-term synaptic plasticity. *J Neurophysiol* 2002;87:2851–2857. [PubMed: 12037188]
- Chen C. ZD7288 inhibits postsynaptic glutamate receptor-mediated responses at hippocampal perforant path-granule cell synapses. *Eur J Neurosci* 2004;19:643–649. [PubMed: 14984414]
- Chen C, Bazan NG. Endogenous PGE2 regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. *J Neurophysiol* 2005;93:929–941. [PubMed: 15653788]
- Chevalere V, Takahashi KA, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. *Ann Rev Neurosci* 2006;29:37–76. [PubMed: 16776579]
- Diana MA, Marty A. Endocannabinoid-mediated short-term synaptic plasticity: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). *Br J Pharmacol* 2004;142:9–19. [PubMed: 15100161]
- Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 2003;83:1017–1066. [PubMed: 12843414]
- Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF. Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci* 2006;26:5628–5637. [PubMed: 16723519]
- Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P. COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proc Natl Acad Sci U S A* 1996;93:2317–2321. [PubMed: 8637870]
- Kim J, Alger BE. Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. *Nat Neurosci* 2004;7:697–698. [PubMed: 15184902]
- Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glycerol prostaglandins by COX-2. *J Biol Chem* 2000;275:33744–33749. [PubMed: 10931854]
- Kozak KR, Crews BC, Morrow JD, Wang LH, Ma YH, Weinander R, Jakobsson PJ, Marnett LJ. Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. *J Biol Chem* 2002;277:44877–44885. [PubMed: 12244105]

- Kozak KR, Prusakiewicz JL, Marnett LJ. Oxidative metabolism of endocannabinoids by COX-2. *Curr Pharmaceut Design* 2004;10:659–667.
- Mackie K. Cannabinoid receptors as therapeutic targets. *Annu Rev Pharmacol Toxicol* 2006;46:101–122. [PubMed: 16402900]
- Murray HJ, O'Connor JJ. A role for COX-2 and p38 mitogen activated protein kinase in long-term depression in the rat dentate gyrus in vitro. *Neuropharmacol* 2003;44:374–380.
- Nirodi CS, Crews BC, Kozak KR, Rorrow JD, Marnett LJ. The glyceryl ester of prostaglandin E₂ mobilizes calcium and activates signal transduction in RAW 264.7 cells. *Proc Natl Acad Sci USA* 2004;101:1840–1845. [PubMed: 14766978]
- Piomelli D. The molecular logic of endocannabinoid signaling. *Nat Rev Neurosci* 2003;4:873–884. [PubMed: 14595399]
- Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc Natl Acad Sci USA* 2002;99:8384–8388. [PubMed: 12060781]
- Sang N, Zhang J, Marcheselli V, Bazan NG, Chen C. Postsynaptically synthesized prostaglandin E₂ modulates hippocampal synaptic transmission via a presynaptic PGE₂ EP₂ receptor. *J Neurosci* 2005;25:9858–9870. [PubMed: 16251433]
- Sang N, Chen C. Lipid signaling and synaptic plasticity. *Neuroscientist* 2006;12:425–434. [PubMed: 16957004]
- Sang N, Zhang J, Chen C. Prostaglandin E₂ glycerol ester, a COX-2 oxidative metabolite of 2-arachidonoyl glycerol, modulates hippocampal inhibitory synaptic transmission. *J Physiol (Lond)* 2006;752:735–745. [PubMed: 16484297]
- Sang N, Zhang J, Chen C. COX-2 oxidative metabolite of endocannabinoid 2-AG enhances excitatory glutamatergic synaptic transmission and induces neurotoxicity. *J Neurochem* 2007;102:1966–1977. [PubMed: 17539917]
- Slanina KA, Schweitzer P. Inhibition of cyclooxygenase-2 elicits a CB1-mediated decrease of excitatory transmission in rat CA1 hippocampus. *Neuropharmacol* 2005;49:653–659.
- Slanina KA, Roberto M, Schweitzer P. Endocannabinoids restrict hippocampal long-term potentiation via CB1. *Neuropharmacol* 2005;49:660–668.
- Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 1997;388:773–777. [PubMed: 9285589]
- Stella N. Cannabinoid signaling in glial cells. *Glia* 2004;48:267–277. [PubMed: 15390110]
- Sugiura, T.; Kishimoto, S.; Oka, S.; Gokoh, M.; Wake, K. Metabolism and physiological significance of anandamide and 2-arachidonoylglycerol, endogenous cannabinoid receptor ligands. In: Fonteh, Alfred N.; Wykle, Robert L., editors. *Arachidonate Remodeling and Inflammation*. Birkhäuser Verlag; Basel, Switzerland: 2004. p. 211–237.
- Sugiura T, kishimoto S, Oka S, Gokoh M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog Lipid.Res* 2006;45:405–446. [PubMed: 16678907]
- Vereker E, Campbell V, Roche E, McEntee E, Lynch MA. Lipopolysaccharide inhibits long term potentiation in the rat dentate gyrus by activating caspase-1. *J Biol Chem* 2000;275:26252–26258. [PubMed: 10856294]
- Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature* 2001;410:588–592. [PubMed: 11279497]
- Wilson RI, Nicoll RA. Endocannabinoids signaling in the brain. *Science* 2002;296:678–681. [PubMed: 11976437]
- Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron* 1993;11:371–386. [PubMed: 8352945]
- Yoshida T, Fukaya M, Uchigashima M, Miura E, Kamiya H, Kano M, Watanabe M. Localization of diacylglycerol lipase- α around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. *J Neurosci* 2006;26:4740–4751. [PubMed: 16672646]

Yu M, Ives D, Ramesha CS. Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. *J Biol Chem* 1997;272:21181–21186. [PubMed: 9261124]

Figure 1.

Increase in COX-2 expression enhances hippocampal LTP. A. Real-time PCR analysis of LPS-induced changes in hippocampal expression of COX-1, COX-2, FAAH and MGL in mice injected with vehicle and LPS (3 mg/kg, i.p.). Analysis of mRNA expression was performed at 4, 12 and 24 hrs after LPS injection. Results are from three independent mice with duplicate wells. B1. Western immunoblot analysis of COX-2 expression in mouse hippocampus. COX protein was analyzed at 4, 12 and 24 hrs after LPS injection. B2. Quantification of COX-2 protein in hippocampal tissue from mice injected with LPS. LPS induces a time dependent elevation of COX-2 expression. C1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in hippocampal slices from animals injected with vehicle and LPS (3 mg/

kg) or LPS plus NS398 (10 mg/kg). C2. Time course of the LPS-induced changes in fEPSP slopes in the absence and presence of NS398. C3. Mean values of the potentiation of fEPSPs averaged from 36 to 40 min following HFS recorded from animals injected with vehicle, LPS and NS398. LTP is significantly elevated at 4 and 12 hrs after LPS injection, and returns to the baseline level at 24 hrs after LPS injection. LPS-enhanced LTP is blocked by NS398 when compared with that vehicle control. * $P < 0.05$, ** $P < 0.01$ compared with the vehicle control. (Numbers in the bar graphs denote the number of observations)

Figure 2.

COX-2 elevation enhances basal synaptic transmission. A1. Representative fEPSP waveforms recorded in hippocampal slices from the animals injected with vehicle, LPS (3 mg/kg) for 12 hrs and LPS+NS398 (10 mg/kg). A2. Input-output function curves under different treatments as described in A1. B1. Representative sweeps of miniature excitatory postsynaptic currents (mEPSCs) recorded in hippocampal slices from the animals injected with vehicle, LPS (3 mg/kg) for 12 hrs and LPS+NS398 (10 mg/kg). The membrane potential was held at -70 mV. Bicuculline (10 μ M) and TTX (0.5 to 1 μ M) were included in the external solution. The synaptic events were analyzed using the MiniAnalysis program. B2. Cumulative probability of mEPSC frequency recorded in slices under different treatments. B3. Mean percentage changes in the

frequency of mEPSCs. B4. Cumulative probability of mEPSC amplitude. B5. Mean percentage changes in the amplitude of mEPSCs. * $P < 0.05$ compared with the vehicle control.

Figure 3.

LTP is not potentiated in COX-2 knockout mice injected with LPS. A1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in the hippocampal slices from wild type animals injected with vehicle and LPS (3 mg/kg) or LPS plus NS398 (10 mg/kg) for 12 hrs. A2. Time courses of the LPS-induced changes in fEPSP slopes in the absence and presence of NS398. A3. Mean values of the potentiation of fEPSPs averaged from 36 to 40 min flowing HFS recorded from wild type animals injected with vehicle, LPS and NS398. LTP is significantly enhanced 12 hrs following LPS injection and is inhibited in the presence of NS398. B1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in the hippocampal slices from COX-2 KO mice injected with vehicle and LPS (3 mg/kg) for 12

hrs. B2. Time course of LPS-induced changes in fEPSP slopes in COX-2 KO animals. B3. Mean values of the potentiation of fEPSPs averaged from 36 to 40 min following HFS recorded from COX-2 animals injected with vehicle and LPS. LTP is not significantly enhanced at 12 hrs following LPS injection. C1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in hippocampal slices derived from Thy-1/ COX-2 transgenic and wild type mice. B2. Time courses of the changes in fEPSP slopes in COX-2 Tg and wild type animals. B3. Mean values of the potentiation of fEPSPs averaged from 36 to 40 min following HFS recorded from COX-2 Tg and wild type animals. LTP is significantly increased in COX-2 Tg mice when compared to their wild type controls. ** $P < 0.01$ compared with the vehicle control.

Figure 4.

Effects of alterations in COX-2 expression on depolarization-induced suppression of inhibition (DSI) at hippocampal perforant path-dentate granule cell synapses. Inhibitory postsynaptic currents (IPSCs) were recorded in hippocampal dentate granule neurons in response to perforant path stimulation at a frequency of 0.5 Hz. DSI was elicited by a 5-sec depolarizing step from a holding potential of -70 to 0 mV. A1. Representative IPSC traces recorded from COX-2 wild type mice in the absence and presence of SR141716 (SR, 1 μ M) or NS398 (20 μ M). Hippocampal slices were incubated with SR and NS398 and continuously perfused during recordings. A2. Time course of averaged IPSCs before and after DSI under different treatments. A3. Mean values of percentage changes in IPSC amplitudes calculated from the first 5 sec

period just after a depolarizing step and normalized to baseline. The inhibition of COX-2 potentiates DSI, and there is a significant difference in DSI between NS398 and the vehicle control ($P<0.01$). B1. Representative IPSC traces recorded from COX-2 KO mice in the absence and presence of SR (1 μ M). B2. Time course of averaged IPSCs before and after DSI in the absence and presence of SR. B3. Mean values of percentage changes in IPSC amplitudes in the absence and presence of SR. C1. Representative IPSC traces recorded from COX-2 KO and their wild type control mice injected with LPS (3 mg/kg) for 12 hrs and from neuronal COX-2 transgenic mice. C2. Time course of averaged IPSCs before and after DSI. C3. Mean values of percentage changes in IPSC amplitudes. DSI is abolished in COX-2 wild type animals that received LPS and in COX-2 Tg mice, but remained the same in COX-2 KO mice that received LPS. * $P<0.05$, ** $P<0.01$ as compared with the vehicle control. ## $P<0.01$ compared with NS398 alone.

Figure 5.

CB₁ receptor antagonist blocks COX-2 inhibition-induced reduction of LTP. A1. Representative fEPSP traces before and after HFS under different treatments. Animals were injected with LPS (3 mg/kg) +SR (5 mg/kg), NS398 (10 mg/kg) and NS398+SR, and recordings were made 12 hrs after injections. A2. Time course of changes in fEPSP slopes under different treatments. A3. Mean values of changes in slope of fEPSPs averaged from 36 to 40 min following HFS under different treatments. SR does not alter the LPS-induced elevation of LTP ($P>0.05$). However, NS398 significantly reduces LTP, and this reduction is blocked by SR ($P<0.01$). B1. Representative IPSC traces recorded from animals injected with and without LPS (3 mg/kg) in the absence and presence of Win 55,212-2 (5 μ M) and/or SR (1 μ M). B2. Mean values of changes in IPSC amplitudes averaged from a 10 min application under different treatments. LPS treatment does not significantly affect Win 55,212-2-induced reduction of IPSCs, suggesting that the CB₁ receptor is still functioning in LPS-injected animals. ** $P<0.01$ compared with the vehicle control.

Figure 6.

Endogenous cannabinoids inhibit and their COX-2 metabolites elevate hippocampal LTP. A1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in the hippocampal slices in the absence and presence of 2-AG (5 μ M), AEA (10 μ M), SR (1 μ M), 2-AG plus SR and AEA plus SR. Slices were pretreated with SR and continuously perfused during the recordings. A2. Time courses of the changes in fEPSP slopes under different treatments. A horizontal bar depicts the duration of eCB application. A3. Mean values of the changes in slope of fEPSPs averaged from 36 to 40 min following HFS under different treatments. 2-AG and AEA significantly reduce LTP ($P < 0.01$, respectively), and the reduction is blocked by SR ($P > 0.05$). In addition, application of SR alone potentiates LTP ($P < 0.01$). B1. Representative traces of fEPSPs recorded before (baseline, black; PG-G application, blue) and after (red) HFS in the hippocampal slices in the absence and presence of PGD₂-G (10 μ M), PGE₂-G (10 μ M).

and $\text{PGF}_{2\alpha}\text{-G}$ (10 μM). B2. Time courses of the changes in fEPSP slopes under different treatments. A horizontal bar depicts the duration of PG-G application. B3. Mean values of the changes in slope of fEPSPs averaged from 36 to 40 min flowing HFS under different treatments. Application of $\text{PGE}_2\text{-G}$ significantly enhances LTP ($P=0.02$), while $\text{PGD}_2\text{-G}$ and $\text{PGF}_{2\alpha}\text{-G}$ do not induce a significant increase in LTP ($P>0.05$). C1. Representative traces of fEPSPs recorded before (baseline, black; PG-EA application, blue) and after (red) HFS in the hippocampal slices in the absence and presence of $\text{PGD}_2\text{-EA}$ (10 μM), $\text{PGE}_2\text{-EA}$ (10 μM) and $\text{PGF}_{2\alpha}\text{-EA}$ (10 μM). C2. Time courses of the changes in fEPSP slopes under different treatments. A horizontal bar depicts the duration of PG-EA application. C3. Mean values of the changes in slope of fEPSPs averaged from 36 to 40 min flowing HFS under different treatments. Application of $\text{PGE}_2\text{-EA}$ and $\text{PGF}_{2\alpha}\text{-EA}$ significantly enhances LTP ($P<0.05$ and $P<0.01$, respectively), while $\text{PGD}_2\text{-EA}$ does not induce a significant increase in LTP ($P>0.05$). * $P<0.05$, ** $P<0.01$ compared with the vehicle control.

Figure 7.

ERK, p38 MAPK, and IP3 mediate PGE₂-G-enhanced LTP. A1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in hippocampal slices in the presence of PD 98059 (20 μ M) and PD 98059 plus PGE₂-G (10 μ M). Slices were pretreated with PD 98059 for 30 min before application of PGE₂-G. A2. Time courses of the changes in fEPSP slopes. A3. Mean values of the changes in slope of fEPSPs averaged from 36 to 40 min flowing HFS. The PGE₂-G-enhanced LTP is blocked by PD 98059 ($P>0.05$). B1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in hippocampal slices in the presence of SB 239063 (10 μ M) and SB 239063 plus PGE₂-G (10 μ M). Slices were pretreated with SB 239063 for 30 min before application of PGE₂-G. B2. Time courses of the changes in fEPSP

slopes. B3. Mean values of the changes in slope of fEPSPs averaged from 36 to 40 min flowing HFS. The PGE₂-G-enhanced LTP is blocked by SB 239063 ($P>0.05$). C1. Representative traces of fEPSPs recorded before (black) and after (red) HFS in hippocampal slices in the presence of 2-APB (20 μ M) and 2-APB plus PGE₂-G (10 μ M). Slices were pretreated with 2-APB for 30 min before application of PGE₂-G. C2. Time courses of the changes in fEPSP slopes. C3. Mean values of the changes in slope of fEPSPs averaged from 36 to 40 min flowing HFS. PGE₂-G-enhanced LTP is blocked by 2-APB ($P>0.05$). D. Western blot analysis of PGE₂-G-induced phosphorylation of ERK and p38 MAPK in hippocampal slices. PGE₂-G (30 μ M) induces a time dependent phosphorylation of ERK and p38 MAPK.