Cyclooxygenase-2 Regulates Prostaglandin E2 Signaling in Hippocampal Long-Term Synaptic Plasticity

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Chen, Chu, Jeffery C. Magee, and Nicolas G. Bazan. Cyclooxygenase-2 regulates prostaglandin E2 signaling in hippocampal long-term synaptic plasticity. J Neurophysiol 87: 2851–2857, 2002; 10.1152/jn.00820.2001. The functional significance of cyclooxygenases (COX-1 and -2), the key enzymes that convert arachidonic acid (AA) to prostaglandins (PGs) in brain, is unclear, although they have been implicated in cellular functions and in some neurologic disorders, including stroke, epilepsy, and Alzheimer’s disease. Recent evidence that COX-2 is expressed in postsynaptic dendritic spines (which are specialized structures involved in synaptic signaling) and is regulated by synaptic activity implies participation of COX-2 in neuronal plasticity. However, direct evidence is lacking. Here we demonstrate that selective COX-2 inhibitors significantly reduced postsynaptic membrane excitability, back-propagating dendritic action potential-associated Ca2+ influx, and long-term potentiation (LTP) induction in hippocampal dentate granule neurons, while a COX-1 inhibitor is ineffective. All of these actions were effectively reversed by exogenous application of PGE2, but not of PGD2 or PGF2α. Our results indicate that COX-2-generated PGE2 regulates membrane excitability and long-term synaptic plasticity in hippocampal perforant path-dentate gyrus synapses.

INTRODUCTION

Cyclooxygenase (COX) is the key enzyme that converts arachidonic acid (AA) to prostaglandins (PGs). Two isozymes of COX have been identified (Vane et al. 1998). COX-1 is constitutively expressed in most tissues and is thought to mediate “housekeeping” functions. On the other hand, COX-2, an inducible enzyme, participates in the injury/inflammatory response (Smith et al. 1996; Vane et al. 1998). Growing evidence, however, suggests that the functional significance of COX-2 is far beyond what was initially revealed (Bazan 2001; Dubois et al. 1998; Ho et al. 1999; Vane et al. 1998). In the brain, COX-2 is expressed in discrete populations of neurons and is enriched in the cortex and hippocampus (Yamagata et al. 1993) and has been implicated in brain functions and in neurologic disorders, including stroke, seizures, and Alzheimer’s disease (Hewett et al. 2000; Ho et al. 1999; Iadicola et al. 2001; Miettinen et al. 1997; Nakayama et al. 1998). However, the mechanisms by which COX and PGs participate in neuronal cell signaling are still not clear.

Unlike in most tissues, “inducible” COX-2 is also constitutively expressed in brain (Yamagata et al. 1993), kidney (Dincuk et al. 1995; Morham et al. 1995), and a few other organs (Dubios et al. 1998; Vane et al. 1998), suggesting that its basal activity is engaged in cellular functions. Gene-deletion studies, for instance, show that mice lacking COX-2 have severe renal abnormalities and a consequently short life span (Dinchuk et al. 1995; Morham et al. 1995; Vane et al. 1998). In the brain, basal expression of COX-2 has been shown to be regulated by synaptic activity, and its expression is upregulated by a high-frequency stimulation (HFS) that is associated with long-term potentiation (LTP) induction (Yamagata et al. 1993). Moreover, COX-2 is localized in neuronal dendritic spines where active synapses are present (Kaufmann et al. 1996). These studies imply that both constitutive and inducible COX-2 may participate in synaptic modifications. However, direct evidence is still lacking. Here we report that selective COX-2 inhibitors significantly reduced postsynaptic membrane excitability, back-propagating dendritic action potential-associated Ca2+ influx, and LTP induction in hippocampal dentate granule neurons, whereas a COX-1 inhibitor is ineffective. All of these actions were effectively reversed by exogenous application of PGE2 but not of PGD2 or PGF2α. Our results indicate that COX-2-generated PGE2 regulates postsynaptic membrane excitability and long-term synaptic plasticity in hippocampal perforant path-dentate gyrus synapses.

METHODS

Hippocampal slice preparation

Hippocampal slices were prepared from male c-57 mice (2–3 mo; body wt, 25–34 g) using standard procedures as described previously (Chen et al. 2001). Briefly, the brain was rapidly removed after decapitation and placed in cold oxygenated (95% O2-5% CO2) low-Ca2+/high-Mg2+ slicing solution composed of (in mM) 2.5 KCl, 7.0 MgCl2, 28.0 NaHCO3, 1.25 NaH2PO4, 0.5 CaCl2, 7.0 glucose, 3 pyruvic acid, 1 ascorbic acid, and 234 sucrose. Then, 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. Slices were 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 the 95% O2-5% CO2-saturated standard ACSF at ~34–35°C. Individual dentate granule cells were viewed with a Zeiss Axioskop microscope, fitted with a ×60 (Olympus) water-immersion objective and differential interference contrast (DIC) optics.

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**Electrophysiologic recordings**

Whole cell patch-clamp recordings were made using an Axoclamp-2B patch-clamp amplifier in bridge mode. Recording pipettes (6–9 MΩ) were pulled from borosilicate glass with a micropipette puller (Sutter Instrument) and fire polished on a microforge (Narishige Scientific Instrument) prior to use. The internal pipette solution contained (in mM) 120 K gluconate, 20 KCl, 4 NaCl, 10 HEPES, 0.5 EGTA, 0.28 CaCl₂, 4 Mg₂ATP, 0.3 Tris₂GTP, and 14 phosphocreatine (pH 7.25 with KOH). Series resistance ranged from 15 to 30 MΩ as estimated directly from the amplifier and was monitored during recordings by injection of a hyperpolarizing current (50 pA) before delivery of a stimulus. The resting membrane potential for recorded cells was around −74 mV. Excitatory postsynaptic potentials (EPSPs) were recorded in response to stimulation of the perforant path at a frequency of 0.05 Hz. Stimuli were elicited via a bipolar tungsten electrode placed in the middle of the molecular layer. The amplitude range of the evoked EPSPs was always adjusted to 2–6 mV (<30% of threshold for generating an action potential). LTP in the perforant path was induced by a HFS (consisting of 8 trains, each of 8 pulses at 200 Hz with an intertrain interval of 2 s) paired with postsynaptic depolarizing current injection (0.5 nA). LTP was operationally defined as >20% increase above baseline for the amplitude of EPSPs from 26 to 30 min after HFS. In experiments where selective COX-1 and -2 inhibitors were applied, slices were pretreated with NS398, nimesulide (Nims), or indomethacin (Indo) for ≥1.5 h and then were continuously perfused with the inhibitors during recordings. All the bath-perfused solutions, including drug solutions, contained 10 μM bicuculline to block ionotropic GABA receptors.

**Ca²⁺ imaging**

Changes in [Ca²⁺]ᵢ in postsynaptic neurons during somatic depolarizing current injection were imaged with the fluorescent dye fura-2 (~100 μM) in the recording pipette as described previously (Magee and Johnston 1997). The internal pipette solution for the Ca²⁺ imaging contained (in mM) 120 K gluconate, 20 KCl, 4 NaCl, 10 HEPES, 4 Mg₂ATP, 0.3 Tris₂GTP, 14 phosphocreatine, and 3 ascorbic acid (pH 7.25 with KOH). A cooled charge-coupled device (CCD) camera (Photometrics, Tucson, AZ) in a sequential frame-transfer mode was used to record high-speed fluorescence images. Relative changes in [Ca²⁺]ᵢ were quantified as changes in ΔF/ΔF₀, where F is fluorescent intensity before stimulation (after subtraction of autofluorescence) and ΔF is the change from this value during neuron firing. The tissue autofluorescence was determined by an equivalent measurement at a parallel location in the slice that was away from the dye-filled neuron. A 380-nm light (13-nm band-pass filter; Omega Optical) was used to excite fura-2. Sequential frame rate for optical recordings was one frame every 25 ms and pixels were binned in a 5-by-5 array. Action potential-induced dendritic Ca²⁺ influx was imaged at cell body and at 25, 50, 100, and 125 μm or beyond from the cell body. Data were presented as means ± SE. Unless stated otherwise, Student’s t-test and one-way ANOVA with Student-Newman-Keuls test were used for statistical comparison when appropriate. Differences were considered significant when P < 0.05. The care and use of the animals reported in this study were approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center.

**RESULTS**

**Selective COX-2 inhibitors, but not those of COX-1, reduce LTP induction in hippocampal dentate granule neurons**

To determine whether COX-2 participates in long-term synaptic plasticity in hippocampal perforant path-dentate gyrus synapses, mouse hippocampal slices were pretreated with a selective COX-2 inhibitor, NS398 (10 μM, a concentration above IC₅₀ for COX-2 but below IC₅₀ for COX-1) (Vane et al. 1998) for ≥1 h and continuously perfused in the recording bath. NS398 did not elicit an effect on baseline EPSPs. However, HFS paired with postsynaptic depolarizing current injection (0.5 nA)-induced enhancement of the EPSP amplitude was significantly reduced from 26 to 30 min after HFS in cells treated with NS398 (141 ± 13%, n = 16) when compared with controls (229 ± 21%, n = 18). The enhancement of the EPSP amplitude was further decreased to 121 ± 16% (n = 9) when the concentration of NS398 was raised to 30 μM (Fig. 1, C and D). To confirm that the NS398-induced inhibition of LTP induction is due to COX-2 inhibition, we employed another selective COX-2 inhibitor, nimesulide (Nims). Similar to the effects of NS398, Nims (30 μM) reduced HFS-induced potentiation of EPSP amplitude to 106 ± 18% (n = 8). We operationally defined LTP induction as >20% increase above baseline amplitude of EPSPs from 26 to 30 min after HFS. Thus it appears that selective COX-2 inhibitors significantly reduced the probability of LTP induction in hippocampal dentate granule neurons (Fig. 1B).

To further assess the significance of COX-2, we used indomethacin (Indo), a relatively effective COX-1 inhibitor (Meade et al. 1993; Yamagata et al. 1993). Application of 1 μM indo (IC₅₀ for COX-1: 0.028 μM and for COX-2: 1.68 μM) had no effect on baseline EPSP amplitude.
PGE₂ selectively reverses COX-2 inhibitor-induced suppression of LTP

If the effects of COX-2 inhibition on LTP induction in hippocampal dentate granule neurons result from blocking the synthesis of PGs, then exogenous application of PGs should restore the COX-2 inhibitor-induced suppression of LTP induction. To test this hypothesis, we individually applied three different PGs in the presence of NS398 (10 μM). As indicated in Fig. 2, bath application of PGE₂ (0.33 μM) significantly reversed NS398 (10 μM)-induced reduction of EPSP potentiation (242 ± 29%, n = 12 vs. 141 ± 13%, n = 16) and probability of LTP induction (100%, 12 of 12 cells vs. 50%, 8 of 16 cells), whereas it had little effect on baseline EPSPs. Bath applications of PGD₂ (0.33 μM) or PGF₂α (0.33 μM) did not significantly increase HFS-induced enhancement of EPSP amplitudes and probability of LTP induction. Because COX-2 converts AA to PGE₂ (Brock et al. 1999) and PGE₂ receptors (EP) are expressed in the hippocampus (Narumiya et al. 1999; Zhang and Rivest 1999), it is likely that PGE₂ is the main messenger in COX-2-mediated activity-dependent synaptic plasticity.

Action of PGE₂ on synaptic modification is not on postsynaptic sites

It has been proposed that the bioactive lipids AA and platelet-activating factor (PAF) are retrograde messengers that modulate hippocampal long-term synaptic plasticity (Bazan 1995; Kato et al. 1994; O’Dell et al. 1991; Williams et al. 1989). To determine whether the role of COX-2 in regulating PG signaling in hippocampal synaptic plasticity is similar to that of other bioactive lipids, we examined the effects of postsynaptic application of PGE₂ on spontaneous miniature EPSPs (mEPSPs). As shown in Fig. 3, when included in the recording pipette solution, 2 μM PGE₂ did not induce significant effects on frequency or amplitude of mEPSPs during 20-min recordings (Kato et al. 1994). We also used paired-pulse protocol (inter-pulse interval, 80–100 ms) to examine the presynaptic effect of COX-2 inhibition (Chen et al. 2001; Zucker 1989). We did not detect differences in the ratios of the paired-pulse facilitation in the absence or presence of COX-2 inhibitors (control: 1.04 ± 0.03, n = 11; 10 μM NS398: 1.01 ± 0.02, n = 16). This indicates that the action of COX-2 reaction products on synaptic modification does not involve presynaptic terminals but postsynaptic sites.

COX-2 inhibition reduces postsynaptic membrane excitability

It has been demonstrated that PGE₂ regulates membrane excitability by modulating K⁺, Iₖ, and TTX-resistant Na⁺ channel currents in sensory neurons (Gold et al. 1998; Ingram and Williams 1996; Nicol et al. 1997). Recent evidence reveals the importance of spatiotemporal correlation of coincidence of postsynaptic firing and EPSPs in synaptic efficacy (Bi and Poo 2001; Koester and Sakmann 1998; Magee and Johnston 1997; Markram et al. 1997). Thus reducing postsynaptic membrane excitability, which causes a decrease in the number of the postsynaptic action potentials (APs) during presynaptic HFS, decreases the probability of LTP induction. To determine whether the inhibition of COX-2 activity altered postsynaptic membrane excitability, we examined the number of APs elicited by presynaptic HFS paired with postsynaptic current injection. As illustrated in Fig. 4, NS398 (10 and 30 μM) and
Nims (30 μM) significantly reduced the number of APs, whereas Indo had little effect on the membrane excitability. Bath application of PGD₂ or PGE₂ (0.33 μM) did not recover NS398 (10 μM)-induced reduction in the number of APs. Correlated to LTP induction, PGE₂ (0.33 μM) significantly reversed NS398 (10 μM)-induced decrease in the number of APs (Fig. 4). These data suggest that basal activity of COX-2 plays an important role in regulating membrane excitability and that COX-2 inhibitor-mediated inhibition of LTP induction may result from reduced postsynaptic membrane excitability. The reduction of postsynaptic membrane excitability caused a decrease in the synchrony of the coincidence of EPSPs and postsynaptic current injection from 0.5 to 0.3 nA during presynaptic HFS. Reduction of postsynaptic depolarizing current injection caused a reduction in the number of APs and consequent LTP induction. Further, we increased postsynaptic depolarizing current injection from 0.5 to 0.8 nA in cells treated with NS398 (10 μM). An increase in the current injection induced an increase in the number of APs and LTP induction (Figs. 2, C and D, and 4, A and B). Therefore a correlation existed between the number of postsynaptic spikes during the tetanus and the magnitude of EPSP potentiation (Fig. 4F). To further examine effects of COX-2-generated PGE₂ on postsynaptic membrane properties, we measured membrane input resistance and the amount of current required to trigger an AP (current threshold). NS398 (10 μM) reduced the membrane input resistance and increased the current threshold. PGE₂ (0.33 μM) significantly reversed NS398-induced decrease in input resistance and increase in current threshold (Fig. 4G). This finding suggests that PGE₂ derived from the tonic activity of constitutive COX-2 contributes to the membrane excitability.

**COX-2 inhibition reduces back-propagating dendritic action potential-associated Ca²⁺ influx**

Active propagation of axonally initiated APs back into the dendrites and associated dendritic Ca²⁺ influx play a critical role in hippocampal LTP induction (Magee and Johnston 1997). To test whether the COX-2 inhibitor-induced decrease in postsynaptic membrane excitability resulted in an inhibition of back-propagating dendritic APs, dendritic [Ca²⁺], changes were imaged during somatic depolarizing current injection. As indicated in Fig. 5, NS398 (10 μM) significantly decreased dendritic Ca²⁺ influx at 100 μm (normalized ΔF/F: 0.54 ± 0.03, n = 8) and >125 μm (0.34 ± 0.08, n = 4) from soma when compared with the control (0.79 ± 0.02, n = 9 and 0.64 ± 0.06, n = 8, respectively). Bath application of PGE₂ (0.33 μM) restored dendritic Ca²⁺ influx to control level (0.75 ± 0.04, n = 11 and 0.56 ± 0.05, n = 9 at 100 and >125 μm, respectively). These optical Ca²⁺-imaging data indicate that NS398 significantly decreased back-propagating dendritic AP amplitudes and associated Ca²⁺ influx and that PGE₂ was able to rescue the COX-2 inhibitor-induced inhibition.

**DISCUSSION**

Our findings provide the first direct evidence that COX-2, but not COX-1, regulates PG signaling in hippocampal long-term synaptic plasticity. Our data also reveal that COX-2-generated PGE₂ is an important signaling molecule in modifying synaptic efficacy because it effectively reversed selective COX-2 inhibitor-induced decreases in the number of APs during HFS, back-propagating dendritic AP-associated Ca²⁺ influx, and LTP induction. It is likely that COX-2 participates in hippocampal synaptic plasticity through regulation of PGE₂ signaling in modulating postsynaptic membrane excitability. Thus the inhibition of COX-2 activity induces the reduction of PGE₂ level that causes a decrease in postsynaptic membrane excitability. This, in turn, reduces the number of APs or back-
propagating dendritic AP amplitude and associated Ca\textsuperscript{2+} influx through voltage-gated Ca\textsuperscript{2+} channels and N-methyl-d-aspartate receptors (NMDA), which is critical for the induction of LTP.

It appears that COX-2 generated PGE\textsubscript{2} does not serve as a retrograde messenger to act on presynaptic sites as AA and PAF do (Bazan 1995; Kato et al. 1994; O’Dell et al. 1991; Williams et al. 1989) because the inhibition of COX-2 with selective COX-2 inhibitors or exogenous application PGE\textsubscript{2} did not elicit changes in the amplitude and frequency of mEPSPs or the ratios of paired-pulse facilitation before and after HFS. However, these manipulations did induce the change in the number of postsynaptic membrane APs during HFS, indicating that COX-2 reaction products may act on the postsynaptic sites.

In the present study, we observed that the number of APs during HFS paired with postsynaptic current injection was reduced in slices pretreated with the COX-2 inhibitor. Because this is a rapid response and the action occurs within a few milliseconds, it is unlikely that this reduction was due to the blockade of new synthesis of enzyme derived from the inducible COX-2. We know that prostanoids are not classic neurotransmitters. They are not stored in or secreted from synaptic vesicles. Once synthesized, they diffuse rapidly to and activate their specific membrane receptors. This means that PGE\textsubscript{2} signaling in this rapid response must be dependent on basal COX-2 expression rather than on the inducible COX-2 expression (Yamagata et al. 1993). This postulation has been supported by evidence that both COX-2 mRNA and protein in unstimulated brain are expressed at relatively high levels in a number of neurons involved in cognitive functions, including hippocampal granule, pyramidal cells, and cortical neurons. In particular, COX-2 protein is expressed at a very high level in dentate granule cells under basal conditions (Yamagata et al. 1993). In addition, COX-2 is consistently localized in dendritic spines of neurons that receive synaptic input (Kaufmann et al. 1996). Our results, that the membrane input resistance and current threshold had altered before HFS in slices pretreated with the COX-2 inhibitor, also provide evidence that basal activity of COX-2-generated PGE\textsubscript{2} may dynamically regulate membrane excitability. Because COX-2 expression is upregulated rapidly by HFS that is associated with LTP induction (Yamagata et al. 1993), it is possible that constitutive COX-2 may contribute to the induction of LTP as we observed in the present study and that inducible COX-2 may contribute to the maintenance of LTP.

One possibility for the COX-2 inhibitor-induced reduction of LTP may be due to its acting on postsynaptic NMDA receptor channels because the perforant path-dentate LTP is dependent on the NMDA receptors. We have tested this possibility and found that the number of APs elicited by HFS in slices pretreated with the COX-2 inhibitor, also provide evidence that basal activity of COX-2-generated PGE\textsubscript{2} may dynamically regulate membrane excitability. Because COX-2 expression is upregulated rapidly by HFS that is associated with LTP induction (Yamagata et al. 1993), it is possible that constitutive COX-2 may contribute to the induction of LTP as we observed in the present study and that inducible COX-2 may contribute to the maintenance of LTP.
that the COX-2 inhibitor-induced changes in membrane excitability and long-term plasticity result from their directly acting on the NMDA or β-adrenergic receptors. Recent progress in synaptic physiology reveals that the back-propagating dendritic APs are a critical element in the induction of long-term synaptic plasticity in hippocampal pyramidal neurons (Magee and Johnston 1997). Postsynaptic APs are initiated in the axon and then propagate back into the dendritic arbor of neurons, evoking an activity-dependent dendritic Ca\(^{2+}\) influx. An elevation of the postsynaptic intracellular free [Ca\(^{2+}\)] contributes to long-lasting changes in the efficacy of glutamatergic synapses.

In hippocampal CA1 pyramidal neurons, transient A-type K\(^+\) channels and hyperpolarization-activated cation channels increase their density with the distance from soma to distal dendrites (Hoffman et al. 1997; Magee 1998). Thus it is likely that selective COX-2 inhibitor- and PGE\(_2\) on K\(^+\) channels and hyperpolarization-activated cation channels increase their density with the distance from soma to distal dendrites (Hoffman et al. 1997; Magee 1998), and they shape EPSPs and modulate the back-propagating dendritic APs via protein kinases A and C (PKA and PKC) (Hoffman and Johnston 1998). AA, the precursor of PGs, has been shown to alter the availability of transient and sustained dendritic K\(^+\) channels that may underlie AA-induced increase in the amplitude of back-propagating dendritic APs (Colbert and Pan 1999).

In sensory neurons, PGE\(_2\) regulates membrane excitability through the cAMP-mediated modulation of ionic conductances, including K\(^+\), Ih, and TTX-resistant Na\(^+\) channel currents (Gold et al. 1998; Ingram and Williams 1996; Nicol et al. 1997). Thus it is likely that selective COX-2 inhibitor-induced decrease in the postsynaptic membrane excitability may result from a relief of PGE\(_2\) modulation of these channels; consequently, reduced postsynaptic membrane excitability would cause decreases in the number and amplitude of back-propagating dendritic APs, resulting in the reduction of the probability of LTP induction in hippocampal dentate granule neurons. Although we did not examine the effects of the COX-2 inhibition or PGE\(_2\) on K\(^+\), Ih, or Na\(^+\) channel activities in the present study, it would be interesting to investigate the COX-2 reaction-product modulation of these channels in hippocampal neurons.

Our data reveal that basal activity of constitutively inducible COX-2 plays an important role in regulating membrane excitability and activity-dependent LTP induction in the hippocampus. These findings will further our understanding of physiologic and pathologic events mediated by COX and the significance of PGs in memory storage and neurologic diseases.

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