Endogenous PGE$_2$ Regulates Membrane Excitability and Synaptic Transmission in Hippocampal CA1 Pyramidal Neurons

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Chen, Chu and Nicolas G. Bazan. Endogenous PGE$_2$ regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. J Neurophysiol 93: 929–941, 2005; doi:10.1152/jn.00696.2004. The significance of cyclooxygenases (COXs), the rate-limiting enzymes that convert arachidonic acid (AA) to prostaglandins (PGs) in the brain, is unclear, although they have been implicated in inflammatory responses and in some neurological disorders such as epilepsy and Alzheimer’s disease. Recent evidence that COX-2, which is expressed in postsynaptic dendritic spines, regulates PGE$_2$ signaling in activity-dependent long-term synaptic plasticity at hippocampal perforant path-dentate granule cell synapses, suggests an important role of the COX-2–generated PGE$_2$ in synaptic signaling. However, little is known of how endogenous PGE$_2$, regulates neuronal signaling. Here we showed that endogenous PGE$_2$ selectively regulates fundamental membrane and synaptic properties in the hippocampus. Somatic and dendritic membrane excitability was significantly reduced when endogenous PGE$_2$ was eliminated with a selective COX-2 inhibitor in hippocampal CA1 pyramidal neurons in slices. Exogenous application of PGE$_2$ produced significant increases in frequency of firing, excitatory postsynaptic potentials (EPSP) amplitude, and temporal summation in slices treated with the COX-2 inhibitor. The PGE$_2$-induced increase in membrane excitability seemed to result from its inhibition of the potassium currents, which in turn, boosted dendritic Ca$^{2+}$ influx during dendritic-depolarizing current injections. In addition, the PGE$_2$-induced enhancement of EPSPs was blocked by eliminating both PKA and PKC activities. These findings indicate that endogenous PGE$_2$ dynamically regulates membrane excitability, synaptic transmission, and plasticity and that the PGE$_2$-induced synaptic modulation is mediated via cAMP-PKA and PKC pathways in rat hippocampal CA1 pyramidal neurons.

INTRODUCTION

Although cyclooxygenases (COXs), the key enzymes that convert arachidonic acid to prostaglandins (PGs; including PGE$_2$, PGD$_2$, PGF$_{2\alpha}$, PGF$_2$, and thromboxane A$_2$), have been implicated in physiological and pathophysiological functions in the CNS, the cellular mechanisms by which COX reaction products are involved have yet to be elucidated. Of three isozymes of COX that have been identified (Bazan and Flower 2002; Chandrasekharan et al. 2002; Vane et al. 1998), COX-2 has received more attention, because it is not only inducible, but is also constitutively expressed in brain and in a few other tissues (Dubois et al. 1998; Vane et al. 1998). The basal expression of COX-2 is regulated by synaptic activity, and its expression is up-regulated 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 synaptic signaling occurs (Kaufmann et al. 1996). Recently we found that selective COX-2 inhibitors, but not those of COX-1, reduce HFS-induced LTP in hippocampal perforant path-dentate granule cell synapses, providing the first direct evidence that COX-2 participates in hippocampal long-term synaptic plasticity (Chen et al. 2002). We also found that the COX-2 inhibitor-induced reduction of LTP can be reversed by exogenous addition of PGE$_2$, but not by PGD$_2$ or PGF$_{2\alpha}$ (Chen et al. 2002). These studies suggest that endogenous PGE$_2$ may play an important role in synaptic modification (Bazan 2001). However, direct evidence is lacking. To address how endogenous PGE$_2$ regulates neuronal signaling, endogenous PGs were eliminated by a selective COX-2 inhibitor. Here we report that the deletion of endogenous PGs significantly reduced membrane excitability of hippocampal CA1 pyramidal neurons, and exogenous application of PGE$_2$ reversed the reduction of membrane excitability and increase of long-term synaptic efficacy and temporal summation that result from blockade of endogenous PG synthesis. These observations are unexpected, because a single COX-2–derived prostaglandin, endogenous PGE$_2$, is involved in regulating membrane excitability, synaptic integration, and plasticity in rat hippocampal CA1 pyramidal neurons. In addition, we discovered that endogenous PGE$_2$ regulation of synaptic activity is mediated via cAMP-PKA and PKC pathways.

METHODS

Hippocampal slice preparation

Hippocampal slices were prepared from 7- to 14-wk-old Sprague-Dawley rats (Chen et al. 2002). Briefly, rats were anesthetized with a mixture of ketamine and xylazine and perfused through the ascending aorta with a cold oxygenated (95% O$_2$-5% CO$_2$) low-Ca$^{2+}$/high-Mg$^{2+}$ solution composed of (in mM) 2.5 KCl, 7.0 MgCl$_2$, 28.0 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 0.5 CaCl$_2$, 7.0 glucose, 3.0 pyruvic acid, 1.0 ascorbic acid, and 234 sucrose. Rats were decapitated, and brains were rapidly removed and placed in the cold oxygenated slicing solution. 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 MgCl$_2$, 25.0 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 2.0 CaCl$_2$, 25.0 glucose, 3 pyruvic acid, and 1 ascorbic acid at 36°C for 0.5–1 h, and maintained in an incubator containing oxygenated ACSF at room temperature (~22–24°C) for >1 h before recordings. Slices were transferred to a recording chamber where they were continuously perfused with the 95% O$_2$-5% CO$_2$-saturated standard ACSF at ~33–34°C. Individual pyramidal neurons were viewed with a Zeiss...
Axioskop microscope, fitted with a 60× (Olympus) water-immersion objective and differential interference contrast (DIC) optics.

**Electrophysiological recordings**

Whole cell patch-clamp recordings were made using an Axoclamp-2B patch-clamp amplifier in bridge mode for the current-clamp recordings. Recording pipettes (3–5 MΩ for somatic and 7–10 MΩ for dendritic recordings) were pulled from borosilicate glass with a micropipette puller (Sutter Instrument). The internal pipette solution contained (in mM) 120 potassium 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). The resting membrane potential for recorded cells was between −62 to −74 mV. Excitatory post-synaptic potentials (EPSPs) were recorded in response to Schaffer-collateral stimulus at a frequency of 0.05 Hz. Paired-pulse stimulation was induced by delivering two pulses, with an interpulse interval of 80–100 ms (Chen et al. 2001; Zucker 1989). Paired-pulse ratio was calculated as P2/P1 (P1, the amplitude of the 1st EPSP; P2, the amplitude of the 2nd EPSP). Antidromic action potentials were elicited via a bipolar tungsten electrode placed in the alveus. To eliminate synaptic activation during antidromic stimulation, the glutamate receptor antagonist DNQX (10 μM) and GABA receptor antagonist bicuculline (10 μM) were added to the bath solutions. Series resistance ranged from 10 to 25 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. EPSPs were recorded in response to Schaffer-collateral stimulus at a frequency of 0.05 Hz via a bipolar tungsten electrode. For whole cell and outside-out macropatch recordings, an Axopatch D patch-clamp amplifier was used for the voltage-clamp experiments at room temperature. Leakage and capacitative currents were digitally subtracted by average null traces or scaling traces of smaller amplitude. The pipettes for voltage-clamp recordings were coated with Silgard. To record K⁺ currents, voltage-gated Na⁺ and Ca²⁺ currents were eliminated by addition of 250 μM CdCl₂ and 1 μM TTX in the external solutions.

**Ca²⁺ imaging**

Changes in [Ca²⁺], in postsynaptic neurons during dendritic depolarizing current injection and back-propagating dendritic action potentials were imaged with the fluorescent dye fura-2 (−100 μM) in the recording pipette as described previously (Chen et al. 2002). The internal pipette solution for the Ca²⁺ imaging contained (in mM) 120 K gluconate, 20 KCl, 4 NaCl, 10 HEPES, 0.5 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⁺], were quantified as changes in ΔF/F, where F is fluorescence 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. Dendritic depolarizing current injection- and back-propagating dendritic action potential-induced dendritic Ca²⁺ influx was imaged at distal dendrites between 250 and 320 μm from the cell body.

**Hippocampal PGE₂ assay**

Quantitative analysis of PGE₂ by liquid chromatography-tandem spectrometry (LC-MS-MS) was performed in rat hippocampal slices (Marcheselli et al. 2003). Hippocampal slices were cut the same as for electrophysiological recordings and pretreated with or without NS398 (20 μM). Hippocampal slices were homogenized in cold methanol and kept under nitrogen at −80°C until purification. Purification was performed by solid-phase extraction technique (Marcheselli et al. 2003). In short, samples pre-equilibrated at pH 3.0 were loaded onto C18 columns (Varian) and eluted with 10 ml 1% methanol in ethyl acetate (EM Science). Samples were concentrated by nitrogen-stream evaporator before LC-MS analysis. Samples were loaded into a Surveyor MS pump (Thermo-Finnegan) equipped with a C18 discovery column (Supelco), 10 cm × 2.1 mm ID, 5-μm internal phase. Samples were eluted in a linear gradient [100% solution A (60:40:0.01 methanol/water/acetic acid) to 100% solution B (99.99:0.01 methanol/acetic acid)] and run at a flow rate of 300 μl/min for 45 min. LC effluents were diverted to an electro-spray-ionization probe (ESI) on a TSQ Quantum (Thermo-Finnegan) triple quadrupole mass spectrometer running on negative ion-detection mode. PGD₂ was used to correct for recovery in the purification of PGE₂ for MS quantitative sensitivity, and PGE₂ standards were used for calibration and optimization. The instrument was run on full-scan mode, to detect parent ions, and selected-reaction mode (SRM) for quantitative analysis, to detect daughter ions simultaneously.

PGE₂ was purchased from Cayman Chemical (Ann Arbor, MI), and oxotremorine M, H-9, wortmannin, SC19290, chelerythrine, and ZD7288 were purchased from Tocris (Ellisville, MO). All other drugs and chemicals were obtained from Sigma-RBI (St. Louis, MO).

Data were presented as mean ± 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**

**PGE₂ regulates somatic membrane excitability**

To eliminate endogenous PGs, slices were pretreated with either indomethacin, a relatively effective COX-1 inhibitor (Meade et al. 1993), or NS398, a selective COX-2 inhibitor, for ≥2 h and were continuously perfused with the inhibitors during recordings. Indomethacin and acetaminophen had very little effect on membrane excitability, while selective COX-2 inhibitors (NS398 or nimesulide) significantly reduced membrane excitability (Chen and Bazan 2003; Chen et al. 2002). Also, because PGE₂ has been reported to be mainly synthesized through a COX-2 pathway in macrophages (Brock et al. 1999), we used NS398 to pretreat slices to deplete endogenous PGE₂. Thus to define the significance of COX reaction products, PGs, in hippocampal CA1 pyramidal neurons, endogenous PGs were eliminated in hippocampal slices by pretreatment with NS398 (20 μM) for at least 2 h. The IC₅₀ for NS398 to inhibit COX-1 is 16.8 μM and to inhibit COX-2 is 0.1 μM (Chen et al. 2002; Vane et al. 1998).

The treatment with NS398 led to a reduction of endogenous PGE₂ by 83% in slices (from control: 5.41 ± 0.63 to 0.92 ± 0.2 PGE₂ pg/mg protein, n = 10, P < 0.001), while there was little change in the levels of PGD₂ or PGF₂α (data not shown), indicating that PGE₂ in the hippocampus is mainly derived from the COX-2 pathway. Removal of endogenous PGs did not significantly affect the resting membrane potential (RMP; control: −66.0 ± 0.5 mV, n = 10; vs. NS398: −65.0 ± 0.6 mV, n = 10, P > 0.05) recorded from cell soma. However, blockade of endogenous PG synthesis significantly reduced membrane input resistance (Rᵢₘ) from 71.3 ± 4.5 to 60.6 ± 3.4
MΩ \((n = 10, P < 0.05)\). In particular, the frequency of depolarizing current-induced action potentials (APs) was greatly reduced (Fig. 1), suggesting that endogenous PGs are required for the maintenance of membrane excitability in hippocampal neurons. If COX-2 inhibitor-induced changes in \(R_{\text{in}}\) and frequency of firing resulted from an elimination of endogenous PGs, exogenous application of PGs should reverse these effects. To test this hypothesis, PGE\(_2\) was applied to slices pretreated with or without NS398. The reason PGE\(_2\) was chosen is that we previously found that PGE\(_2\), but not PGD\(_2\) or PGF\(_2\alpha\), modulates activity-dependent long-term synaptic plasticity in hippocampal dentate granule cells, and also that PGE\(_2\) is the main COX-2 reaction product. It seems that PGE\(_2\) \((0.5 \mu\text{M})\) induced a transient depolarization of the RMP of about 1–2 mV, which then returned to baseline, and did not significantly increase \(R_{\text{in}}\) from 71.3 ± 5.5 to 75 ± 6.0 MΩ \((n = 6, P > 0.05)\) and the frequency of spikes in control slices (Fig. 1). These observations indicate that exogenous addition of PGE\(_2\) did not exert a profound effect on membrane properties of pyramidal neurons. However, in NS398-treated slices, PGE\(_2\) restored \(R_{\text{in}}\) to baseline \((60.6 ± 3.4 \text{ to } 71.7 ± 4.0 \text{ MΩ, } n = 10, P < 0.05)\) and significantly increased the frequency of firing (Fig. 1). PGE\(_2\)-induced increases in the frequency of spikes were greater in slices treated with the COX-2 inhibitor than those in control slices, indicating that endogenous PGE\(_2\) is an important signaling molecule in maintaining somatic membrane excitability.

**PGE\(_2\) regulates dendritic membrane excitability**

COX-2 has been shown to be localized in neuronal dendritic spines (Kaufmann et al. 1996), where synaptic signaling occurs. To determine whether endogenous PGE\(_2\) influences dendritic membrane excitability, recordings were made from dendrites at 240–300 μm from the soma in slices treated with and without the COX-2 inhibitor. As in the soma, the elimination of endogenous PGs did not significantly change RMP \((\text{control: } 69.0 ± 1 \text{ mV, } n = 7; \text{vs. NS398: } 68.0 ± 2 \text{ mV, } n = 9, P >\)
moved up when endogenous PGE2 was eliminated by the frequency relationship (Figs. 1 and 2). The stimulus threshold and this increase can be reversed by application of PGE2 in dentate granule neurons (Chen et al. 2002). Since endogenous PGE2 influences dendritic membrane excitability, it would be of interest to see whether PGE2 altered back-propagating dendritic action potentials. Antidromic APs were elicited by stimulating the axons through an electrode placed in the alveus in slices treated with or without NS398. The elimination of endogenous PGE2 did not induce a change in back-propagating dendritic AP amplitudes (dendritic recordings at 240–300 μm from the soma). Exogenous addition of PGE2 also did not significantly increase back-propagating AP amplitudes (Fig. 3).

**PGE2 modulates voltage-gated K⁺ channel currents**

It has been shown that PGE2 modulates a hyperpolarization-activated current (Ih), delayed rectifier-like K⁺ channels, a TTX-resistant Na⁺ current, and Ca²⁺ currents in sensory and sympathetic neurons (Evans et al. 1999; Gold et al. 1998; Ingram and Williams 1996; Nicol et al. 1997). To determine which ion channels were modulated by PGE2 that underlay PGE2-induced increase in membrane excitability in hippocampal CA1 pyramidal neurons, we determined the effects of PGE2 on Ih and voltage-gated K⁺ currents of pyramidal neurons in hippocampal slices. We first examined the effect of PGE2 on Ih under the whole cell configuration. The Ih current was elicited by a hyperpolarizing voltage step to −120 mV from a holding potential of −50 mV. As indicated in Fig. 4A, PGE2 (1 μM) slightly enhanced a hyperpolarizing step-induced inward current (5 ± 0.4%, n = 6). To confirm that the hyperpolarizing step-induced inward current was the Ih, we used ZD7288, a selective Ih blocker (IC50: 10.6 μM; Gasparini and DiFrancesco 1997). ZD7288 (20 μM) almost eliminated the hyperpolarizing step-induced relaxation current (Fig. 5B). This result indicates that PGE2 at a concentration of 1 μM has very little effect on the Ih. Then we examined the effect of PGE2 on a muscarinic K⁺ current (Im), since the Im has been shown to

**FIG. 2.** Endogenous PGE2 regulates dendritic membrane excitability in hippocampal CA1 neurons. A: representative responses recorded from a control dendrite (at a distance of 250 μm from the soma) in the absence or presence of PGE2 during injections of currents (−0.35, −0.1, 0.1, and 0.3 nA). B: representative responses recorded from a dendrite (at a distance of 250 μm from the soma) treated with a selective COX-2 inhibitor, NS398 (20 μM), and application of PGE2 during injections of currents (−0.35, −0.1, 0.1, and 0.3 nA). C: elimination of endogenous PGE2 with the COX-2 inhibitor reduces dendritic Rin and exogenous application of PGE2 (0.5 μM) restores Rin to the baseline. **P < 0.01. D: elimination of endogenous PGE2 significantly reduces dendritic frequency of spikes. E: percentage changes in firing frequencies following application of PGE2 (0.5 μM) in control and NS398-treated slices.
A-type current and a 22.5 mV from dendrites of 2 different neurons at 250 and 280 A-type and delayed rectifier K in primary cultured hippocampal neurons. The transient inhibition was mainly on the delayed rectifier K current component (Fig. 4, PGE2). It seems that PGE2 (1 μM) induced an inhibitory effect on the IM current component (7.3 ± 2.1%, n = 7, P < 0.05) when there was a hyperpolarizing step to −50 mV from a holding potential of −20 mV (Fig. 4, F and H). The IM current component was confirmed by application of oxotremorine M, (Oxo-M, 20 μM), a muscarinic receptor agonist (Fig. 4, G and H). Finally, we determined the effect of PGE2 on K+ currents. The recordings were made in outside-out macropatches to examine the PGE2 modulation of K+ currents. As indicated in Fig. 4, D and E, PGE2 inhibited 12 ± 6% of the transient current component and 24 ± 7% of the sustained component (n = 5, P < 0.01). To confirm the PGE2-induced inhibition of the K+ currents, the whole cell recordings were made in primary cultured hippocampal neurons. The transient A-type and delayed rectifier K+ currents were isolated by current subtractions derived from the depolarizing steps from −90 to +50 mV and from −30 to +50 mV. We found that PGE2 produced a 4.7 ± 2.4% (n = 3, P < 0.05) reduction of the A-type current and a 22.5 ± 1.5% (n = 4, P < 0.01) reduction of the delayed rectifier current, suggesting that PGE2-induced inhibition was mainly on the delayed rectifier K+ current.

**PGE2 increases dendritic Ca2+ influx**

We have previously observed that NS398 reduced somatic depolarizing current injection-induced dendritic Ca2+ influx, and that the reduction was reversed by exogenous addition of PGE2 (Chen et al. 2002). It had been presumed that the changes in dendritic Ca2+ influx induced by the COX-2 inhibitor and PGE2 resulted from an alteration in back-propagating dendritic action potential amplitudes. However, we did not see that PGE2 induced an increase in back-propagating dendritic action potential amplitude in the pyramidal neurons directly recorded at the distal dendrites from the slices treated with and without NS398. To resolve this discrepancy, we measured dendritic Ca2+ influx elicited by dendritic depolarizing current injections or back-propagating dendritic action potentials induced by stimulating the axons (Chen et al. 2002). The neurons were filled with the calcium indicator fura-2 (~100 μM) via a dendritic whole cell recording pipette. Ca2+ influx was imaged at the distal dendrites from 260 and 320 μm (Fig. 5, A and B). As shown in Fig. 6C, PGE2 (0.5 μM) increased the number of spikes and fluorescence changes (ΔF/F) induced by dendritic depolarizing current injections. Figure 5D indicates that PGE2 (0.5 μM) increased the fluorescence elicited by back-propagating dendritic action potentials. The average of PGE2-induced changes of ΔF/F elicited by back-propagating dendritic action potential-induced Ca2+ influx was 29.6 ± 10.7% (n = 6, P < 0.05), and that elicited by dendritic current injections was 72 ± 19.3% (n = 6, P < 0.05). We observed that PGE2 increased the rest of action potential amplitudes when a train (5 or 10) of action potentials was elicited. There were 13 ± 3.2% (n = 8) and 17 ± 5.6% (n = 6) increases in action potential amplitudes at 5th and 10th action potentials in the presence of PGE2.
suggested that the increase in back-propagating dendritic action potential-induced Ca\(^{2+}\) influx by PGE\(_2\) resulted from its increasing dendritic membrane excitability, although PGE\(_2\) did not increase single back-propagating dendritic action potential amplitude. To determine whether PGE\(_2\) had an effect on voltage-gated Ca\(^{2+}\) currents in CA1 pyramidal neurons, Ca\(^{2+}\) currents were elicited by a depolarizing step to 0 mV from a holding potential of –70 mV and monitored in the absence and presence of PGE\(_2\). It seemed that PGE\(_2\) (1 \(\mu\)M) produced an inhibition of the Ca\(^{2+}\) currents by 11 ± 4.3% \((n = 6)\). This means that the directly effect of PGE\(_2\) on the Ca\(^{2+}\) channel currents is inhibitory.

**PGE\(_2\) modulates synaptic transmission**

COX-2–synthesized PGE\(_2\) modulates activity-dependent long-term synaptic plasticity in hippocampal perforant path-dentate granule cell synapses (Chen et al. 2002). To define the actions of endogenous PGE\(_2\) on synaptic transmission in pyramidal neurons, the effects of exogenous application of PGE\(_2\) on EPSPs in response to Schaffer-collateral stimulus were compared in neurons treated with or without NS398. As shown in Fig. 6, exogenous application of PGE\(_2\) (0.5 \(\mu\)M) induced a long-lasting enhancement of EPSP amplitude (LTP) in control slices; however, this enhancement was more pronounced in slices where endogenous PGE\(_2\) had been eliminated with NS398 (average increase in EPSP amplitudes recorded from the control slices: 116.5 ± 15% of base, \(n = 7\) vs. the NS398-treated slices: 145.1 ± 11.8% of base, \(n = 10\); \(P < 0.05\)), suggesting that endogenous PGE\(_2\) is a modulator of synaptic efficacy and plasticity. Then we examined the effect of PGE\(_2\) on the temporal summations of EPSPs in response to a train of five stimuli at a frequency of 100 Hz. As shown in Fig. 6, exogenous application of PGE\(_2\) only slightly increased summations of EPSPs in control slices \((n = 5)\); however, this caused a significant enhancement of the summations in slices treated with the COX-2 inhibitor \((n = 5)\), suggesting that endogenous PGE\(_2\) is essential for synaptic integration.

To determine whether PGE\(_2\)-induced enhancement of EPSPs resulted from its acting on presynaptic or postsynaptic sites, we recorded mEPSCs in the absence and presence of PGE\(_2\) in slices treated with NS398. It seems that PGE\(_2\) did not significantly induce changes in both frequency and amplitude of mEPSCs (Fig. 7). Meanwhile, we measured CV\(^2\) from the EPSP data (Fig. 6) before and after PGE\(_2\). There is no correlation between 1/CV\(^2\) and mean EPSP amplitudes. To further examine the possible mechanisms, a paired-pulse facilitation (PPF) protocol was used (Chen et al. 2001; Zucker 1989). As shown in Fig. 8, there were no changes in ratios of PPF before or after application of PGE\(_2\) both in the control \((1.30 ± 0.05\) to 1.31 ± 0.07; \(n = 7)\) and in NS398-treated slices \((1.30 ± 0.05\) to 1.21 ± 0.04; \(n = 10; P > 0.05\)). Thus these data did not provide information as to whether PGE\(_2\)-induced enhancement of evoked EPSPs resulted from the presynaptic or postsynaptic mechanisms.

**Cyclic AMP/PKA and PKC pathways mediate PGE\(_2\)-induced increase in EPSPs**

The physiological and pathophysiological actions of PGE\(_2\) are mediated through interaction with a family of distinct G protein–coupled receptors with the typical seven-hydrophobic-transmembrane-segment architecture, designated as EP\(_{1-4}\) (Boie et al. 1997; Breyer et al. 2001; Narumiya et al. 1999). These PGE\(_2\) receptors (EPs) mediated different signal transduction pathways. To define which signal transduction pathways mediate the PGE\(_2\)-induced enhancement of EPSPs in pyramidal neurons, we first used H-9, a selective PKA inhibitor. Bath application of H-9 (20 \(\mu\)M) blocked PGE\(_2\)-induced enhancement of EPSPs (116 ± 19% of base, \(n = 5, P > 0.05\)), suggesting that endogenous PGE\(_2\) is a modulator of synaptic efficacy and plasticity.

**FIG. 4.** PGE\(_2\) modulates voltage-gated K\(^+\) currents in pyramidal neurons. A: representative current traces in the absence and presence of PGE\(_2\) (1 \(\mu\)M) and ZD7288 (20 \(\mu\)M) recorded at a hyperpolarizing step to –120 mV from a holding potential of –50 mV under the whole cell configuration. B: ZD7288-sensitive current subtracted from before and after ZD7288. C: mean changes of the hyperpolarizing step-induced inward current in the presence of PGE\(_2\). D: representative current traces in the absence and presence of PGE\(_2\) recorded at a depolarizing step to +50 mV from a holding potential of –90 mV in outside-out macropatches. E: mean values of the PGE\(_2\)-induced inhibition of outward K\(^+\) currents. External solution contained TTX (1 \(\mu\)M) and CdCl\(_2\) (250 \(\mu\)M). F: representative current traces in the absence and presence of PGE\(_2\) recorded at a hyperpolarizing step to –50 mV from a holding potential of –20 mV under the whole cell configuration. G: representative current traces in the absence and presence of oxotremorine M (Oxo-M) recorded at a hyperpolarizing step to –50 mV from a holding potential of –20 mV. H: mean values of PGE\(_2\)- and Oxo-M–induced decreases of the current.

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suggesting that the cAMP/PKA pathway is involved in PGE₂ signaling in synaptic transmission and plasticity. However, H-9 at a concentration of 20 μM could have an effect on PKC andPKG. Therefore H-89, a highly selective PKA inhibitor, was employed. As indicated in Fig. 9, H-89 (1 μM) delayed the effect of PGE₂, but did not eliminate PGE₂-induced EPSP potentiation (123 ± 18% of base, n = 8, P < 0.05). In addition, the EPSPs were still potentiated following the wash-out of PGE₂ even in the presence of H-89, suggesting that there may be other factors involved. Recent evidence indicates that a PI₃K signaling pathway may be involved in the PGE₂-mediated effects in addition to the cAMP-PKA pathway (Fujino and Regan 2003; Fujino et al. 2003). To test whether PGE₂-induced potentiation of EPSPs was also mediated by this pathway, we used both H-89 and wortmannin (1 μM), a PI₃K inhibitor, to block both cAMP and PI₃K pathways. However, the EPSPs were still enhanced following application of PGE₂ (126 ± 18% of base, n = 7, P < 0.05). Thus it seems that the PI₃K pathway may not mediate the PGE₂-induced increase in EPSPs. To test this possibility, we employed chelerythrine (3 μM), a PKC inhibitor. PGE₂ still enhanced EPSPs in the presence of chelerythrine. However, the PGE₂-induced effect was completely blocked in the presence of H-89 plus chelerythrine, (91 ± 5% of base, n = 5, P > 0.05), indicating an involvement of PKC. In addition, we also examined the effect of PGI₂ on synaptic transmission at CA3-CA1 synapses. It appeared that PGI₂ (0.5 μM) produced little effect on EPSPs in hippocampal slices treated with NS398 (105 ± 12% of base, n = 4, P > 0.05), suggesting that PGI₂ may not contribute to hippocampal synaptic transmission.

**DISCUSSION**

In this study, we have examined the consequences of eliminating endogenous PGE₂ on membrane biophysical properties. We show a significant reduction of the membrane input resistance and frequency of firing under the condition of deleted endogenous PGE₂, and that exogenous application of PGE₂ reversed this reduction of membrane excitability both in soma and apical dendrites of rat hippocampal CA1 pyramidal neurons. Moreover, exogenous application of PGE₂ produced a
greater enhancement of EPSPs and temporal summation in slices where endogenous PGE2 had been eliminated with NS938 compared with those in control slices. This is the first demonstration that endogenous PGE2 plays an important role in dynamically maintaining membrane excitability, synaptic transmission, integration, and plasticity in the hippocampus.

We observed in this study that the COX-2 inhibitor, NS398 (20 μM), reduced PGE2 by 83% in slices treated for 2 h, whereas there were no significant changes in the levels of PGD2 and PGF2α. This indicates that PGE2 is mainly synthesized through the COX-2 pathway. This is consistent with the report that PGE2 is the main product of COX-2 in macrophages (Brock et al. 1999). Since PGI2 is another main product from COX-2 as reported in macrophages (Brock et al. 1999), and there is evidence indicating a novel subtype of PGI2 receptor expressed in the CNS that may be involved in neuronal survival (Satoh et al. 1999; Takechi et al. 1996), we found that PGI2 and its receptors do not participate in COX-2-mediated synaptic modification in the hippocampus.

It has been shown that exogenous PGE2 increases membrane excitability in rat dorsal root ganglion neurons (Evans et al. 1999; Nicol et al. 1997). We observed from this study that exogenous application of PGE2 produced a greater increase in the frequency of spikes in slices where endogenous PGE2 synthesis is blocked by the COX-2 inhibitor than that in the control slices. Insofar as the mechanism(s) involved, it is possible that PGE2-induced increases in the input resistance and frequency of firing may result from a cAMP-PKA pathway-mediated inhibition of delayed rectifier-like K+ channels, or modulation of a hyperpolarization-activated current (Ih, Evans et al. 1999; Ingram and Williams 1996; Nicol et al. 1997). To test whether changes in membrane excitability induced by endogenous PGE2 resulted from the modulation of active conductance, we have determined the effects of PGE2 on Ih and K+ currents in pyramidal neurons. It seems that PGE2 at a concentration of 1 μM induced an inhibitory effect mainly on the delayed rectifier K+ current component and a small inhibition on the transient component, and slightly increased the Ih.
Four genes encoding $I_h$ have been cloned, termed HCN1-4, for hyperpolarization-activated cyclic nucleotide-sensitive cation channels (Ludwig et al. 1998; Santoro et al. 1998; Seifert et al. 1999), and these four HCN channels that carry $I_h$ currents exhibit distinct activation kinetics and responses to cAMP (Kaupp and Seifert 2001; Santoro et al. 2000). Both HCN1 and 2 are expressed in hippocampal CA1 pyramidal neurons, but the density of HCN1 is much higher than that of HCN2. HCN1 channels display the fastest activation kinetics, but with a minimal response to cAMP (Santoro et al. 1998). HCN2 channels activate slowly and are modulated strongly by cAMP (Ludwig et al. 1998). This may be reason why PGE₂ had a small effect on the $I_h$ current, although PGE₂ increases cAMP. The inhibition of the delayed rectifier K⁺ current observed in this study is consistent with what was observed in sensory neurons (Evans et al. 1999; Nicol et al. 1997). This may be the major contributor for the PGE₂-induced increase in membrane excitability. Since a muscarinic K⁺ current ($I_M$) may contribute to membrane firing and passive properties such as input resistance and may be present in hippocampal CA1 pyramidal neurons (Hu et al. 2002; Marrion...
1997; Shah et al. 2002), we examined the effect of PGE$_2$ on the $I_M$. It seems that PGE$_2$ does have an inhibitory effect on the $I_M$, but small. This inhibition of the $I_M$ may contribute to the increased input resistance and enhanced membrane excitability. In addition, the elimination of endogenous PGE$_2$ did not affect the back-propagating dendritic AP amplitudes (dendritic recordings at 240–300 $\mu$m from the soma), suggesting that PGE$_2$ does not significantly affect dendritic voltage-gated Na$^+$ channels in hippocampal CA1 pyramidal neurons even though there are reports that PGE$_2$ modulates the TTX-resistant Na$^+$ current in cultured rat DRG neurons (Gold et al. 1998). Since the Na$^+$ currents in adult rat pyramidal neurons can be eliminated by TTX (1 $\mu$M), this suggests that there is very little, if any, TTX-resistant Na$^+$ current in this type of neuron (Magee and Johnston 1995). In addition, DRG and hippocampal pyramidal neurons are two different types of neurons; therefore, the effect of PGE$_2$ on the TTX-resistant Na$^+$ current in DRG neurons may not be applied to the pyramidal neurons.

We have found previously that NS398 reduced somatic depolarizing current injection-induced dendritic Ca$^{2+}$ influx and that this reduction could be restored by exogenous application of PGE$_2$ in hippocampal dentate granule neurons (Chen et al. 2002). These changes in dendritic Ca$^{2+}$ influx were assumed to result from alterations in back-propagating dendritic action potential amplitudes. However, in this study, we did not observe an increase in single back-propagating dendritic action potential amplitude in the presence of PGE$_2$. To determine whether PGE$_2$ could alter dendritic Ca$^{2+}$ influx in CA1 pyramidal neurons, experiments were conducted to detect dendritic depolarizing current injection- and back-propagating dendritic action potential-induced Ca$^{2+}$ influx at distal dendrites in the absence and presence of PGE$_2$ in slices treated with NS398. We found that PGE$_2$ increased back-propagating dendritic action potential-induced Ca$^{2+}$ influx by 72%. Although PGE$_2$ did not increase single back-propagating dendritic action potential amplitude, it increased the rest of action potential amplitudes when a train (5 or 10) of action potentials was elicited. There are ~13 and 17% increases in action potential amplitude at the 5th and 10th action potentials in the presence of PGE$_2$. We have examined the effect of PGE$_2$ on voltage-gated Ca$^{2+}$ currents in CA1 pyramidal neurons and found that PGE$_2$ inhibits Ca$^{2+}$ currents. This is in harmony with the previous reports that PGE$_2$ decreases voltage-gated
Ca
2+ currents (Borgland et al. 2002; Ikeda 1992). Therefore it is likely that the effects of PGE2 on the outward potassium currents may be responsible for the changes in membrane excitability and enhanced Ca
2+ influx. Thus there is a similarity in PGE2-induced increase in dendritic Ca
2+ influx between dentate granule neurons and CA1 pyramidal neurons. It is apparent, however, that there is a difference in the magnitude of the back-propagating dendritic action potential-induced Ca
2+ influx in the presence of PGE2. We showed previously that somatic current injection-induced Ca
2+ influx decreases with the distance from soma to dendrites in dentate granule neurons (Chen et al. 2002); however, this decrease in Ca
2+ influx was more pronounced in pyramidal neurons. Indeed, there exist differences between the two types of neurons in terms of geometry, ion channel distributions, and filtering properties. For instance, it has been well documented that the back-propagating dendritic action potential amplitude and associated Ca
2+ influx decrease with the distance from soma to distal dendrites. This is due mainly to a linear increase in A-type K+ current density in rat CA1 pyramidal neurons (Hoffman et al. 1997). It is not clear whether there is a similar phenomenon in dentate granule neurons. At present there is no available information for the linear increase in A-type K+ current density from the soma to distal dendrites of granule neurons. In addition, there is a linear increase in Ih current density from soma to apical dendrites observed in CA1 pyramidal neurons (Magee 1998), while the Ih channel expression in granule neurons is very low (Chen 2004; Santoro et al. 1998). Thus it is possible that there may be a discrepancy in back-propagating dendritic action potential-associated Ca
2+ influx between the two types of neurons.

Of three COX isozymes that have been identified, COX-2 has been demonstrated to be involved in hippocampal long-term synaptic plasticity (Chen et al. 2002; Yamagata et al. 1993). We found previously that COX-2 inhibitors reduce HFS-induced long-term potentiation, while the COX-1 inhibitor has no effect on LTP induction in the hippocampal perforant path. In particular, application of PGE2, but not of PGD2 or PGF2α, reverses the COX-2 inhibitor-induced reduction of LTP (Chen et al. 2002). Because PGE2 is the main COX-2 reaction product, it is likely that PGE2 is the major signaling molecule in COX-2-mediated hippocampal synaptic plasticity. In addition, COX-2 expression is localized in neuronal dendritic spines, specialized structures where cell-to-cell communications take place (Kauffman et al. 1996), indicating that COX-2-synthesized PGE2 may play an important role in synaptic signaling. In this study, we observed that PGE2 produced a long-lasting enhancement of EPSPs and increased temporal summation at CA3-CA1 synapses, confirming that endogenous PGE2 regulates synaptic activity. However, the mechanisms by which PGE2 enhances synaptic transmission are still not clear. We found that PGE2 did not alter both the frequency and amplitude of mEPSCs in pyramidal neurons from the slices treated with the COX-2 inhibitor. In addition, there was no significant change in the paired-pulse facilitation in slices treated with or without NS398. Thus the exact action sites for the PGE2-induced enhancement of synaptic transmission remain to be elucidated. Nevertheless, these results do suggest that endogenous PGE2 synthesized by COX-2 regulates synaptic transmission, integration, and plasticity through EP receptor(s). In fact, the consequence of increased or decreased COX-2 expression is ultimately dependent on the amount of PGs synthesized and the functioning of PG receptors. Four subtypes of PGE2 receptors (EPs) have been cloned and termed EP1-4 (Boie et al. 1997; Breyer et al. 2001; Narumiya et al. 1999). These EP receptors have seven-hydrophobic-transmembrane-segment architecture typical of G protein–coupled receptors, and evoke cellular responses via distinct intracellular mechanisms (Breyer et al. 2001; Narumiya et al. 1999). EP3 and EP4 receptors couple with the G
i•-AC-CAMP pathway and increase cAMP levels. In contrast, activation of EP3 receptors inhibits CAMP generation via a pertussis toxin-sensitive G
i•-coupled mechanism. There is also one splice variant of EP3 that positively couples to adenyllylclase. EP1 receptors couple with the G
s•-PKC-PLC-IP3 pathway. In rat spinal dorsal horn neurons, PGE2-induced increase in membrane excitability is through a direct effect on membrane depolarization and inhibition of glycineergic neurotransmission. These effects are mediated via postsynaptic EP3-like receptors (Ahmadi et al. 2002; Baba et al. 2001). To elucidate which signal transduction pathways mediate the PGE2-induced increase in synaptic efficacy in hippocampal CA1 pyramidal neurons, we used H-89, a selective PKA inhibitor. While a blockade of the PKA activity by H-89 did not completely eliminate the PGE2-induced enhancement of EPSPs, chelerythrine, a PKC inhibitor, together with H-89 completely blocked the PGE2-induced effect, indicating an involvement of both PKC and PKA activities. This was further confirmed by use of H-9 at a concentration of 20 μM, where both PKA and PKC were inhibited.

Growing evidence suggests that the functional significance of the COX-2 and PGs is far beyond what was initially apparent. Our results suggest that endogenous PGE2 dynamically regulates membrane excitability, temporal summation, and synaptic efficacy in hippocampal pyramidal neurons. These important physiological functions are tonically regulated by endogenous PGE2 in the hippocampus under physiological conditions. COX reaction products have been proposed to act as volume transmitters rather than as synaptic transmitters. It is possible that COX-2-synthesized PGE2 acts on the PG receptors within the same neuron (autocrine) or in neighboring neurons (paracrine). For instance, PGE2 release from astrocytes that is neurotransmitter (glutamate)-dependent can act on neighboring neurons (Zonta et al. 2003). PGE2 also stimulates glutamate release from astrocytes (Bezzi et al. 1998). In particular, COX-2 is expressed in dendritic spines; thus local released PGE2 may act on EPs on dendrites to regulate dendritic membrane excitability and synaptic transmission. Our results suggest that this is the case. Since COX-2 is inducible as well as constitutively expressed in brain, up- or down-regulation of its expression resulting in increased or decreased levels of PGE2 may not only profoundly influence physiological functions, but also be associated with pathological conditions. For instance, a single seizure causes a 10-fold increase in COX-2 expression, whereas repeated seizures result in a 70-fold induction in the hippocampus (Bazan 2001; Marcheselli and Bazan 1996). It can be reasonably postulated that there is a significant amount of PGE2 being produced when the COX-2 expression is significantly elevated during seizure. The increased PGE2, thus will contribute to epileptic activities. In addition, COX-2 expression is significantly increased in patients with Alzheimer’s disease and in experimental stroke, suggesting that COX-2 and its reaction products are involved.
in several neurological disorders (Hewett et al. 2000; Ho et al. 1999; Iadecola et al. 2001; McCullough et al. 2004; Miettinen et al. 1997; Nakayama et al. 1998). Therefore the significance of our findings is in providing a new insight into the roles of specific COX-2–mediated neuronal signalling and its reaction products in physiological and pathological functions in the hippocampus.

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