Profiling of Eicosanoid Production in the Rat Hippocampus during Kainic Acid-induced Seizure

DUAL PHASE REGULATION AND DIFFERENTIAL INVOLVEMENT OF COX-1 AND COX-2

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Kainic acid (KA)-induced seizure in rat involves eicosanoid production in the brain, but their production mechanism and biological functions are poorly understood. We profiled the eicosanoid production during KA-induced seizure by a comprehensive lipids analysis using liquid chromatography-tandem mass spectrometry. Systemic KA administration caused production of large amounts of prostaglandin (PG) F2_

\[ \text{e} \] and PGD2 in the hippocampus, with smaller amounts of other PGs and hydroxyeicosatetraenoic acids. The production was biphasic, which consisted of an initial burst in the first 30 min and a sustained late phase production. The initial phase was specific to the hippocampus and was blocked by intracerebroventricular administration of KA receptor antagonists. A selective cyclooxygenase (COX)-2 inhibitor, NS398, completely inhibited the initial phase productions, except for PGD2 and thromboxane B2, whose productions were also dependent on COX-1. These results suggest that KA signals directly stimulate the arachidonic acid cascade in the initial phase and that COX-1 and COX-2, both constitutively expressed at low levels, differentially contribute to PG productions. In the late phase, a sustained PG production in hippocampus appears due to the increased COX-2 levels even with a limited arachidonic acid supply. The present study demonstrates a dual phase regulatory mechanism of eicosanoid production during KA-induced seizure, providing a biochemical basis for understanding the biosynthesis and roles of eicosanoids in the brain.

The pathology of intractable temporal lobe epilepsy involves glutamate excitotoxicity: an activation of glutamate receptors by high concentration of glutamate causes neuronal damages in the brain (1). An excitotoxic amino acid, kainic acid (KA),4 stimulates KA receptors, which are the members of non-N-methyl-D-aspartate ionotropic glutamate receptors. When systemically administrated to rats, KA induces a rapid and recurrent epileptic seizure, followed by a neuronal degeneration in specific brain areas, including hippocampus (2). KA-induced seizure has been thus studied as one of the animal models of human temporal lobe epilepsy (3).

Eicosanoids such as prostaglandins (PGs) and leukotrienes (LTs) are arachidonic acid-derived lipid mediators produced from membrane phospholipids, which exert diverse biological activities as intercellular signaling molecules through their cognate G protein-coupled receptors (4, 5). Involvement of eicosanoids in the KA-induced seizure has been suggested; KA stimulates eicosanoid production in the rat brain (6), and expression levels of enzymes such as cyclooxygenase (COX)-2 (7–12) and 5-lipoxygenase (8) are affected by KA. However, the roles of eicosanoids as well as their production mechanisms are poorly understood. In the present study, we profiled the eicosanoid production in the rat hippocampus during KA-induced seizure. Taking advantages of the simultaneous quantification method using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) (13, 14), we carried out a comprehensive analysis of lipid mediators in the rat hippocampal tissues. The time course of COX-2 up-regulation and the profile of lipid mediator production suggested a dual phase regulatory mechanism; a direct KA action may cause a transient burst PG production in the initial phase, whereas increased COX-2 levels mediate the late phase production under limited arachidonic acid supply. We also demonstrate a contribution of COX-1 in the production of PGD2, and TxB2, in the hippocampus, as well as a role of constitutively expressed COX-2, suggesting a diversity of PG production pathways in the brain.

EXPERIMENTAL PROCEDURES

Animals—Three-week-old male Wistar rats, 3-day-old male rats, and embryonic day 18 rats (SLC, Hamamatsu, Japan) were used in the study. All animal studies were conducted in accordance with the Guidelines for Animal Research at the University of Tokyo and were approved by the Animal Ethics Committee of the University of Tokyo.

Reagents—Kainic acid, NS398, and indomethacin were purchased from Wako (Osaka, Japan). UBP296, UBP301, and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were from Tocris (Bristol, UK). [α-32P]CTP was from PerkinElmer Life Sciences. Rabbit polyclonal anti-cytosolic phospholipase A2α (cPLA2α) antibody was from Cell Signaling Technology (Beverly, MA), and rabbit polyclonal anti-COX-2 antibody was from Cayman Chemical (Ann Arbor, MI). Horseradish peroxidase-conjugated donkey anti-rabbit IgG was from Amersham Biosciences. All eicosanoid primary standards and deuterium-labeled eicosanoid internal standards for quantification were purchased from Cayman Chemical (Ann Arbor, MI).

Drug Administration, Tissue Collection, and Lipid Extraction—Rats were euthanized at indicated periods after intraperitoneal KA administration (10 mg/kg, dissolved in saline). Hippocampi (70–80 mg) and cerebral cortex (70–100 mg) were dissected, immediately frozen under liquid nitrogen, and stored at −80 °C until use. The tissues were crushed...
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to powder with an SK-100 mill (Tokken, Chiba, Japan) without thawing, and lipids were extracted with 1 ml of ethanol with a mixture of deuterium-labeled internal standards of eicosanoids. The extracts were further cleaned up with Oasis HLB extraction cartridges (30 mg, Waters, Milford, MA) as described (14).

For the intracerebroventricular administration of KA receptor antagonists, CNQX, UBP296, and UBP301 were dissolved in phosphate-buffered saline (PBS)/Me2SO (95:5, v/v) at a concentration of 10 mM (50 nmol in 5 μl). 5 μl of the solutions was injected into both of the lateral ventricles by a 10-μl Hamilton microsyringe fitted with a 27-gauge × 3/4-inch length needle with the following coordinates: anterior/posterior = −1 mm behind bregma, medial/lateral = 1 mm, and an injection depth of 3.5 mm. For COX inhibitor administration, indomethacin and NS398 were dissolved in Me2SO (5 mg/ml) and intraperitoneally injected at a dose of 10 mg/kg, 30 min before KA administration.

Quantification of Eicosanoids—Eicosanoids in the brain were quantified as described (14). Briefly, a TSK-7800 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) ion source (Thermo Electron, Waltham, MA) was operated in negative-ESI and selected reaction monitoring (SRM) mode. A reversed phase high performance liquid chromatograph system, consisting of four Shimadzu (Kyoto, Japan) LC-10A pumps and a Shimadzu CTO-10 column oven, an electrically controlled 6-port switching valve (Valco, Houston, TX), and a 3033 HTS autosampler (Shiseido, Tokyo, Japan), was connected to the MS instrument and used for the rapid resolution of PGs with Shiseido Capcellpak C18 MG53 (1 × 100 mm) column under 10 μl/min. For the accurate quantification, an internal standard method was used. As internal standards, a mixture of deuterium-labeled eicosanoids was used. Automated peak detection, calibration, and calculation were carried out by the use of Xcalibur 1.2 software package (Thermo Electron).

Reverse Transcription-PCR—For the preparation of first-strand cDNA templates from rat hippocampus, total RNA was isolated by Isogen reagent (Nippon Gene, Toyama, Japan), and mRNA was isolated by using a μMACS mRNA isolation kit (Miltenyi Biotec, Gladbach, Germany). Oligo(dT)-primed reverse transcriptions were performed with SuperScript II enzyme (Invitrogen). Following primers were used for PCR: cPLA2α (forward, 5'-TTTGGGCGATATGCTGGACACTCCTG-3'; reverse, 5'-GAGGGAAAACAGACAAGAGGATGGGG-3'), COX-1 (forward, 5'-GGAGTCTGCTGCTGATTTCCCT-3'; reverse, 5'-GGAGACATAGGGCAGGAAAAC-3'), PGI2 (forward, 5'-ATGCGCAAAATCTTGTGCTTCCA-3'; reverse, 5'-CACCCTGGTTGATGTTGCTTCTT-3'), microsomal PGE synthase-1 (mPGS-1, forward, 5'-TTCCTGTGGGTGGATAGGAAAC-3'; reverse, 5'-ACAGGCAGAATCTGGGCAGAACACTAG-3'), lipocalin-type PGD synthase (forward, 5'-CTTGGATTTCCACAGGACCCAG-3'; reverse, 5'-ACTTATCCGTTGAGTGGCAGAAA-3'), PGI synthase (forward, 5'-ATGCGCAAAATCTTGTGCTTCCA-3'; reverse, 5'-GGCTGACATGCAGAACGACTATC-3'), and glyceroldehyde-3-phosphate dehydrogenase (forward, 5'-GAAGAGTCTGTTGTAACGGATTGTCG-3'; reverse, 5'-TTGTCAATTGGAAGAATGCGAACC-3'). Expected PCR product sizes were 2088, 1733, 1701, 409, 511, 1319, and 906 bp for cPLA2α, COXI, COX-2, mPGS-1, lipocalin-type PGD synthase, PGI synthase, and glyceroldehyde-3-phosphate dehydrogenase, respectively. PCR conditions were as follows: initial denaturation at 94 °C for 2 min, followed by 30 cycles of 96 °C for 15 s, 65 °C for 30 s, and 68 °C for 2 min (for cPLA2α, COX-1, COX-2, and PGI synthase) or 1 min (for mPGS-1, lipocalin-type PGD synthase, and glyceroldehyde-3-phosphate dehydrogenase), and final extension at 68 °C for 5 min. The amplified products were confirmed by 2% agarose gel electrophoresis.

Northern Blot Analysis—Total RNA was separated in 1.2% agarose gel containing 5% formaldehyde. Total RNA samples were prepared from pooled tissues of at least 3 rats, 25 μg/lane (for cPLA2α), 50 μg/lane (for COX-1), and 20 μg/lane (for COX-2) of which were loaded onto the gels. The RNA was transferred onto Hybond-N* membrane for 16 h and cross-linked with ultraviolet light. [α-32P]CTP-labeled riboprobes were used for hybridization. Antisense riboprobes (1.0 × 106 to 1.0 × 107 cpm/μg of RNA) were synthesized by transcription of cDNA fragments subcloned in a pGEM-3Zf(−) vector using T7 and SP6 RNA polymerases (Promega, Madison, WI). cDNA sequences used were, nucleotides 1254–1538 and 1539–2099 for cPLA2α, 201–796 for COX-1, and 660–1044 for COX-2 (nucleotide enumerations are based on sequences of GenBank™ accession numbers BC070940, NR03388, and AF233596, respectively). All the probe sequences were selected within the open reading frames of respective cDNA sequences.

Hybridizations with given labeled riboprobes were performed at 57 °C for 16 h using 1 × 107 cpm/ml of probes in a hybridization buffer containing 50% formamide, 5 × Denhardt’s solution (Wako, Osaka, Japan), 10% dextran sulfate, 5 × SSPE (0.75 M NaCl, 50 mM sodium phosphate buffer, 2.5 mM EDTA, pH 7.4), 50 mM potassium phosphate buffer (pH 7.4), 1% sodium dodecyl sulfate, 0.01 mg/ml denatured salmon sperm DNA, and 50 mM dithiothreitol. After several washes under stringent conditions, radioactive signals were visualized with a BAS-2000 imaging analyzer (Fuji film, Tokyo, Japan).

Western Blot Analysis—Hippocampi were homogenized in a lysis buffer (0.34 M sucrose, 150 mM NaCl, 80 mM potassium phosphate buffer, 10 mM MgCl2, pH 7.4) containing a Complete™ EDTA protease inhibitor mixture (1 tablet/20 ml, Calbiochem, San Diego, CA). 8 μg/lane of lysates was subjected to a 10% SDS-polyacrylamide gel electrophoresis and transferred onto an Immob-Blot polyvinylidene difluoride membrane (Bio-Rad). The membrane was blocked overnight at 4 °C with BlockACE (Yukiurushi, Sapporo Japan) and incubated for 1 h at room temperature with rabbit polyclonal antibody for cPLA2α or COX-2 diluted 1:1000 in 0.1% Triton X-100/PBS (T-PBS). After wash-
ing with T-PBS, the membrane was incubated with donkey anti-rabbit IgG conjugated with horseradish peroxidase for 1 h. After washing, immunoreactive signals were visualized with an ECL Plus Western blotting detection system (Amersham Biosciences).

Statistical Analysis—Statistical analyses were carried out with GraphPad Prism 4.0.3 package (GraphPad Software, San Diego, CA), and *p* values of <0.05 were considered statistically significant.

RESULTS

Biphasic PG Production in the Rat Hippocampus after Systemic KA Administration—A comprehensive analysis of eicosanoids in the rat hippocampal tissue was carried out with a LC-ESI-MS/MS multiplex quantification system. Fig. 1 shows typical chromatograms for hippocampal tissues 1 h after intraperitoneal KA injection and those from saline-injected control rats. In our system, five major PGs, 6-keto-PGF1α (a stable metabolite of PGI2), TxB2 (a stable metabolite of TxA2), PGF2α, PGE2, and PGD2, were readily detected even in the untreated rats (Fig. 1A) and dramatically increased after KA administration (Fig. 1B). Three major LTs, LTB4, LTC4, and LTD4, were undetectable. Among hydroxyeicosatetraenoic acid (HETE) isomers, two major peaks of 11-HETE and 12-HETE were observed, which increased after KA administration.

Using deuterium-labeled internal standards, PGs were quantified up to 24 h after KA treatment. As shown in Fig. 2A, hippocampal tissue produced PGF2α and PGD2 as two major PGs, followed by PGE2, TxB2, and 6-keto-PGF1α in this order. The PG levels reached rapidly a peak within 30 min to 1 h after KA administration and then decreased to lower levels. The production levels remained higher than the basal levels at 3 h and sustained up to 24 h. All PG species monitored showed a similar time course (Fig. 2A). These results demonstrated that hippocampal PG production after systemic KA administration was biphasic; an initial burst was followed by a sustained late phase production. Interestingly, the initial response to KA within 30 min was not observed in the cerebral cortex, suggesting that PG production at this phase is specific to hippocampus (Fig. 2B).

Primary eicosanoids produced in the hippocampal tissue can be further converted to biologically active or inactive metabolites. To evaluate this, metabolites were quantified for three major eicosanoids produced in the hippocampus, PGF2α, PGD2, and PGE2. As shown in Fig. 2 (C and D), considerable amounts of 13,14-dihydro-15-keto-PGF2α and 13,14-dihydro-15-keto-PGE2 were detected proportionally to respective precursors both in the initial phase and in the late phase, suggesting that PGF2α and PGE2 are readily metabolized to these inactive compounds by 15-hydroxyprostaglandin dehydrogenase (PGDH). The levels of 13,14-dihydro-15-keto metabolite of PGD2 as compared with its precursor PGD2 were much smaller than those of PGF2α and PGE2, suggesting that PGD2 is rather stable against PGDH-mediated PG inactivation (Fig. 2, C and D). PGD2 can also be converted to PGJ2, 12,14-PGJ2, and/or 15-deoxy-Δ12,14-PGJ2, which are reportedly bioactive metabolites (15), or 11β-PGF2α (16). The amounts of these metabolites were almost undetectable or very small throughout the time course, suggesting they are not major metabolites of PGD2 in the rat hippocampus (data not shown).

![FIGURE 2. Biphasic PG productions in the hippocampus after KA administration. A, time course of hippocampal PG levels after KA administration. Hippocampal tissues were dissected at indicated periods after KA administration, and PG levels were quantified (n = 6 for each time point). B, PG levels in the cerebral cortex and hippocampus before and 30 min after (filled columns) KA administration (intraperitoneal). C and D, levels of 13,14-dihydro-15-keto (DHK) metabolites in the hippocampus before (control groups, open columns) and 30 min after (filled columns) KA administration (intraperitoneal). *, p < 0.001 versus respective control group in Tukey’s multiple comparison test after analysis of variance. Data are expressed as mean ± S.D.; n, ranged from 3 to 6 for each experimental group.](image-url)
Inhibition of the Initial Phase PG Production by KA Receptor Antagonists—To examine if the initial phase hippocampal PG production was mediated by KA receptors in the hippocampus, rats were pretreated (intracerebroventricularly) with CNQX (a non-N-methyl-D-aspartate receptor antagonist), UBP296 (a selective antagonist for GluR5 subunit-containing KA receptors), or UBP301 (a selective antagonist for KA receptors) 30 min prior to the systemic KA administration (intraperitoneally). UBP296 and UBP301 have been reported as potent selective antagonists for KA receptors (reported $K_i$ values of $\sim 1 \mu M$ and $\sim 6 \mu M$, respectively; see Ref. 17). As shown in Fig. 3 (A–E), all of the PG productions were significantly suppressed by UBP296. CNQX and UBP301 were less potent than UBP296, because they inhibited only PGF$_{2\alpha}$ production with statistical significance (Fig. 3, A–E). When much smaller amounts of KA (1 nmol/rat) were intracerebroventricularly administrated, the rats exhibited immediate epileptic response within several minutes, much earlier than those observed by systemic administration (45–60 min). PG profiles after intracerebroventricular KA administration were similar to those by systemic KA administration (data not shown). Collectively, these results suggest that immediate burst PG productions in the hippocampus were mediated directly by activation of KA receptors in the brain, probably by those expressed in the hippocampus.

Changes of Enzyme Levels in the Hippocampus after Systemic KA Administration—To understand the mechanism of KA-induced PG productions, we measured the levels of enzymes involved in the arachidonic acid cascade at mRNA and protein levels. Reverse transcription-PCR analysis of hippocampal tissue samples after KA treatment demonstrated an increase in the COX-2 and mPGES-1 mRNAs after KA treatment (Fig. 4A). Northern blot analyses were performed for cPLA$_2$, COX-1, and COX-2 (Fig. 4B). COX-2 mRNA increased transiently, reaching a peak 3–6 h after KA treatment. For contrast, cPLA$_2$ and COX-1 mRNAs did not show significant changes. Western blot analysis showed a low level expression of COX-2 protein in unstimulated control rats, and a significant increase was observed 3 h after KA administration, which lasted for 24 h (Fig. 4C). The amount of cPLA$_2$ protein did not show significant changes (Fig. 4C). These results demonstrated that KA administration stimulates COX-2 up-regulation through KA receptors; however, the time course was delayed from the initial burst PG production (Fig. 2A). Rather, the COX-2 up-regulation was concomitant with the late phase PG production, suggesting a role of increased COX-2 in the late phase production.

Effects of COX Inhibitors on the Initial Phase PG Production—To further analyze COX isoform dependence of the initial phase PG production, effects of COX inhibitors were examined. As shown in Fig. 5, pretreatment of rats with a COX-2-selective inhibitor, NS398, effectively suppressed the initial phase PG production. NS398 inhibited the production of PGD$_2$ and TxB$_2$ only by 50–70%, whereas other PGs (PGF$_{2\alpha}$, PGE$_2$, and 6-keto-PGF$_{1\alpha}$) were almost completely inhibited. Indomethacin, a non-selective COX inhibitor, inhibited all of the PG productions. These results demonstrate that COX-2 is constitutively present in the brain and plays a major role in PG productions, and that COX-1 contributes to a lesser extent in the production of PGD$_2$ and TxB$_2$.

**DISCUSSION**

In the present study, we profiled the eicosanoid production of rat hippocampal tissues during KA-induced seizure using the LC-ESI-MS/MS multiple quantification method (13, 14). One of the advantages
of our strategy is that multiple eicosanoids can be measured simultaneously. From the method, a novel insight is now provided for PG production in KA-induced seizure model.

**Dual Phase Regulation of Eicosanoid Productions in the Hippocampus**—Systemic KA administration caused biphasic PG production (Fig. 2). Initially, an immediate burst of PG production occurred within 30 min after KA administration. Subsequently, late phase PG production sustained to 24 h, with levels being lower than the initial phase but higher than the untreated basal levels. The prominent productions of PGF2α and PGD2 in the initial phase (Fig. 2) agree well with a previous report (6). In addition, the lack of LT synthesis in our observation (Fig. 1) illustrates that the COX pathway is dominant in the hippocampus during this period. Kawaguchi et al. (12) reported an increased PGE2 production in the rat hippocampus 24 h after KA administration. Our results, however, demonstrate that PGF2α and PGD2 rather than PGE2 are major metabolites even 24 h after KA stimulation.

Analysis of the metabolic fate of major PGs showed that PGF2α and PGE2 are converted to the 13,14-dihydro-15-keto form in the hippocampus, whereas PGD2 is rather resistant to this inactivation pathway (Fig. 2, C and D). This result agrees with the substrate specificity of NAD+-dependent 15-PGDH (18–20). It is not likely that PGD2 is converted to other metabolites such as PGJ2, 12,14-DPGJ2, 15-deoxy-Δ12,14-PGJ2, and/or 11β-PGF2α, because they were hardly or very weakly detected in the hippocampal tissue (data not shown). The time course of the production of 13,14-dihydro-15-keto metabolites was similar to those of precursor PGs, which reached a peak at 30 min to 1 h after KA administration (data not shown). Thus, we suppose that both PGs and their metabolites can be removed from the hippocampal tissue, possibly by blood flow or diffusion.

The biphasic profile of PG production can be explained by the mode of KA action and the enzyme levels of the arachidonic acid cascade (Fig. 2). In the initial phase, large amounts of PGs are produced rapidly without enzyme levels apparently changed (Figs. 2 and 4). In addition, this initial robust production was not observed in the cortex, suggesting that it is hippocampus-specific (Fig. 2B). Further, intracerebroventricular

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**FIGURE 5. Effects of COX inhibitors on the initial phase PG productions.** A–E, effects of NS398 pretreatment on the productions of 6-keto-PGF2α (A), TxB2 (B), PGF2α (C), PGE2 (D), and PGD2 (E). Hippocampal tissues were dissected at indicated periods (0–180 min) after KA administration (intraperitoneal). In all panels (A–E), effects of KA administration were significant (*p* < 0.0001, by two-way analysis of variance). F, effects of indomethacin pretreatment on the production of PGs. Hippocampal tissues were dissected 30 min after KA administration (intraperitoneal). *p* < 0.0001 by Student’s unpaired *t* test, versus respective KA administration group. Open columns, vehicle (Me2SO) pretreatment 30 min prior to KA administration; filled columns, inhibitor pretreatment 30 min prior to KA administration. IND, indomethacin. Data are expressed as mean ± S.D.; n, ranged from 4 to 8 for each experimental group.

**FIGURE 6. Dual phase hippocampal PG productions in KA-induced seizure model.** A, dual phase PG production after KA administration. Our results suggest that hippocampal PG production consists of two distinct phases: the initial phase and the late phase productions that occur within 30 min and within several hours to 24 h after systemic KA administration, respectively. Induction of COX-2 occurs concomitantly with the late phase PG productions, suggesting that COX-2 up-regulation is involved in the late phase mechanism, whereas the initial phase mechanism is independent of COX-2 up-regulation. B and C, possible mechanisms for the initial phase (B) and the late phase (C) productions. In B, KA receptor signals in hippocampal PG-producing cells (e.g. neurons or astrocytes) directly stimulate arachidonic acid cascade through intracellular calcium increase. Both COX-1 and COX-2 contribute to PG production in this phase, with differential coupling to the production of each PG species: PGD2 and TxA2 are produced by both COX-1 and COX-2, whereas PGF2α and PGE2 are mostly COX-2-dependent. In C, increased COX-2 levels determine the late phase PG production profiles. With lower or no KA receptor signals, arachidonate supply is much limited. Increased COX-2 activity pulls up the PG production from the basal production levels; unchanged COX-1 levels suggest that COX-1 has minimal contribution to PG productions in the late phase.
administration of KA receptor antagonists blocked the PG productions in the hippocampus. All these results suggest that this process is mediated by KA receptor in the hippocampus (Fig. 3), consistent with the high density distribution of KA receptors in the hippocampus (21, 22). The most probable mechanism is that activation of KA receptor increases intracellular \([ \text{Ca}^{2+} ] \) (23), causing translocation of cPLA2 to the membrane, as reported in various types of cells (24–26) (Fig. 6B).

The mechanism of the late phase PG production may much differ from that of the initial phase. In the late phase, the COX-2 levels were increased by KA treatment (Fig. 4), as reported (7–12). The COX-2 up-regulation can result in an increased production of PGs, but the levels of late phase productions were much lower than the initial phase. This can be due to the limited supply of arachidonic acid: KA receptor signals might no longer stimulate arachidonic acid release as in the initial phase (Fig. 6C). The constitutive PG productions observed in the untreated rats (Figs. 1 and 2A) suggest that, in the hippocampus, there is a significant supply of arachidonic acid in the steady state. Thus, increased COX-2 levels with this limited arachidonic acid supply may illustrate the nature of the late phase production (Fig. 6C). In contrast to the initial phase PG production that was specific to the hippocampus, we observed the late phase PG production also in the cortex (data not shown). Since it has been reported that COX-2 up-regulation is seen in the cortex as well as hippocampus (7, 10), the late phase PG production mechanism may be common in hippocampus and cortex. The mechanism of KA-induced COX-2 up-regulation may involve an increased transcription and/or a stabilization of COX-2 mRNA (27). KA-induced intracellular signaling processes activate extracellular signal-regulated kinase (28, 29) and p38 mitogen activated protein kinase (29, 30) in the brain; these can cause COX-2 up-regulation via transcriptional activation (27, 31, 32) and mRNA stabilization (27, 33, 34), respectively. In addition, cAMP-response element-binding protein phosphorylation is affected in KA-stimulated (28) and epileptic (35) hippocampus, which may also modulate COX-2 mRNA transcription (27). Further investigations are required to elucidate the precise mechanism and physiological roles of COX-2 up-regulation by KA in the brain.

Differential Involvement of COX-1 and COX-2 in the Initial Phase PG Productions—A critical and important finding from this study is that COX-1 and COX-2 differentially contribute to PG productions in the initial phase. Our results with a COX-2-selective inhibitor, NS398, showed that COX-1 might also contribute to productions of PGD2 and TXB2 during KA-induced seizure (Figs. 5 and 6B). The mechanism of the differential COX-1/COX-2 contribution can be explained by the coupling efficiency of terminal PG synthases to each COX isoform. A functional coupling of COX-2 to PGF synthase (36, 37) as well as COX-1 to PGD and thromboxane synthases has been reported (38, 39). However, PGD2 and TXB2 productions seem dependent also on COX-2 in the brain, because NS398 significantly reduced their production (Fig. 5). It is possible that platelets and immune cells also produce PGD2 and thromboxane via COX-1. In such cases, it is unlikely that KA directly stimulates these cell types, because KA effects are localized to hippocampus (Fig. 2B). Rather, KA may indirectly stimulate these cells through activating neurons or glial cells.

In Vivo Significance of the Dual Phase PG Production—Although the present study was not aimed to directly address the function of eicosanoids in epileptic seizure or neuronal degeneration, our results provide an important concept on such studies, that there are distinct mechanisms of PG productions serially occurring, i.e. the initial phase and the late phase productions. There are controversial reports on the effect of COX inhibitors; in some COX inhibition was protective to KA-induced seizure (40), and in others COX inhibition rather aggravated symptoms (41, 42). Most of the studies were performed by using pretreatment with inhibitors, which blocked the initial phase PG production. Because the effective periods of inhibition may vary depending on the inhibitor and the route of administration, it is difficult to evaluate the significance of the late phase production in KA-induced seizure by a single-dose pre-treatment with inhibitors. To discriminate the initial phase and the late phase PG productions, administration method of inhibitors should be well designed. Such methods may include a post-treatment with inhibitors, which conserves the initial phase intact. A recent report by Gobbo et al. (43) demonstrated that post-treatment with COX-2-selective inhibitors enhanced the functional recovery from KA-induced neuronal degeneration, whereas pretreatment aggravated the seizure symptoms, suggesting the diversity of PG roles at the beginning and at the later periods. Similar experiments with detailed monitoring of PG amounts will help in understanding the causal relations of each phase of PG production and the outcomes.

In conclusion, the present study demonstrated a dual phase regulatory mechanism of eicosanoid production in the rat hippocampus during KA-induced seizure. In addition to the temporal and spatial regulation, our results also revealed the diversity of PG-producing pathways to which COX-1 and COX-2 differentially contribute. A detailed understanding of the mechanism of lipid mediator production will provide a biochemical basis for understanding the roles of lipid mediators in seizure-related pathologies. Furthermore, recent studies reporting novel lipid mediators in the brain (44–46) raise the possible involvement of non-eicosanoid lipid mediators in excitotoxic degeneration. Thus, our strategy using LC-ESI-MS/MS will serve as a universal tool for understanding the roles of lipid mediators in the brain.

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