Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus

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Introduction

The acute insult of status epilepticus (SE) is followed by a latent period characterized by the functional or morphological changes of the hippocampus over several days to weeks, before the onset of spontaneous recurrent seizures (SRS) (Motte et al., 1998; Dube et al., 2001). Epilepsy results from long-lasting plastic changes in the brain involving the axonal or dendritic reorganization, dispersion of the granule cell layer (GCL) or inhibitory interneuronal loss. Epileptic insult also increases the cell proliferation in the subgranular layer (SGL) or subventricular zone (SVZ), and then produces the abnormal granular neurons in the hilus and astrogliosis in CA1 (Parent et al., 1997; Scharfman et al., 2000, 2001; Jung et al., 2004). Several evidences suggest that ectopic granule cells and astrogliosis might be key factors that switch the nonepileptic brain to epileptic brain (Dashtipour et al., 2001; Parent, 2002; Pitkanen and Sutula, 2002; Jung et al., 2004; Tian et al., 2005; Vessal et al., 2005; Parent et al., 2006).

Upstream mechanism involved in the abnormal proliferation and migration response is currently unknown. As the generation of new neurons or glia within the hippocampus is instructed by local signaling (Luskin, 1998), the alterations in the microenvironment of the stem cell, such as microglial inflammation and cyclooxygenase-2 induction may cause ectopic neurogenesis or astrogliosis. Here, we examined if inflammatory blockade with celecoxib, a selective cyclooxygenase-2 inhibitor, could modulate the altered microenvironment in the epileptic rat brain. Celecoxib attenuated the likelihood of developing spontaneous recurrent seizures after pilocarpine-induced prolonged seizure. During the latent period, celecoxib prevented neuronal death and microglia activation in the hilus and CA1 and inhibited the generation of ectopic granule cells in the hilus and new glia in CA1. The direct inhibition of precursor cells by celecoxib was further demonstrated in human neural stem cells culture.

These findings raise the evidence of COX-2 induction to act importantly on epileptogenesis and suggest a potential therapeutic role for COX-2 inhibitors in chronic epilepsy.

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Keywords: Epileptogenesis; Celecoxib; Ectopic granule cell; Inflammation; COX-2; Neurogenesis; Microglia; Spontaneous recurrent seizure; Epilepsy
proinflammatory mediators, including cyclooxygenase-2 (COX-2) (Aarum et al., 2003).

COX-2 is expressed in discrete populations of neurons and is enriched in the cortex and hippocampus (Yamagata et al., 1993; Hurley et al., 2002). It can be induced in migratory immune cells, glia, and neurons by electrical stimulus-, kainate-, or pilocarpine-induced seizures (Chen et al., 1995; Sandhya et al., 1998; Voutsinos-Porche et al., 2004). COX-2 participates in the inflammatory response (Seibert et al., 1994), neuronal death (Nakayama et al., 1998), neuronal hyperexcitability (Wilingale et al., 1997), and astrocytic activation (Voutsinos-Porche et al., 2004). COX-2 is also implicated in cell proliferation. COX-2 induces the growth and progression of a variety of tumor types (Gupta and Dubois, 2001; Shono et al., 2001; Thun et al., 2002), and COX inhibition by nonsteroidal anti-inflammatory drug treatment causes a reduction of neuronal birth in the dentate gyrus after acute global ischemia (Kumihashi et al., 2001).

We have recently reported a potential role of newly generated cells in epileptogenesis (Jung et al., 2004). COX-2 induction may act on epileptogenesis through the progressive loss of hippocampal neurons, ectopic neurogenesis, and astrogliosis in the epileptic focus. In this study, we tried to correct the epileptic microenvironment in the pilocarpine-induced SE model by a selective COX-2 inhibitor, celecoxib throughout the latent period. The development of SRS was monitored and seizure-induced hippocampal changes were evaluated. We also utilized neural stem cell (NSCs) culture in vitro, which allowed us to examine the direct effect of celecoxib on proliferating progenitor cells.

Materials and methods

Lithium-pilocarpine-induced status epilepticus model

All the procedures were performed under an institutional approval in accordance with NIH Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (12 weeks old; Genomics), weighing 200–220 g, were used for epileptic rats (n = 72) and normal controls (n = 9). Epileptic rats were generated by lithium-pilocarpine-induced SE, as described elsewhere (Olien et al., 2001; Chu et al., 2004a; Jung et al., 2004). Lithium chloride (127 mg/kg, i.p., Sigma) was injected 24 h prior to the administration of pilocarpine. Rats were pretreated with methylscopolamine-bromide (1 mg/kg, Sigma, 30 min prior to pilocarpine), and then single dose of pilocarpine (30 mg/kg, i.p., Sigma) was administered. Seizures were scored by Racine (1972)'s scale. The beginning of SE was defined as the onset of continuous generalized seizure activity without regaining normal behavior between the seizures. For the rats developing SE, we controlled the duration of SE by i.p. injection of diazepam (10 mg/kg, Sanjin). Normal control rats were treated with the lithium-methylscopolamine, and saline instead of pilocarpine, and then only a single diazepam injection at 2 h after saline injection. All rats received i.p. injections of 0.9% saline: 5 ml twice (in the morning and evening) for 2 days after SE. Starting on the third day after SE, all rats were tube fed with crushed pellets using 20-mm feeding needles (Popper and Sons Inc.) for 3 days on average until the rats commenced to eat their normal pellets.

Drug administration

One day after SE (considering the recovery time from SE), rats were fed with celecoxib (prescription formulation; Pfizer Inc.) using 20-mm feeding needles (Popper and Sons Inc.), dissolved in phosphate-buffered saline, 20 mg/kg (n = 36) daily until the day of sacrifice. Epileptic rats fed with vehicle alone served as epilepsy-only group (n = 36), and normal rats fed with vehicle alone (n = 9) served as normal control group. Also, 5-bromo-2'-deoxyuridine-5'-monophosphate (BrdU, 100 mg/kg, diluted in saline) was injected i.p. once a day for 14 consecutive days from the day of SE. The rats were sacrificed at 14 and 28 days after SE for analysis of neuronal loss, microglial activation, hippocampal cell proliferation, and migration and differentiation of BrdU-positive cells (n = 6 for epilepsy-celecoxib, epilepsy-only, and normal control groups at each timing).

SRS monitoring from D28 to D42

Since the frequency and duration of SRS in the pilocarpine model were much higher during the light (diurnal) than the dark (nocturnal) period (Chu et al., 2004a; Jung et al., 2004), all recordings for SRS were done during the light period. Epileptic rats (n = 9 for the two epilepsy groups) were video-monitored for at least 12 h/day (up to 84 h/week) from 28 to 42 days after SE. For video monitoring, digital video camcorders were used which allowed recording of nine rats simultaneously (three rats per camera). The recordings were analyzed by independent observers for group allocation. The frequency and duration of stage 4 or 5 seizures were recorded, and for rating of seizure severity, Racine’s scale was used.

Determination of COX-2 expression by Western blotting

Additional epileptic rats were sacrificed at 1, 4, 7, 14, and 28 days after SE (n = 3 for each group at each timing). After the sacrifice, the brain was removed and frozen in liquid nitrogen. The frozen tissues were homogenized in 30 mM Tris–Cl, pH 7.5, 100 μM phenylmethylsulfonyl fluoride (PMSF). Proteins (25 μg) from whole cell lysates were separated by SDS-PAGE and transferred onto nitrocellulose membrane. The blots were blocked overnight with 5% nonfat dry milk in Tris-buffered saline (TBS), followed by incubation for 1 h with rabbit anti-murine polyclonal antiserum to COX-2 (1:1000, Caymen Chemicals, Inc.) at a dilution of 1:500. After washing with TBS, the blots were incubated with a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody room temperature. Anti-β-actin antibody (Santa Cruz, USA) was used as a control. The blots were developed via enhanced chemiluminescence (Pierce, Rockford, IL, USA), digitally scanned (GS-700, Bio-Rad, Hercules, CA, USA) and analyzed (Molecular Analysts, Bio-Rad).

Immunohistochemistry and histology

The dorsal hippocampus was chosen for comparison between epilepsy-celecoxib and epilepsy-control groups because the previous studies (Chu et al., 2004a; Jung et al., 2004) demonstrated that the dorsal hippocampus got damaged more severely and contained more numerous BrdU-positive cells than other limbic areas. We removed the brains and either cryopreserved tissue blocks for cryostat sectioning (30 μm thickness) or embedded them in paraffin for sectioning (7 μm thickness). The coronal sections through the dorsal hippocampus (at the level corresponding to 2.8–4.5 mm posterior to bregma) were selected, and every 7th section of the hippocampus (6 sections per animal) was examined for quantitative immunohistochemical analysis. Immunohisto-
chemistry was processed as described previously (Chu et al., 2004a; Jung et al., 2004). The specimens were fixed in 4% paraformaldehyde in PBS (pH 7.5) for 40 min, washed with PBS, incubated for 2 h with blocking buffer (2% horse serum/1% BSA/0.1% Triton X-100 in PBS, pH 7.5), incubated overnight at 4°C with primary antibodies, and then incubated for 1 h at room temperature with secondary antibodies. Primary antibodies were as follows: rabbit anti-murine polyclonal antiserum to COX-2 (1:1000, Caymen Chemicals, Inc.); monoclonal anti-BrdU (1:300, Pharmingen); sheep polyclonal anti-BrdU antibodies (1:300, BioDesign); anti-NeuN (1:200, Chemicon); anti-Calbindin (CB, 1:200, Sigma). Mouse anti-rat OX42 primary antibodies (1:500, Chemicon) were used as markers for microglia. Biotinylated goat anti-mouse IgG (ABC, sigma) and FITC-conjugated anti-sheep IgG (1:100, Biodesign) or Cy3-conjugated anti-mouse IgG antibodies (1:300, Jackson Immunoresearch) were used for the secondary antibodies. The colocalization was analyzed using a laser scanning confocal microscopy with a Bio-Rad MRC 1024.

Fig. 1. COX-2 expression in the rat brain after status epilepticus. (A) COX-2 immunoreactivity (green) is detected in a large number of neurons (red) throughout the hippocampus, especially in the dentate gyrus. (B) It is also observed in a number of smaller process bearing cells which are GFAP-positive (red) in the CA1 area. The insets are higher magnification of COX-2-immunoreactive neurons or astrocytes. (C) Western blotting documents a time course of COX-2 expression after SE. SE causes an upregulation in COX-2 expression. It peaks at 1 day after SE and declines thereafter. (D) Relative optical density of COX-2 expression in the epilepsy-only is 1.5 times higher than that of epilepsy-celecoxib group at 14 days after SE. Scale bar: 100 μm. Data are mean ± SD values of three independent experiments. *P < 0.05 compared with epilepsy-control group (n = 3, Mann-Whitney U test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
(argon and krypton). The fluorescence signals were detected at excitation/emission wavelengths of 550/570 nm (Cy3, red) and 488/522 (FITC, green). Biotin signals were detected with 3,3′-diaminobenzidine (DAB; brown) and Vector VIP (purple). Nissl staining was also performed for the evaluation of hippocampal neuronal loss after SE.

Cell quantification

Quantitative analysis of the positively stained cell number was performed in a predefined field of the hippocampal regions with optimal magnification to discriminate the cell outline; CA1 stratum pyramidale: $15 \times 10^{-3}$ mm$^2$, ×400; CA3 stratum pyramidale: $15 \times 10^{-3}$ mm$^2$, ×400; CA2 stratum pyramidale: $15 \times 10^{-3}$ mm$^2$; GCL: $1 \times 10^{-3}$ mm$^2$, ×600; hilus of the dentate gyrus, SGL and caudal SVZ: whole area, ×400 (Jung et al., 2004). We selected for comparisons corresponding coronal sections, determined using the rat atlas of Paxinos and Watson (1997). Hippocampal neurons were counted in 7-μm-thick Nissl-stained sections (6 sections per animal; $n = 6$ for each group). Profiles with smaller somas than 3 μm were considered as glial or as necrotic cells, and they were excluded (Coggeshall and Lekan, 1996). BrdU- or OX42-positive cells in the SGL, GCL, caudal SVZ, hilus, and CA1 and CA3 areas of the hippocampus (6 sections per animal; $n = 6$ for each group) were also counted. Only morphologically intact cells were counted at ×400 and ×600 magnification. The cell profiles were expressed as average number of cells within a predefined surface area/ microscopic field. Since there was no obvious difference in cell profiles between hemispheres, values for right and left hemisphere were averaged in each rat. Values were expressed as mean ± standard deviation (SD).

Human neural stem cells culture

All experimental procedures were approved by the Care of Experimental Animals Committee of Seoul National University Hospital and by institutional review board for the human cell use. Primary dissociated cell cultures were prepared from embryonic human brains of 15 weeks gestation as described previously in detail (Flax et al., 1998; Cho et al., 2002; Ourednik et al., 2002; Jeong et al., 2003; Chu et al., 2003, 2004a,b,c; Imitola et al., 2004). The cerebrum cultures were retrovirally transduced with the v-myec oncogene and subsequently cloned (H1 clone). H1 cells were cultured in poly-L-lysine-coated culture dishes or flasks as single cells or large clusters, which could be subcultured and passaged weekly over a period of 6 months. Cells were seeded in a serum-free medium consisting of Dulbecco’s modified Eagle’s medium (DMEM) containing insulin (10 μg/ml), transferrin (10 μg/ml),...
sodium selenite (30 nM), hydrocortisone (50 nM), and triiodothyronine (0.3 nM). The celecoxib was added to cultures for 72 h at various concentrations.

Assay for cell proliferation

Cell proliferation was assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) absorbance and by counting cells on photomicrographs of 4',6-diamidino-2-phenylindole (DAPI, Vector Laboratories). For MTT assays, cultures were incubated with a stock solution of MTT (5 mg/ml in PBS, pH 7.4, Sigma) at 37°C for 4 h at the final concentration of 1 mg/ml, and absorbance at 570 nm was measured on the ELISA reader. For cell counting, the number of intact DAPI-stained nuclei in five 200× microscope fields per well (at the 3-, 6-, 9-, and 12-o’clock positions and in the center) was recorded. In both cases, results were expressed as a percentage of values obtained in control cultures not treated with celecoxib.

BrdU labeling in vitro

Cells were plated on 12 mm round Aclar plastic coverslips previously coated with 10 μg/ml polylysine and housed in 35-mm dishes. BrdU (50 μg/ml; Sigma) was added for 72 h to the cultures, exposed to celecoxib or not. BrdU-positive cells in culture were counted in five fields per well (center and at 3, 6, 9, and 12 o’clock). Cells containing densely red-stained nuclei were considered BrdU-positive. Results were expressed as a percentage of the number of intact DAPI-stained nuclei obtained in the same fields.

Statistical analysis

Data were analyzed by unpaired Student’s t test if they were normally distributed (Kolmogorov-Smirnov test). Otherwise, we used the Mann-Whitney U test. Repeated measures analysis of variance (ANOVA) followed by post hoc test (Turkey B) was used for the analysis of immunohistochemical study data using SPSS 11.0 program. P values of less than 0.05 were considered to indicate a statistically significant difference.

Results

Outcome of pilocarpine-induced status epilepticus model

Seventy-eight rats injected with lithium and pilocarpine developed SE which was characterized by continuous motor-limbic seizures accompanied by intermittent rearing and falling with a mean latency of 22 ± 4 min (mean ± SD), and six rats out of these died from SE. The duration of SE was controlled as 60 min.

Reduction in a likelihood of developing spontaneous recurrent seizures by celecoxib

From 28 to 42 days after SE, SRS were observed in 8 (89%) of 9 vehicle-treated rats and 5 (56%) of 9 celecoxib-treated rats. The frequency of observed seizures was 1.92 ± 0.60 seizures per day (n = 9; ranging from 0 to 3.30) in epilepsy-only group and 0.68 ± 0.32 (n = 9; ranging from 0 to 1.55) in epilepsy-celecoxib group. The duration of observed seizures was 14.82 ± 3.50 seconds per seizure in epilepsy-only group and 7.12 ± 2.82 in epilepsy-celecoxib group. The frequency and duration of SRS in epilepsy-celecoxib group were significantly reduced compared with epilepsy-only group (P < 0.01, Mann-Whitney U test).

Inhibition of COX-2 expression in the epileptic rat brain by celecoxib

COX-2 immunoreactivity was observed in the perinuclear region of neurons in either normal control or epileptic rats (Fig. 1A). Notably, COX-2 immunoreactivity was also expressed in GFAP-positive reactive astrocytes in the hilus, CA3 and CA1 after SE, but not in normal control animals (Fig. 1B). The COX-2 protein was expressed in a time-dependent manner after SE (Fig. 1C). It peaked at 1 day after SE and declined thereafter. Celecoxib significantly attenuated the COX-2 expression in the rat brain over the latent period after SE (Fig. 1D, P < 0.01, Mann-Whitney U test).

Neuroprotective effect of celecoxib following SE

Nissl-stained sections through the dorsal hippocampus were used to assess the SE-induced neuronal loss. Nissl staining of 14-day preparations from the two epilepsy groups demonstrated a substantial
neuronal loss in the hilus and CA1 and CA3 areas of the hippocampus, but there was no apparent neuronal loss in the GCL and CA2 (Fig. 2). From the quantitative analysis, the pyramidal cell loss in the CA1 was evident at 14 days after SE (−71% of normal control), while that in the CA3 was less severe (−45% of normal control). Neuronal loss was also severe in the hilus of the dentate gyrus (−70% of normal control). Celecoxib prevented the neuronal loss in the CA1, CA3, and hilus following SE (−32%, −12%, −14% of normal control; n = 6 for each group; ANOVA followed by post hoc test).

Suppression of activated microglia at sites of neurodegeneration by celecoxib

Activated microglial cells immunostained with OX42 were observed in the damaged hippocampal areas, notably in the hilus, CA1, and CA3, 14 days after SE (Fig. 3). The number of OX42-positive cells significantly increased at 14 days after SE. From the quantitative analysis, epilepsy-celecoxib group showed a reduced number of activated microglial cells, as compared with epilepsy-control group (hilus: epilepsy-celecoxib: 50 ± 12 cells; epilepsy-control: 124 ± 25 cells; CA1: epilepsy-celecoxib: 115 ± 37 cells; epilepsy-control: 180 ± 24 cells; P = 0.005, t test).

Inhibition of hippocampal cell proliferation after SE by celecoxib

We investigated the effect of COX-2 inhibition on the hippocampal cell proliferation by BrdU immunohistochemistry at 14 days after SE. In normal control group, BrdU-positive cells were unevenly distributed along the SGL and SVZ. In epilepsy-control group, the cell proliferation was upregulated with subsequent expansion of the SGL and SVZ. Celecoxib treatment for 14 days suppressed the cell proliferation in these areas. From the quantitative analysis, SE increased BrdU-positive cells compared with normal control group by 5-fold in the SGL and SVZ, while celecoxib treatment caused a 35% decrease of BrdU-positive cells compared with epilepsy-control group (Fig. 4, n = 6 for each group; P < 0.01, ANOVA followed by post hoc test).

Inhibition of hilar neurogenesis and CA1 gliogenesis after SE by celecoxib

In epilepsy-only group, BrdU-positive cells were distributed in more extensive damaged areas, hilus, CA1, and CA3 at 28 days after SE. A few BrdU-positive cells were found in these areas of epilepsy-celecoxib group at the same time period (Fig. 4).
Hippocampal distribution of BrdU-positive cells in epilepsy-only group was significantly different from that in epilepsy-celecoxib or normal control groups (Fig. 4, \( n = 6 \) for each group; \( P < 0.01 \), ANOVA followed by post hoc test). Celecoxib treatment caused a considerable decrease of BrdU-positive cells differentiated to neurons in the hilus (epilepsy-celecoxib: \( 11 \pm 4 \) cells; epilepsy-vehicle: \( 19 \pm 3 \) cells), and astrocytes in the CA1 area (epilepsy-celecoxib: \( 49 \pm 7 \) cells; epilepsy-vehicle: \( 94 \pm 15 \) cells), as compared with the epilepsy-control group.

Discussion

Selective COX-2 inhibitor, celecoxib, was administered to correct the microenvironment in the hippocampus over a latent period from the induction of SE. Celecoxib dramatically suppressed the neuronal death, microglia activation and abnormal neurogenesis/gliogenesis, and then attenuated the likelihood of developing SRS. The inhibition of precursor cells by celecoxib was further demonstrated in celecoxib-treated NSCs culture. This suggests that the activation of COX-2 may have a central role in the genesis of epilepsy, as well in the pathways targeted by new anti-epileptogenic drugs.

COX-2 is expressed constitutively in discrete populations of neurons (Seibert et al., 1994) and can also be induced in a variety of cells such as migratory immune cells and glia by glutamate or proinflammatory stimuli (Bazan et al., 1994; Sandhya et al., 1998; Hirst et al., 1999; Manev et al., 2000; Hurley et al., 2002; Desjardins et al., 2003). It has been suggested that COX-2 may play a role in epilepsy. COX-2 expression was induced in hippocampus after kindling, kainate-induced seizures and pilocarpine-induced seizures and also in the genetically susceptible El mice (Chen et al., 1995; Sandhya et al., 1998; Hirst et al., 1999; Okada et al., 2001; Voutsinos-Porche et al., 2004). Furthermore, the induction of astrocytic COX-2 was observed in epilepsy patients with hippocampal sclerosis, and the concentrations of prostaglandins (PGs) increased in the cerebrospinal fluid of these patients (Desjardins et al., 2003). Our findings confirm the previous observations that the COX-2 expression in the rat brain is increased markedly following SE.

The increase in COX-2 activity contributes to neurodegeneration by glutamate excitotoxicity, oxidative stress, or the neurotoxic actions of PGs such as PGEs (Bezzi et al., 1998). The drugs that inhibit COX-2 activity, such as indomethacin and selective COX-2 inhibitors could reduce hippocampal cell death and seizure frequencies in several animal models of epilepsy (Baran et al., 1994; Paolelli et al., 1998; Kunz and Oliw, 2001; Okada et al., 2001). However, NMDA antagonist, dizocilpine which led to the neuroprotection in the limbic areas after the epileptic insult was not
effectiveness for preventing the development of SRS (Brandt et al., 2003). It is therefore, postulated that other factors, i.e., inhibition of altered neurogenesis and gliogenesis, than neuroprotection are implicated in anti-epileptogenic effects of celecoxib.

The effect of COX inhibitors in animal models of epilepsy has been contradictory. The previous studies with nonselective COX inhibitors showed mixed results with anticonvulsant (Wallenstein and Mauss, 1984; Paoletti et al., 1998) or proconvulsant effects (Seregi et al., 1984). COX-2 inhibitor, SC58125, attenuated the and Mauss, 1984; Paoletti et al., 1998) or proconvulsant effects (Seregi et al., 1984). COX-2 inhibitor, SC58125, attenuated the seizure-induced increase of the major COX-2 product, PGE2, and improved neuronal survival in the hippocampus of rats with kainate-induced seizures (Kawaguchi et al., 2005). Rofecoxib was found to reduce seizure frequencies and hippocampal cell death in kainate-induced epilepsy model (Kunz and Oliw, 2001), and celecoxib improved functional outcome (as measured by Morris Water Maze performance) following kainate administration (Gobbo and O’Mara, 2004). In contrast, NS-398 increased neuronal injury and mortality in mice, resulting in a paradoxical increase in PGE2 (Baik et al., 1999). The comparison and interpretation of these studies are complicated by differences in the selectivity of the study drugs as well as differences in the treatment protocols and outcome measures. In this study, we attempted to treat the epileptic rats by selective COX-2 inhibitor, celecoxib, throughout the latent period in the lithium-pilocarpine-induced SE model and to investigate various parameters such as hippocampal neurogenesis, astroglialosis, microglia infiltration, neuronal death, and development of SRS occurring during or following that period.

Prolonged seizure can induce the endogenous cell proliferation in the adult rodent hippocampus (Parent et al., 1997; Scharfman et al., 2000; Dashtipour et al., 2001; Ekdahl et al., 2003). Newly generated cells from the dentate gyrus after prolonged seizure differentiate into basket cells in granule cell layer (Hattiangady et al., 2004) or migrate abnormally into the ectopic location, the hilus, differentiating to granule cell-like neurons (Parent et al., 1997; Scharfman et al., 2000; Parent, 2002; Jung et al., 2004; Parent et al., 2006). These ectopic cells have three times more total synapses on their somata than mature granule cells in the GCL, but they lack the inhibitory synapses on the somata and proximal dendrites (Traub et al., 1999; Dashtipour et al., 2001). The ectopic hilar cells can discharge synchronously with spontaneous epileptiform bursts of CA3 pyramidal cells (Scharfman et al., 2000; Scharfman et al., 2001). On the other hand, astroglialosis is another prominent feature of the epileptic brain (Rothstein et al., 1996; Tashiro et al., 2002). While prolonged seizure accelerates the proliferation of neuronal-lineage restricted precursors in the SGL (Hattiangady et al., 2004), glial-lineage restricted precursors increase in the caudal SVZ. Animal studies have shown that kindled seizures upregulate the expression of GFAP (Hansen et al., 1990; Torre et al., 1993) and cause glial cell hypertrophy and proliferation (Khurgel et al., 1992). The application of Lo-AA toxin to suppress astrocytes selectively in fully kindled rat brains could attenuate behavioral, physiological, and anatomical responses to further stimulation (Vessal et al., 2005). In our previous study (Jung et al., 2004), the suppression of these new neurons and glia by cytosine-b-D-arabinofuranoside infusion led to the attenuation of SRS frequency, suggesting the epileptogenic role of the altered neurogenesis or astroglialosis. The present findings extend theses previous observations to therapeutic trial to correct the upstream pathway of altered cell generation.

As the generation of new neurons within the hippocampus is instructed by local signaling, the alterations in the microenvironment of stem cell niches may cause ectopic neurogenesis or even block essential neurogenesis leading to deficits in learning and memory (Monje et al., 2003; Ekdahl et al., 2003; Hattiangady et al., 2004). The hallmark of neuroinflammation is the activation of microglial cells. Several recent studies have shown that neural precursor cells preferentially migrate to sites of inflammation in animal models of multiple sclerosis and more acute injury (such as ischemia and intracerebral hemorrhage), and that these new cells differentiate into oligodendrocytes or neurons (Picard-Riera et al., 2002; Chu et al., 2003, 2004a,b,c; Jeong et al., 2003, 2005). A gradient of microglial cells could induce the migration of precursor cells, and soluble factors from microglia affect the differentiation of precursor cells towards neuronal lineage (Aarum et al., 2003; Imitola et al., 2004). We speculate that the suppression of ectopic hilar neurogenesis is partially due to a decreased gradient of microglial inflammation and proinflammatory cytokines between the hilus and SGL.

COX-2 is an important modulator inducing proliferation of progenitor cells (Sasaki et al., 2003). PGE2 receptor, EP3 is highly expressed in the dentate gyrus (Nakamura et al., 2000), and PGE2, one of the major products of COX, is known to regulate the proliferation and growth of astrocytes (Konger et al., 1998; Hirst et al., 1999). In addition, it has been demonstrated that COX-2 activity is increased in human cancers and that the COX-2-generated PGs promote tumor cell proliferation, survival, and angiogenesis (McGinty et al., 2000; Nzeako et al., 2002). The COX-2 inhibitors are known to show anti-tumor activity through COX-2 enzyme inhibition and COX-independent apoptosis induction, i.e., facilitating the dephosphorylation of Akt and ERK2 (Lim et al., 1999). In order to examine the direct effect of celecoxib on precursor cells, we employed MTT assay and BrdU labeling in human NSCs cultures. Celecoxib decreased the NSCs number and the incorporation of BrdU into the cells. The suppression of ectopic neurons and astroglialosis by celecoxib might not only result from an indirect effect via correction of inflammatory environment but from a direct effect on progenitor cells.

Collectively, our data suggest that COX-2 inhibition may prevent the epileptogenesis. Additional studies delineating the molecular mechanisms by which COX-2 and its inhibitors modulate the epileptogenesis will likely provide important future therapeutic implications.

Acknowledgments

This study was supported by a Korean Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund, KRF-2005-E00182).

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