Little is known about the effect of epigallocatechin-3 gallate (EGCG), a major constituent of green tea, on the expression of cyclooxygenase (COX)-2. Here, we studied the role of phospholipase D (PLD) isozymes in EGCG-induced COX-2 expression. Stimulation of human astrocytoma cells (U87) with EGCG induced formation of phosphatidylbutanol, a specific product of PLD activity, and synthesis of COX-2 protein and its product, prostaglandin E₂ (PGE₂). Pretreatment of cells with 1-butanol, but not 3-butanol, suppressed EGCG-induced COX-2 expression and PGE₂ synthesis. Furthermore, evidence that PLD was involved in EGCG-induced COX-2 expression was provided by the observations that COX-2 expression was stimulated by overexpression of PLD1 or PLD2 isoforms and treatment with phosphatidic acid (PA), and that prevention of PA dephosphorylation by 1-propranolol significantly potentiated COX-2 expression induced by EGCG. EGCG induced activation of p38 mitogen-activated protein kinase (p38 MAPK), and specific inhibition of p38 MAPK dramatically abolished EGCG-induced PLD activation, COX-2 expression, and PGE₂ formation. Moreover, protein kinase C (PKC) inhibition suppressed EGCG-induced p38 MAPK activation, COX-2 expression, and PGE₂ accumulation. The same pathways as those obtained in the astrocytoma cells were active in primary rat astrocytes, suggesting the relevance of the findings. Collectively, our results demonstrate for the first time that PLD isoforms mediate EGCG-induced COX-2 expression through PKC and p38 in immortalized astroglial line and normal astrocyte cells.

Cyclooxygenase (COX) is the key enzyme in the metabolic pathway leading to prostaglandin (PG) and thromboxane A2 formation from arachidonic acid (1). Two isoforms have been identified, COX-1 and COX-2 (2). COX-1 is constitutively expressed in nearly all normal mammalian tissues and mediates the synthesis of PGs required for physiological tissue homeostasis. In contrast, COX-2 expression is rapidly induced in response to various stimuli, including inflammatory signals, mitogens, cytokines, and growth factors in a wide variety of cells such as macrophages, microglia, and astrocytes (3–5). COX-2 has also been in normal brain, in discrete populations of neurons (6, 7). The function of basal prostanooid production in brain is unclear. As COX-2 expression appears to be up-regulated by physiological synaptic activity, it has been suggested that normal COX-2 expression in neurons may be related to regulation of the sleep/wake cycle, hormone release, and neuronal signaling (6, 8). The expression of COX-1 and COX-2 has been reported to be associated with complex changes observed during a variety of diseases of the brain. Prostanoids are considered important mediators for various brain functions and have recently been implicated in the pathogenesis of cerebral ischemia, seizures, and other neural injuries (6, 10–13). The regulation and function of COX-2 and prostaglandin synthesis in the central nervous system are not completely understood.

Polyphenolic compounds in green tea have recently received increasing attention as preventive agents against hippocampal neuronal damage following transient global ischemia, cardiovascular disease and cancer (14–17). Green tea polyphenols, which comprise 30% of the dry weight of green tea leaves, include epigallocatechin-3-gallate (EGGC), epigallocatechin, epicatechin-3-gallate, and epicatechin. EGC is the most abundant of these catechins and it has been attributed many healthful benefits.

However, a recent study demonstrates that EGCG up-regulates COX-2 expression and prostaglandin E₂ (PGE₂) production in Raw 264.7 macrophage cells (18), suggesting that EGC may enhance inflammatory processes. However, the downstream effectors linking EGC stimulation with COX-2 expression and PG production remains unidentified, although the effects of EGC on COX-2 expression and PG production are well documented. PLD, PLD1 and PLD2, have been cloned and characterized for regulation and cellular function (20). However, segregated roles of the two PLD isoforms in cellular responses are still poorly understood. Activation of PLD occurs through interactions of the ARF and Rho families as well as with protein kinase C (PKC) (20). The relative contribution of these factors to PLD activation is highly dependent on the cell type and signaling model examined. Several lines of evidence
have suggested a functional role for PLD in COX-2 regulation during cell activation (21, 22). However, the role of PLD isoforms in EGCG-induced COX-2 expression has not been studied in any biological system. We therefore investigated a role of PLD in the regulation of COX-2 expression in glial cells treated with EGCG. We selected the normal astrocyte cell and immortalized astroglial cell line (U87) for several reasons. 1) Astrocytes are the major cell population in the nervous system (23), and reports using astrocytes are lacking. 2) PG and lipid metabolites formed by astrocytes may contribute to central nervous system physiology and pathology (24, 25). In this study, we demonstrate that EGCG activates PLD through an upstream protein kinase C to elicit p38 activation and finally induce COX-2 expression in normal rat astrocyte cells and glioma cells. This is the first study to show the involvement of PLD isoforms in mediating EGCG-induced COX-2 expression in any biological system.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum, and LipofectAMINE were purchased from Invitrogen. Phospho-ERK1/2, ERK, phospho-p38, and p38 antibodies were from Cell Signaling. Rabbit polyclonal COX-2 antibody and goat anti-actin antibody were from Santa Cruz Biotechnology. A polyclonal antibody that recognizes both PLD1 and PLD2 was generated as previously described (26). Phosphatidylbutanol (PtdBut) standard was from Avanti Polar Lipid. 1-propanol, 1-butanol, diacetyl PA, and anti-β-tubulin antibody were from Sigma, and PD98059, SB203580, and G6976 were from Biomol (Plymouth Meeting, PA). [3H]Myristate was obtained from PerkinElmer Life Sciences. Silica gel 60 Å thin layer chromatography plates were from Whatman. Horseradish peroxidase-conjugated anti-mouse IgG and anti-rabbit IgG were from Kirkegaard & Perry Laboratories (Gaithersburg, MD). Enhanced chemiluminescence (ECL) reagents and the PGE2 enzyme immunoassay kit were from Amersham Biosciences.

Cell Culture and Transfection—U87 MG human astroglia was obtained from the American Type Culture Collection. Cells were maintained in DMEM supplemented with 10% (v/v) fetal bovine serum under 5% CO2. U87 MG cells were transiently transfected for 40 h with expression plasmid encoding empty vector or a catalytically inactive mutant of p38 MAPK (T180A, Y182F), using LipofectAMINE according to the manufacturer’s instructions. U87 cells stably expressing PLD isoform were obtained by transfection, using LipofectAMINE. Transfected cells were selected with G418 (700 μg/ml) for 6–8 weeks. Western Blot—Cells were harvested with 0.1% Triton X-100, 1% sodium deoxycholate, 0.2% SDS, 200 mM NaCl, 1 mM Na2VO3, 1 mM NaF, 10% glycerol, 10 μg/ml leupeptin, 10 μg/ml protamin, 1 mg phosphomethylsulfon fluoride). The resulting cell lysates were spun at 15,000 × g in an Eppendorf microcentrifuge for 10 min at 4 °C to pellet the unbroken cells. Protein concentrations were determined using Bradford method with bovine serum albumin as a standard (4). Protein samples were analyzed by SDS-polyacrylamide gel electrophoresis on 8% gels and were transferred to a nitrocellulose membrane. The blots were then blocked with 5% nonfat milk in Tris-buffered saline-Tween 20 (25 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20) and incubated with appropriate primary antibodies followed by incubation with horseradish peroxidase-conjugated secondary anti-body. Immunoreactive bands were detected using enhanced chemiluminescence, resuspended in sample buffer.

PGE2, Production Assay—PGE2 levels were determined using an enzyme immunoassay kit according to the manufacturer’s instructions. Briefly, 50 μl of standard or sample was pipetted into the wells of a 96-well plate. Aliquots of mouse polyclonal PGE2 antibody and PGE2 conjugated to alkaline phosphatase were then added to each well, and the plate was incubated at room temperature for 1 h. After incubation, the wells were washed six times with 200 μl of PBS containing 0.05% Tween 20, and the TMB substrate was added. Wells were read at 670 nm with an enzyme-linked immunosorbent assay reader 30 min after adding substrate.

Reverse Transcription-Polymerase Chain Reaction—Total RNA was isolated using RNAzol B (TEL-TEST, Inc. Friendwood, TX), and cDNA was prepared using reverse transcriptase that originated from Avian Myeloblastosis Virus (Takara, Japan) according to the manufacturer's instructions. PCR was performed with 20 cycles of sequential reactions as follows: 94 °C for 30 s, and 55 °C for 30 s, and 72 °C for 30 s. Oligonucleotide primers were obtained from Bioneer (Seoul, Korea). The sequence of PCR primers are as follows: The PCR primers for the COX-2 gene were 5′-ACACTCTATCTAGCATCC-3′ (sense primer) and 5′-GAAGGAGACCTTCTCATAC (antisense primer). 5′-AGATCC- ACAACGATACATT-3′ and (forward) 5′-TCTCTCAAGATTGTGCAC-GAA-3′ for glyceraldehyde-3-phosphate dehydrogenase. PCR products were separated by electrophoresis in a 1.5% agarose gel and detected under UV light.

RESULTS

EGCG Induces COX-2 Expression and PGE2 Production—We investigated whether EGCG, a major compound of green tea, is involved in the signal transduction pathways leading to COX-2 expression and PGE2 production in U87 MG human astroglia cells. Treatment of the cells with EGCG resulted in significantly increased levels of COX-2 expression in a dose- and time-dependent manner (Fig. 1A). The increase in COX-2 expression was apparent 12 h after 50 μM EGCG treatment, reaching a maximal level at 100 μM EGCG. Furthermore, the induction of COX-2 appeared in a time-dependent manner, and 100 μM EGCG treatment showed an increase in COX-2 protein within 6 h, which peaked at 18 h and then sustained up to 24 h after treatment. Because COX-2 catalyzes biosynthesis of PGs, we examined whether this enzyme was responsible for EGCG-induced PGE2 production in the culture media of cells stimulated with EGCG. As shown in Fig. 1B, COX-2 protein expression induced by EGCG was accompanied by an increase in PGE2 accumulation in a dose-dependent manner. The results indicate that EGCG can lead to COX-2 protein expression and subsequently PGE2 biosynthesis in U87 MG human astroglia cells.

PLD Mediates EGCG-induced COX-2 Expression—We next investigated how EGCG-induced COX-2 protein expression is regulated during glioma cell activation. There is also evidence that a PLD-derived signaling pathway is involved in the generation of PGs in several cell types (21, 22). Therefore, to address the possible involvement of PLD activation in EGCG-induced COX-2 expression and PGE2 biosynthesis, cells prelabeled with [3H]myristate were stimulated with 100 μM EGCG for various times, and PLD activity was measured by the formation of [3H]PtdBut from 1-butanol, a product specific to PLD activity. As shown in Fig. 2, EGCG induced PtdBut formation, in a time-dependent manner. Although the experiments showed that PLD was activated in EGCG-treated human astrocytes, the specific role of PLD in COX-2 regulation remains to be clarified.

PLD activity was measured by the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29). PLD activity was assessed by measuring the formation of [3H]phosphatidylbutanol (PtdBut), the product of PLD described previously (29).
oma cells, they provided no direct evidence that PLD was involved in the induction of COX-2 expression. A role for PLD in the pathway leading to COX-2 expression received further support when 1-butanol was used to block PA production by PLD, by virtue of the formation of phosphatidylbutanol through the transphosphatidylation reaction. U87 MG cells were stimulated with EGCG in the presence of 1% 1-butanol or 3-butanol, and then release of PGE2 was measured from supernatants as described in “Experimental Procedures.” The values shown for PGE2 production are the mean ± S.E. of three independent experiments.

PKC Is Involved in the COX-2 Protein Expression and PGE2 Formation Induced by EGCG—It has been suggested that PKC is an important regulator of PLD. Moreover, PKC is known to regulate COX-2 expression and PGE2 production by various agonists (19, 30). Using Western blot analysis and confocal immunofluorescence microscopy, we have found that EGCG induces translocation of PKC-α from the cytosol to the membrane (data not shown), which is well documented as a measure of PKC activation. Therefore, we investigated whether EGCG-induced PLD activation and COX-2 expression is regulated by PKC. As shown in Fig. 5, Ca2+-dependent PKC-specific inhibitor, Go6976, significantly inhibited not only EGCG-induced PLD activation but also EGCG-induced COX-2 expression, and subsequently PGE2 biosynthesis, in a dose-dependent manner, suggesting that PKC might be involved in EGCG-induced PLD activation, COX-2 expression, and PGE2 formation.

p38 Mediates EGCG-induced PLD Activation, COX-2 Expression, and PGE2 Synthesis—It has been reported that COX-2 hydrolase (PAP) inhibitor. Fig. 3B shows that propranolol potentiated EGCG-induced COX-2 expression in a dose-dependent manner. To further establish that PA was involved in the induction of COX-2 expression, cells were treated with various concentrations of dioctanoyl PA. Fig. 4 shows that PA significantly induced COX-2 expression in a dose- and time-dependent manner. These results demonstrate that PLD activity and the intracellular accumulation of PA are importantly involved in EGCG-induced COX-2 expression in U87 MG human astroglia cells.
expression can be regulated by p38 MAPK or p42/44 MAPK (31, 32). Therefore, we evaluated the ability of p38 and p42/44 inhibition to block COX-2 expression and PGE2 production in astroglia cells. The p38 MAPK-specific inhibitor, SB203580, but not the ERK upstream inhibitor PD98059, dramatically suppressed EGCG-induced COX-2 expression and PGE2 biosynthesis (Fig. 6, A and B), suggesting that the p38 kinase signaling pathway may be involved in the EGCG-induced COX-2 expression and PGE2 production. In addition, we found that SB203580, an inhibitor specific for p38, caused a dose-dependent decrease in EGCG-stimulated PLD activity (Fig. 7A). Moreover, expression of a catalytically inactive mutant of p38 MAPK (T180A/Y182F) significantly attenuated EGCG-induced PLD activation in U87 MG cells (Fig. 7B), suggesting that p38 is involved in EGCG-induced PLD activation.

EGCG Induces Activation of p38 MAPK via PKC—We next determined whether EGCG activates ERK and p38 MAPK in human astroglia cells. As shown in Fig. 6C, the phosphorylation of ERK by EGCG peaked at 10 min and decreased thereafter. EGCG stimulation significantly induced activation of p38 MAPK. The phosphorylation of p38 MAPK induced by EGCG peaked at 30 min and then declined. Reproducing the immunoblot with anti-ERK or anti-p38 MAPK antibodies showed the equal loading of proteins in each lane. To examine whether p38-mediated COX-2 expression is exerted via PKC, we stimulated the cells with EGCG in the presence of PKC inhibitors and determined the change of phosphorylation of p38. As shown in Fig. 8, pretreatment with the PKC inhibitor Go6976 suppressed EGCG-induced p38 phosphorylation, suggesting that EGCG induces activation of p38 via PKC.

Both PLD1 and PLD2 Mediate EGCG-induced COX-2 Expression via PKC and p38 MAPK Pathway—To investigate which isozyme of PLD is involved in EGCG-induced COX-2 expression, we generated U87 MG cells stably overexpressing vector, PLD1, or PLD2 (Fig. 9A). In unstimulated cells, overexpression of PLD1 or PLD2 led to higher basal expression of COX-2 protein (Fig. 9B). In EGCG-stimulated cells, overexpression of PLD 1 or 2 dramatically increased COX-2 expression, compared with that of control cells (Fig. 9B), suggesting that overexpression of PLD1 or PLD2 significantly enhances EGCG-induced COX-2 expression. In addition, inhibitors of PKC and p38 MAPK abolished EGCG-induced COX-2 expression in cells overexpressing PLD1 and PLD2 (Fig. 9C). COX-2 expression in PLD1-expressing cells (U87-PLD1) was more sensitive to PKC inhibition than that in PLD2-expressing cells (U87-PLD2). COX-2 expression in both PLD1- and PLD2-expressing cells showed similar response to inhibitor of p38 MAPK. These results suggest that both PLD1 and PLD2 isozymes mediate EGCG-induced COX-2 expression via same pathway, PKC and p38.

PLD Also Mediates EGCG-induced COX-2 Expression through PKC and p38 in Primary Astrocyte Cells—U87 cells are transformed glial cells. The relevance of the findings are questionable in that experiments were done in an immortalized astroglial line that may or may not mimic the in vivo situation. Therefore, we tried to ascertain whether treatment with EGCG stimulates COX-2 expression and the same pathways as those obtained in the glioma cells are active in primary astroglial cell cultures. Rat primary astrocytes and microglial cells were stimulated with EGCG or PA for the indicated times, and total RNA was extracted for reverse transcriptase-PCR analysis. As shown in Fig. 10, A and B, addition of EGCG rapidly increased the mRNA levels of COX-2 in both primary astrocytes and microglia. Moreover, treatment with PA significantly induced transcription of the COX-2 gene in these cells. Treatment of the primary rat astrocytes with EGCG significantly resulted in increased levels of COX-2 expression in a dose- and time-de-
pendent manner (Fig. 10 C). These findings demonstrate that EGCG induces the COX-2 in normal glial cells. We next investigated how EGCG-induced COX-2 protein expression is regulated during normal astroglial cell activation. Pretreatment of normal glial cells with 1% 1-butanol caused great inhibition of EGCG-induced COX-2 expression, but the same concentration of 3-butanol, an inactive analogue for PLD-mediated PA formation, had no significant effect (Fig. 11 A). Pretreatment with pranolol, an inhibitor of PA phosphatase, also stimulated EGCG-induced COX-2 expression (Fig. 11 B). These data indicate that EGCG induces COX-2 expression by PLD-mediated pathway in primary astroglial cells. In addition, EGCG stimulated PLD activity and pretreatment with p38 or PKC-specific inhibitor suppressed both EGCG-induced PLD activation and COX-2 expression in normal astrocyte cells.

DISCUSSION

Green tea is the most popular and widely consumed beverage in the world, after water, and is reported to possess beneficial health effects for humans (33). These beneficial health effects have attracted considerable diseases, including cancer and cardiovascular and neurodegenerative diseases (34–36). Studies
have verified that polyphenols in green tea are potent antioxidants, with majority of beneficial effects elicited by EGCG, one of the main constituents of green tea. However, the present study demonstrates that EGCG up-regulates COX-2 expression and PGE2 production in astroglioma cells.

The major finding of this study is that PLD is a vital component of the signal transduction pathway induced by EGCG that leads to the expression of COX-2 in human astroglioma cells. To the best of our knowledge, this is the first study to link PLD isozymes to EGCG-induced COX-2 expression in any cell system.

Some reports have described an inhibitory effect of EGCG on COX-2 up-regulation induced by agonists such as PMA, N-nitrosomethylbenzylamine, or interleukin-1β (IL-1β) (37–39). However, in human chondrocytes, treatment of EGCG alone showed a moderate increase in COX-2 expression (~4-fold relative to control) and COX-2 activity (PGE2 formation) when compared with the untreated control (39). In addition, Park et al. (18) have reported that in the macrophage cell line, Raw 264.7, COX-2 expression, and PGE2 production are increased by EGCG treatment. Therefore, it seems that the effects of EGCG on COX-2 expression and PGE2 production still remain uncertain.

Little is known about how COX-2 protein levels are regulated in glial cells, the PLDs responsible for such a regulation, and the molecular mechanisms involved. We have studied signal transduction pathways involved in EGCG-induced COX-2 expression in U87 MG human astroglialoma cells. EGCG stimulated COX-2 protein expression and PGE2 production as well as PLD activity.

Some studies have implicated a PLD-derived signaling pathway in the generation of prostaglandins in many cell types (19). However, PLD-independent signaling pathways must exist for the production of prostaglandins because EGF, which has been shown to induce COX-2, was demonstrated not to activate PLD in the studies by Sciorra and Daniel (21). Kaneki et al. (40) showed that PMA-induced COX-2 expression in osteoblast-like UMR-106 cells was dependent upon PLD activity. As a control for nonspecific effects of butanol, the secondary and tertiary forms of butanol have often been employed. The evidence identifying PLD activity as important in functional events relies on
the use of alcohols. Importantly, inhibition of PA formation through the addition of the primary alcohol 1-butanol blocked EGCG-induced COX-2 expression, whereas 3-butanol, which does not participate in transphosphatidylidation, did not. Furthermore, direct addition of PA to the cells was very effective in stimulating COX-2 expression. Interestingly, propranolol, an inhibitor of PA phosphatase, strongly potentiated EGCG-induced COX-2 expression. These results demonstrate that PLD activity and the intracellular accumulation of PA are importantly involved in EGCG-induced COX-2 expression.

It is well known that PKC acts as a mediator of a broad spectrum of effects, including the activation of PLD (19). Activation of PKC has been suggested to be a key event in the signaling pathway leading to COX-2 expression (19, 30, 31). In the present study, we found that EGCG-induced PLD activation and COX-2 expression were reduced by a PKC inhibitor, indicating that PKC activation is involved in the signal transduction leading to PLD activation and COX-2 expression by EGCG.

Recently, it has been reported that COX-2 expression can be regulated through different MAP kinase signaling pathways and that the particular signaling pathway involved is dependent on the type of stimuli (41, 42). In U87 astroglial cells, EGCG induced phosphorylation of ERK and p38 MAP kinase. The observed increase in phosphorylated ERK levels by EGCG was transient with peak enhancement of phosphorylation being evident at 10 min post-stimulation. By contrast, p38 MAPK activity and the intracellular accumulation of PA are importantly involved in EGCG-induced COX-2 expression.

Our results suggest that EGCG may act on two pathways to enhance COX-2 synthesis in U87 cells, i.e. via activation of PKC and p38 MAPK. Very recently, Kim et al. (43) demonstrated that sphingosine-1-phosphate in amniotic fluid modulated COX-2 expression via ERK, but not p38 kinase in human amnion-derived WISH cells. In addition, S1P-induced COX-2 expression was not affected in the presence of 1-butanol. It was reported that induction of COX-2 by interleukin-1 is mediated by both ERK and p38 kinase in human astrocytes (30). These results suggest that the intracellular signaling pathways involved in the modulation of COX-2 by sphingosine-1-phosphate or interleukin-1 differ from those involved in the modulation of COX-2 by EGCG. Fiebich et al. (30) demonstrated a similar role for PKC and p38 MAPK activation of COX-2 in SH-N-SK human neuroblastoma cells.

However, there is no direct evidence to date that indicates direct regulation of COX-2 expression by PLD protein levels. Our current observation that overexpression of PLD1 or PLD2, caused by ectopic expression in cells, leads to increased expression of COX-2, clearly indicates a positive role for PLD isoforms in COX-2 expression in human U87 MG astroglial cells. To the best of knowledge, this is the first direct indication that suggests regulation of COX-2 expression by PLD protein levels. We and other groups (44–46) have reported the link between p38 and PLD activity. The idea that both PLD1 and PLD2 can couple to regulation of COX-2 expression by EGCG is an interesting one that might fit with the findings that both PKC and p38 are involved. COX-2 expression in PLD1 express-
ing cells was more sensitive to PKC inhibition than that in PLD2-expressing cells. It might be because of more responsiveness of PLD1 to PKC. We further used cultured primary rat astroglial and microglial cells to corroborate our findings. EGCG induced COX-2 gene expression in primary rat astrocyte and microglial cells, in line with the increase observed in human astrogliaoma cells. Moreover, we found that PLD mediated EGCG-induced COX-2 expression through PKC and p38 in normal astrocyte cells. Thus, the same pathways as those obtained in the astrocytoma cells were active in primary rat astrocytes, suggesting the relevance of the findings.

COX-2 expression is regulated in not only a cell type-specific manner but also a species-specific manner. Induction of COX-2 expression may represent a novel mechanism by which EGCG mediates its diverse actions within the central nervous system. The conclusion that EGCG mediates COX-2 expression may seem surprising or even paradoxical, because the actions of EGCG and COX-2 are generally thought to be beneficial (14–17) and detrimental, respectively (47, 48). Specifically, induction of COX-2 is believed to play a role in inflammation, toxic shock, cancer, and apoptosis (52–57). However, there is evidence suggesting physiologically important or salutary actions of COX-2 in other situations (42, 51–56). For example, COX-2 protects cardiomyocytes against oxidative stress (40), and exerts anti-apoptotic actions in various cell types (51–53). The finding that genetic disruption of COX-2 results in cardiac fibrosis (57) also suggests that COX-2 expression may be protective. Interestingly, the clinical experience accumulated with COX-2 inhibitors suggests that COX-2 exerts protective effects in patients with cardiovascular disease (58). A concept is emerging that COX-2 induction represents an important compensatory mechanism to defend against vascular injury (59). Furthermore, it is now recognized that COX-2 is constitutively expressed in the kidney and brain, and plays an important role in maintaining renal function and in modulating neural responses (58). Recently, it was proposed that the pathophysiological roles of COX-2 are much more complex than hitherto appreciated, and that this enzyme may exert either beneficial or deleterious effects depending on the intensity of its induction, the pathophysiological setting, and the ability of specific cells to metabolize PGH₂ produced by COX-2, into cytoprotective prostanooids (55). The pathological role that COX-2 plays may depend on a number of factors, among which the cell types and their inherent prostanooid synthetic pathways appear to be the key determinants. It is suggested that throughout adult life, COX-2 might remain an important modulator of specific neural responses (53). EGCG has been demonstrated to pass the blood-brain barrier and reach the brain parenchyma in animal studies, and detection of EGCG in rat brain suggests polyphenols can modulate neuronal activity (43). COX-2 also seems to play an essential role in neural development and adaptation (53). At present, the role of COX-2 in human brain function and the potential impact of specific COX-2 inhibitors are unknown and requires evaluation, especially in view of the well known negative impact of nonspecific COX inhibitors on cognitive function in the elderly (53). To the best of our knowledge, this is the first report to show that EGCG might activate PLD through an upstream protein kinase C to elicit p38 activation and finally induce COX-2 expression.

In summary, the involvement of PLD1 and PLD2 isoforms in EGCG-induced COX-2 expression has been explored. Since this study is the only report upon the potential role of EGCG in glial cells, further studies on the physiological roles of EGCG are required and necessary to determine overall signal transduction pathways that are associated with EGCG-induced COX-2 regulation.

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Role of PLD in EGCG-induced COX-2 Expression

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Phospholipase D Isozymes Mediate Epigallocatechin Gallate-induced Cyclooxygenase-2 Expression in Astrocyte Cells
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