Cyclooxygenase (COX)-2 and COX-1 Potentiate β-Amyloid Peptide Generation through Mechanisms That Involve γ-Secretase Activity*

Weiping Qin, Lap Ho, Patrick N. Pompl, Yuanenzhen Peng, Zhong Zhao, Zhongmin Xiang, Nikolaos K. Robakis‡, Junichi Shioi, Jason Suh, and Giulio Maria Pasinetti§

From the Neuroinflammation Research Laboratories, ‡Department of Psychiatry, Mount Sinai School of Medicine, New York, New York 10029

Received for publication, July 16, 2003, and in revised form, September 23, 2003 Published, JBC Papers in Press, September 24, 2003, DOI 10.1074/jbc.M307699200

In previous studies we found that overexpression of the inducible form of cyclooxygenase, COX-2, in the brain exacerbated β-amyloid (Aβ) neuropathology in a transgenic mouse model of Alzheimer’s disease. To explore the mechanism through which COX may influence Aβ amyloidosis, we used an adeno viral gene transfer system to study the effects of human (h)COX-1 and hCOX-2 isoform expression on Aβ peptide generation. We found that expression of hCOXs in human amyloid precursor protein (APP)-overexpressing (Chinese hamster ovary (CHO)-APPswe) cells or human neuroglioma (H4-APPsw) cells resulting in 10–25 nM prostaglandin (PG)-E2 concentration in the conditioned medium coincided with an 1.8-fold elevation of Aβ-(1–40) and Aβ-(1–42) peptide generation and an 1.8-fold induction of the C-terminal fragment (CTF-γ) cleavage product of the APP, an index of γ-secretase activity. Treatment of APP-overexpressing cells with the non-selective COX inhibitor ibuprofen (1 μM, 48 h) or with the specific γ-secretase inhibitor L-685,458 significantly attenuated hCOX-1- and hCOX-2-mediated induction of Aβ peptide generation and CTF-γ cleavage product formation. Based on this evidence, we next tested the hypothesis that COX expression might promote Aβ peptide generation via a PG-E2-mediated mechanism. We found that exposure of CHO-APPswe or human embryonic kidney (HEK-APPsw) cells to PG-E2 (11-deoxy-PG-E2) at a concentration (10 nM) within the range of PG-E2 found in hCOX-expressing cells similarly promoted (1.8-fold) the generation of the CTF-γ cleavage product of APP and commensurate Aβ-(1–40) and Aβ-(1–42) peptide elevation. The study suggests that expression of COXs may influence Aβ peptide generation through mechanisms that involve PG-E2-mediated potentiation of γ-secretase activity, further supporting a role for COX-2 and COX-1 in Alzheimer’s disease neuropathology.

A large number of epidemiological studies have indicated that the use of non-steroidal anti-inflammatory drugs (NSAIDs) may prevent or delay the clinical features of Alzheimer’s disease (AD) (1–6). However, recent therapeutic studies with both NSAIDs (7–9) and steroids (10) have been unable to confirm this epidemiological evidence. The pharmacological activity of NSAIDs is generally attributed to the inhibition of COXs, which are rate-limiting enzymes necessary for the production of prostaglandins (PGs). Both COX-1 and COX-2, the constitutive and inducible forms of COX, respectively (11–13), are known to be involved in inflammatory responses and normal neuronal functions (14–16).

We (17–19) and others (15, 20–24) have shown that COX-2 expression in the brain and PG-E2 content in the cerebrospinal fluid (24) are elevated in AD and further that COX-2 protein content in the brain correlates with the severity of amyloidosis and clinical dementia (19). Moreover there is evidence that COX-1 expression is also elevated in the AD brain, raising the possibility that both COX-1 and COX-2 may contribute to AD neuropathology (21, 24, 25). Thus, the characterization of COX activities and subsequent PG generation in the brain as well as their potential roles in amyloidosis is receiving a great deal of attention.

Further studies implicating COX in neuronal dysfunction in vivo include work byAndreasson et al. (26), demonstrating that COX-2-overexpressing transgenic mice developed memory dysfunction, neuronal apoptosis, and astrocytic activation in an age-dependent manner. Moreover a recent study has shown that overexpression of human (h)COX-2 in neurons of PSAPP transgenic mice (a transgenic mouse model of AD expressing both mutant amyloid precursor protein (APPsw) and mutant presenilin-1 (A246E-PS1)) significantly potentiated amyloidogenic Aβ peptide generation and amyloid plaque deposition in the brain (27), indicating that conditions of elevated COX expression can promote neuronal dysfunction and amyloidosis in vivo.

In this study, we continued to explore the role of COXs in AD amyloidogenesis by testing the hypothesis that COXs may directly influence Aβ peptide generation in vitro. We found that a mechanism through which COX may promote amyloidogenic generation of Aβ peptides might involve PG-E2-mediated promotion of γ-secretase activity. Understanding the apparent mechanistic relationship of COXs and Aβ generation is highly relevant to the successful development of COX inhibitors and other NSAID-based therapeutic strategies for AD.

* This work was supported by NIA, National Institutes of Health Grant AG13799 and by the Dana Foundation for Brain Research (to G. M. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Neuroinflammation Research Laboratories, Dept. of Psychiatry, Mount Sinai School of Medicine, Box 1230, One Gustave L. Levy Place, New York, NY 10029. E-mail: giulio.pasinetti@mssm.edu

§ The abbreviations used are: NSAID, non-steroidal anti-inflammatory drug; COX, cyclooxygenase; Aβ, β-amyloid; h, human; CHO, Chinese hamster ovary; PG, prostaglandin; APP, amyloid precursor protein; sAPP, soluble APP; CTF, C-terminal fragment; HEK, human embryonic kidney; m.o.i., multiplicity of infection; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MOPS, 4-morpholinepropanesulfonic acid; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)]-ethylglycine; ELISA, enzyme-linked immunosorbent assay; 11-dPG-E2, 11-deoxy-PG-E2.
EXPERIMENTAL PROCEDURES

Generation of hCOX-1 or hCOX-2 Adenoviruses—hCOX-1 and hCOX-2 cDNA constructs (described previously by our laboratories (28)) were introduced into the AdenoX™ genome introduced to the recombinant Adeno-X virus according to the Adeno-X expression system manual (Clontech). In brief, the full-length hCOX-1 cDNA was subcloned into the pShuttle vector cassette via MluI and ApaI, and the full-length hCOX-2 cDNA used for generation of hCOX-2 transgenic mice (28) was cloned into the pShuttle vector cassette via ApaI and XbaI. Both pShuttle-hCOX-1 and pShuttle/hCOX-2 were then transfected into the Adeno-X viral DNA via I-CEl and PstI-cleaved sites; the identity of the hCOX-1 or hCOX-2 Adeno-X viral DNA was confirmed by nucleotide sequencing (not shown). The recombinant viruses were then packaged by transfecting Pacl-linearized recombinant viral DNA into human embryonic kidney (HEK) 293 cells with the aid of LipofectAMINE (Invitrogen). HCOX-1 or hCOX-2 Adeno-X viral titer was determined by the tissue culture infectious dose 50 (TCID50) method (29). This identical strategy was used to generate recombinant LacZ adenovirus (Clontech) expressing the β-galactosidase gene (Clontech), which served as a negative control.

Cell Cultures and Treatments—Chinese hamster ovary (CHO) cells expressing human embryonic precursors of the β-secretase (BACE1) and γ-secretase (γ-Secretase) (HeLa-BACE1/γ-Secretase) (provided by Dr. G. Schenk) were grown in McCoy’s 5A medium supplemented with 10% fetal bovine serum, 1% streptomycin/penicillin, and 200 μg/ml G418 (Invitrogen). Human H4 neuroglioma cells with constitutive APPswe expression (H4-APPswe), a gift from Dr. B. Millan (University of Nijmegen, Nijmegen, The Netherlands), were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, 100 μg/ml penicillin, 100 μg/ml streptomycin, 200 μg/ml G418, 150 μg/ml hygromycin, and 150 μg/ml puromycin. HEK293 cells carrying human APPswe (HEK-APPswe, a gift of Dr. Brian M. Austen) were grown in Dulbecco’s modified Eagle’s medium supplemented with 1% streptomycin-penicillin (Invitrogen) and 800 μg/ml G418 (Invitrogen).

For viral infection, CHO-APPswe, or H4-APPswe cells were seeded at 4 × 10^4 cells/cm² and cultured at 37 °C in the presence of 5% CO₂. Following 48 h of incubation, cultures (50% confluence) were infected with recombinant hCOX-1, hCOX-2, or LacZ adenovirus with doses of virus defined as 10 multiplicities of infection (m.o.i./c) (29). Conditioned medium was collected 48 h postinfection for Aβ detection. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay of the cell lysate as described previously (30).

Buprofen and 11-deoxyprostaglandin E₂ (Cayman Chemical, Ann Arbor, MI) and I-685,458 (a gift from Merck Sharp and Dohme Research Laboratories, Terlingua Park, Harlow, UK) were stored at −20 °C (in Meso). Disposable aliquots of Meso (final concentration, 0.01%) were also stored at −20 °C to mimic freeze-thaw conditions in vehicle-treated cultures. All cultures and reagents were demonstrated to be free of endotoxin (<10 pg/ml) by Limulus lysate assay (Sigma) (not shown). hCOX-1, hCOX-2, and APP Immunodetection—Following adenoviral infection and/or incubation with drugs for 48 h, conditioned media were collected, and tissue cultures were lysed in RIPA buffer (1× phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 0.1 M EDTA) in the presence of a protease inhibitor mixture (Sigma) on ice and stored at −20 °C. For immunoblot analysis, protein content was determined by the Bradford method (Bio-Rad), samples were boiled and centrifuged, and proteins were resolved electrophoretically by SDS-PAGE (10%). Proteins were transferred to nitrocellulose membranes (Bio-Rad) and immunoreacted with appropriate antibody. In these studies immunoreactivities were visualized by fluorescence autoradiography using enhanced chemiluminescence detection (SuperSignal chemiluminescent detection kit, Pierce).

Polyclonal C8 antibody (raised against amino acids 676–695 of human APP, a gift from Dr. Selkoe) was used for detection of human APP was used to quantify total soluble (s)APP released into the conditioned medium of CHO-APPswe, cells. Monoclonal 6E10 antibody (Senetek, St. Louis, MO) recognizing amino acids 1–17 of the Aβ domain of APP (a site that constitutes the C terminus of APP peptide) was used to quantify the level of sAPPβ released into the conditioned medium.

For detection of hCOX-2 (or hCOX-1) expression in transfected CHO-APPswe cells, specific antibodies raised against a synthetic peptide derived from the carboxyl region of hCOX-2 or hCOX-1 sequence were used (Cayman Chemical). Specificities of hCOX-2 and hCOX-1 antibodies were reported previously by our laboratories (28), and β-actin immunoreactivity (anti-β-actin, Sigma) was used to control for variations in gel loading.

C-terminal Fragment (CTF)-γ Assay—In hCOX-2- or hCOX-1-infected cells, levels of CTF-γ cleavage product of APP were assessed from membrane preparations as described previously (31–33). In brief, cell monolayers were rinsed twice with ice-cold phosphate-buffered saline on ice, scraped from tissue culture dishes, and centrifuged (1,500 rpm, 10 min, 4 °C). Cell pellets were then resuspended (0.5 ml/10-cm dish) in homogenization buffer (10 mM MOPS, pH 7.0, 10 mM potassium chloride, 1× Complete protease inhibitor (Roche Applied Science) and homogenized by passing cell suspensions through a 23-gauge needle 10 times. Supernatents were then centrifuged (2,500 rpm, 15 min, 4 °C) to remove unbroken cells and nuclei. The supernatant (membrane and postnuclear supernatant) was then centrifuged (14,000 rpm, 20 min, 4 °C) and rinsed in homogenization buffer. Membranes were then resuspended in assay buffer (150 mM sodium citrate, pH 6.4, 1× Complete protease inhibitor) and incubated for 2 h at 37 °C in 25 μl of incubation buffer/assay sample to allow for generation of the CTF-γ cleavage product; negative control samples were maintained on ice. CTF-γ cleavage products (as well as CTF-α and CTF-β) were resolved electrophoretically in 10–20% Tris-Tricine gels (Bio-Rad) and identified using the anti-APP polyclonal C8 antibody. Immunoreactivities were visualized autoradiographically using a chemiluminescence detection kit (SuperSignal, Pierce).

Aβ Peptide Enzyme-linked Immunosorbent Assay (ELISA)—For detection of Aβ peptide generation, conditioned media from CHO-APPswe, or LacZ cells were centrifuged (3,500 × g, 10 min, 4 °C) to remove cellular debris. Aβ1–40 or Aβ1–42 was assayed by sandwich ELISA according to manufacturer’s instructions (BioSource, Camarillo, CA). PG-E2 Assays—PG-E2 content was assessed in the same conditioned medium used for Aβ peptide determinations using a commercially available ELISA (Cayman Chemical) as described previously (34). In brief, conditioned medium was applied to 96-well plates precoated with goat anti-mouse IgG and incubated (18 h at 4 °C) with PG-E2 monoclonal antibody and a recovery tracer. After incubation with PG-E2 monoclonal antibody, plates were rinsed five times with wash buffer and developed (1 h at room temperature) using Ellman’s reagent. Specific PG-E2 concentration was determined spectrophotometrically and calculated by plotting (percentage of sample or standard bound/mass bound) the protein standard versus PG-E2 concentration in standard.

Statistical Analysis—All values are expressed as means ± S.E. Differences between means were analyzed using a two-tailed Student’s t-test. In all analyses, the null hypothesis was rejected at the 0.05 level. All statistical analyses were performed using the Prism Stat program (GraphPad Software, Inc., San Diego, CA).

RESULTS

Adenovirus-mediated hCOX-1 or hCOX-2 Delivery System—In control studies the dose-dependent adenoviral mediated expression of hCOX-1 or hCOX-2 at 20, 40, and 40 m.o.i. 48 h postinfection was confirmed in CHO-APPswe and H4-APPswe, cells by Western blot analysis (Fig. 1, a and b and d and e, respectively). No apparent endogenous COX-1 or COX-2 immunoreactivity was found in LacZ adenovirus-infected CHO-APPswe or H4-APPswe, cells (Fig. 1, a and β); although endogenous expression of COX(a) in LacZ adenovirus-infected cells could also be detected at longer exposure time (not shown).

The functional expression of hCOX-1 and hCOX-2 in CHO-APPswe, (Fig. 1c) and H4-APPswe, (Fig. 1f) cells was monitored by PG-E2 generation by ELISA. We found that hCOX-1 or hCOX-2 infection at 10 m.o.i. resulted in 10–25 nm PG-E2, in the conditioned medium, which is within the physiological concentration of PG-E2 described previously in the human brain (35). Consequently a virus titer of 10 m.o.i. was selectively utilized throughout this study to assess the role of hCOXs in Aβ peptide generation. In control adenoviral LacZ infection studies (using β-galactosidase staining as an indicator of adenoviral infection) we found that 10 m.o.i. resulted in 90% efficiency of infection with no apparent cytotoxicity as assessed by MTT assay 48 h postinfection (data not shown).
hCOX-1 or hCOX-2 infection of CHO-APP_swe cells results in functional elevation of PG-E2. a, b, d, and e, gel immunoblot analysis of hCOX-1 or hCOX-2 protein expression in CHO-APP_swe (a and b) or H4-APP_751 (d and e) cells following LacZ, hCOX-1, or hCOX-2 adenoviral infection 48 h postinfection at 10, 20, and 40 m.o.i. c, f, quantification of PG-E2 content in the conditioned medium of each respective cell line as indicated 48 h following adenoviral infection (10 m.o.i.) by ELISA. Values represent means ± S.E. of determinations made in three separate culture preparations; n = 3 per culture. *, p < 0.01 versus LacZ control group.

hCOX-1 or hCOX-2 Infection of CHO-APP_swe Cells Promotes Aβ-(1–40) and Aβ-(1–42) Generation—hCOX-1 or hCOX-2 adenoviral infection (10 m.o.i.) of CHO-APP_swe (Fig. 2, a and b) or H4-APP_751 (Fig. 2, c and d) cells resulted in ~1.8-fold elevation of Aβ-(1–40) and Aβ-(1–42) content in the conditioned medium relative to LacZ-infected cells 48 h postinfection. In parallel studies, MTT assay revealed no cell toxicity in hCOX-1, hCOX-2, or LacZ adenovirus-infected CHO-APP_swe or H4-APP_751 cell cultures 48 h postinfection at 10 m.o.i. (data not shown).

hCOX-1 or hCOX-2 Promotes CTF-γ Generation in CHO-APP_swe Cells That Is Prevented by COX Inhibition—Based on the evidence that both hCOX-1 and hCOX-2 expression promotes Aβ-(1–40) and Aβ-(1–42) generation, we next examined the influence of expression of hCOXs on APP processing by assessing the generation of CTF-γ APP cleavage product (known index of γ-secretase activity). For this study, fresh membranes from CHO-APP_swe cells were isolated and then incubated at 37°C for 2 h to allow generation of CTFS. Unlike other secretase assays, this in vitro γ-secretase assay detects enzymatic cleavage under physiologic conditions wherein CTF-γ is generated by cleavage of membrane-bound APP.

In this experiment, we found that, compared with the LacZ control group, both hCOX-1 and hCOX-2 adenosine virus infection in CHO-APP_swe cells leads to a functional >3-fold induction of PG-E2 content in the conditioned medium (Fig. 3g). Elevation of PG-E2 content in the conditioned medium coincided with potentiation of the ~6-kDa CTF-γ generation (Fig. 3, a and c, lanes 3 and 4, respectively, and b and d, relative to steady-state content of holo-APP) from fresh membrane preparations 48 h postinfection, strongly suggesting hCOX-1 and hCOX-2 overexpression leads to an induction of γ-secretase activity. Increased CTF-γ signal further coincided with significant elevation of Aβ-(1–40) and Aβ-(1–42) content in the conditioned medium of these same cultures (Fig. 3, e and f, respectively). As expected, in control studies, no detectable CTF-γ cleavage product was detected in membrane preparations from either hCOX-1- (Fig. 3a, lanes 1 and 2) or hCOX-2 (Fig. 3c, lanes 1 and 2)-transfected CHO-APP_swe cells kept on ice during the entire period of the reaction time.

To further address the physiological role of hCOX-1- and hCOX-2-mediated promotion of Aβ peptide generation, we next examined the role of the non-selective COX inhibitor ibuprofen on generation of CTF-γ and Aβ-(1–40) and Aβ-(1–42) peptides. We found that hCOX-1- and hCOX-2-mediated promotion of the ~6-kDa CTF-γ generation was prevented by co-treatment of CHO-APP_swe cells with the non-selective COX inhibitor ibuprofen (Fig. 3, a and c, lane 4 versus lane 6, and b and d) at 1 μM, which coincided with the reduction of Aβ-(1–40) and Aβ-(1–42) peptides (Fig. 3, e and f, respectively) and hCOX-1- and hCOX-2-mediated promotion of PG-E2 content (Fig. 3g) to LacZ levels as assessed by ELISA of the conditioned medium 48 h postinfection.

No significant alteration of CTF-γ generation (Fig. 3, a and c, lane 3 versus lane 5, and b and d) or Aβ-(1–40) and Aβ-(1–42) peptide content (Fig. 3, e and f) was found in LacZ adenovirus-infected CHO-APP_swe cells treated with 1 μM ibuprofen relative to vehicle-treated LacZ control CHO-APP_swe cells. Moreover no detectable cell toxicity was found following ibuprofen treatment (1 μM) in either LacZ-, hCOX-1-, or hCOX-2-infected CHO-APP_swe cultures as assessed by MTT assay (not shown). Finally in this assay no detectable change in total holo-APP (105 kDa) was observed in either hCOX-1 or hCOX-2 CHO-APP_swe cells relative to LacZ adenovirus-transfected cells (Fig. 3, a and c, lane 3 versus lane 4) irrespective of ibuprofen treatment (Fig. 3, a and c, lane 5 versus lane 6) (quantification not shown).

hCOX-1- and hCOX-2-mediated Induction of Aβ Peptide Generation Is Prevented by the γ-Secretase Inhibitor L-685,458—To further assess the specific role of hCOX-1- and hCOX-2-medi-
ated induction of γ-secretase activity, in parallel studies we explored the role of the selective γ-secretase inhibitor L-685,458 in hCOX-mediated promotion of Aβ peptide generation. We found that co-treatment of CHO-APPswe with the γ-secretase-specific inhibitor L-685,458 at 20 nM selectively prevented hCOX-1- and hCOX-2-mediated induction of Aβ-(1–40) (Fig. 4a) and Aβ-(1–42) (Fig. 4b) peptide contents in the conditioned medium of CHO-APPswe cells. However, as expected, we found that treatment of CHO-APPswe with 50–100 nM L-685,458 further inhibited endogenous CHO-APPswe Aβ-(1–40) (Fig. 4a) and Aβ-(1–42) (Fig. 4b) contents in the conditioned medium as reported previously (35).

In control studies, we found that blockade of hCOX-1- and hCOX-2-mediated induction of Aβ generation by the selective γ-secretase inhibitor L-685,458 at 20–100 nM was highly specific and did not influence the activities of hCOXs as suggested by a lack of change in PG-E2 content (Fig. 4c) in the conditioned medium 48 h after treatment. Moreover no detectable cell toxicity in response to L-685,458 at any concentration tested relative to vehicle (Me2SO, 0.01%) was found in LacZ, hCOX-1, or hCOX-2 adenovirus-infected CHO-APPswe cells as assessed by MTT assay (not shown).

hCOX-1 and hCOX-2 Expression Do Not Influence the Non-amyloidogenic sAPPα Pathway in CHO-APPswe Cells—To further confirm that hCOX-1 and hCOX-2 selectively promoted Aβ generation through mechanisms involving γ-secretase activity, we next assessed the potential role of hCOX-1 and hCOX-2 in non-amyloidogenic pathways by measuring sAPP secreted in the conditioned media.

We found that sAPPα content (defined as 6E10-immunoreactive sAPP) in the conditioned medium, see “Experimental Procedures” for more information), which is assumed to be an α-secretase-cleaved form of APP, was not affected by hCOX-1 or hCOX-2 expression in CHO-APPswe cells (Fig. 5a, inset) relative to the LacZ-infected control group. Similarly no detectable change in total sAPP content (defined as 22C11-immunoreactive sAPP) was found in the conditioned medium of hCOX-1- and hCOX-2-infected CHO-APPswe cells (Fig. 5a, inset) relative to LacZ-infected cells. Finally the sAPPα/total sAPP ratio, indicative of α-secretase activity (Fig. 5a), was not altered in response to hCOX-1 or hCOX-2 infection (identical cultures were used for detection of sAPPα/total sAPP ratio, Aβ peptides, and CTF-γ cleavage product generation discussed above). Collectively this evidence suggests that the non-amyloidogenic α-secretase activity is not altered by expression of hCOXs in vitro. In further control studies we also confirmed that total steady-state holo-APP content (defined as C8-immunoreactive APP) in the cell lysate of CHO-APPswe cells was not altered by hCOX-1 or hCOX-2 adenovirus infection (Fig. 5b) relative to LacZ adenovirus-infected cells.

11-Deoxy-PG-E2 Treatment Promotes Aβ Peptide Generation in CHO-APPswe Cells and HEK-APPswe Cells—Based on the evidence that hCOX-1 and hCOX-2 can promote CTF-γ and Aβ peptide generation in vitro, we tested whether PG-E2, the major product of the COX enzymatic pathway, could promote similar responses at a concentration comparable to that elicited by hCOX-1 or hCOX-2 viral expression.

Similar to responses observed following infection with hCOXs, we observed that exposure to the PG-E2 analog 11-deoxy-PG-E2 (11-dPG-E2; 10 nM) significantly potentiated the generation of the ~6-kDa CTF-γ (relative to holo-APP content) in membrane preparations from CHO-APPswe cells (Fig. 6, a and b) 48 h posttreatment. Further 11-dPG-E2-mediated induction of CTF-γ cleavage product in CHO-APPswe cells coincided with an approximate ~1.8-fold elevation of Aβ(1–40) and Aβ(1–42) content in the conditioned medium of the same cultures. No detectable change in holo-APP was observed in both CHO-APPswe (Fig. 6c, quantification not shown) cells following 11-dPG-E2 treatment.

Moreover we found that 11-dPG-E2-mediated induction of CTF-γ generation and subsequent elevation of Aβ(1–40) and
Aβ-(1–42) peptides in CHO-APPsw cells was highly selective and occurred in the absence of detectable alterations to “non-amyloidogenic” APP pathways as indicated by a lack of change in the sAPPα/total sAPP ratio in the conditioned medium or total cellular holo-APP contents relative to vehicle-treated CHO-APPsw cells (Fig. 6, d and e), respectively. Finally the 11-dPG-E2 (10 nM)-mediated induction of CTF-γ generation and subsequent elevation of Aβ-(1–40) and Aβ-(1–42) peptides were confirmed in HEK-APPsw cells in the absence of detectable changes in holo-APP and sAPPα levels (data not shown).

DISCUSSION

The accumulation and aggregation of Aβ peptides in the brain is believed to be an early event in the pathogenesis of AD (1). Aβ peptides are generated by the sequential proteolytic cleavage of APP by β- and γ-secretase, and consequently inhibitors of these secretases are under investigation as potential Aβ-lowering strategies for AD. Recent studies have suggested that ibuprofen among other NSAIDs may selectively reduce Aβ-(1–42) production in vitro via direct modulation of γ-secretase activity (36–38) and not via the COX-inhibiting features characteristic of this class of drugs. However, the evidence that the expression of both the inducible and constitutive forms of COX (15, 17–24) as well as PG-E2 (39) are elevated in AD raises the possibility that COXs may contribute to AD pathology and that COX inhibition may be therapeutically relevant. This evidence is further supported by our previous work showing that hCOX-2 expression in PSAPP mice induced potentiation of brain parenchymal amyloid plaque formation coincidental with a 2-fold increase in PG-E2 production (40). The goal of this study was to further test the hypothesis that COX may directly influence amyloidogenesis and explore the mechanisms through which COX-1 and COX-2 may promote Aβ peptide generation in vitro.

We found that hCOX-1 or hCOX-2 infection in CHO-APPsw and H4-APPswe cells resulting in 10–25 nM PG-E2, which is within the range of PG-E2 concentrations observed in the hu-
man brain (35), coincided with a significant elevation of Aβ(1–42) and Aβ(1–40) peptide generation and increased generation of the γ-CTF product of APP. This evidence strongly supports the hypothesis that COX-1 and COX-2 may have promoted Aβ generation via a novel mechanism resulting in potentiation of γ-secretase activity. Consistent with this hypothesis, we found that the selective γ-secretase inhibitor L-685,458 prevented hCOX-1- and hCOX-2-mediated promotion of Aβ peptide generation without altering PG-E2 production. This finding coupled with the evidence that hCOX-1 and hCOX-2 overexpression did not influence sAPPα secretion into the conditioned medium supported the hypothesis that COX may have promoted Aβ peptide generation via downstream activation of γ-secretase activities rather than interfering (inhibiting) with non-amyloidogenic secretory APP pathways.

In light of the observed relationship between PG-E2 production (a major product of the COX enzymes) and Aβ production following hCOX infection, we hypothesized that increased PG-E2 synthesis (as found in cerebrospinal fluid of AD (39)) may have exacerbated Aβ peptide generation by specifically influencing γ-secretase activity. Consistent with our observations that hCOX-1 and hCOX-2 overexpression promoted Aβ generation, we found that treatment of CHO-APPsw cells and HEK-APPsw cells with PG-E2 (11-dPG-E2) at a concentration comparable to that elicited by hCOX-1 or hCOX-2 viral expression achieved similar potentiation of both Aβ peptide generation and γ-secretase activity. This novel finding tentatively suggested that expression of COXs in the brain might influence amyloidogenesis through activation of signal transduction pathways downstream of the PG receptor. In view of the evidence that endogenous EP4 PG-E2 receptors are positively coupled to adenyl cyclase (41) in CHO cells, it may the case that the EP4 receptor represents a novel target for blocking Aβ generation in the brain. However, given that characterization of PGs and their receptor families in the brain remains in developmental stages (42), the therapeutic utility of PG receptor antagonists, at present, may be limited.

Previous evidence indicated that ibuprofen (a non-selective COX inhibitor) can decrease AD type amyloid burden in a mouse model (43, 44). Based upon this evidence and the fact that ibuprofen can selectively lower Aβ(1–42) levels in the brain and in APP-overexpressing cells (36, 37), we further
evaluated the role of ibuprofen in blocking $\alpha$-secretase activity mediated by COXs in vitro. We found that ibuprofen (at a low 1 $\mu$m concentration) significantly prevented hCOX-2- and hCOX-1-mediated promotion of the generation of $\alpha$-secretase products in control CHO-APP$\text{sw}$ cells as reflected by inhibition of the $\alpha$-CTF product of APP generation (and decreased PG-E2 content). Given that we did not observe any changes in “basal-endogenous” $\alpha$-secretase activity in CHO-APP$\text{sw}$ cells treated with ibuprofen at this concentration (consistent with previous evidence (36, 37)), we suggest that at a low 1 $\mu$m dose ibuprofen might modulate generation of $\alpha$-secretase products by inhibiting responses mediated by COXs/PGE$_2$. However, it remains indisputable that ibuprofen (among other NSAIDs) at dosages higher than that used in our study can directly influence $\gamma$-secretase activity and eventually selectively lessen $\beta$-secretase generation (36, 37). However, we also note that recent in vitro evidence indicates that ibuprofen may significantly decrease both $\beta$-secretase (1–40) (−19%) and $\beta$-secretase (1–42) (−63%) content in the brain of a mouse model of AD (45), consistent with the possibility that ibuprofen may nonspecifically inhibit generation of both $\alpha$-secretase products under certain conditions in vivo. Finally we note that other studies also suggest an additional potential antiamyloidogenic role for ibuprofen via mechanisms that impact APP processing and favor sAPP-$\alpha$ formation in neuroblastoma SH-SYSY cells (46).

Despite the implications of the present findings and growing evidence from our (17–19, 40) and other (15, 20–25, 47) labo-
The Role of Cyclooxygenases in Alzheimer’s Disease

50977

ratories implicating COX-1 and COX-2 in the pathophysiology of AD and models of AD type neuropathology, the role of COX in the clinical progression of AD is little understood (48, 49). Moreover it remains that clinical trials with NSAIDs in AD have had marginal success (7–9) when applied therapeutically in moderate-severe AD. However, given that multiple in vitro studies now show that COX-inhibiting NSAIDs can mitigate cognitive impairment and amyloidosis in mouse models of AD type neuropathology (prophylactically) (36, 43, 44) and that epidemiological studies continue to support a beneficial role for NSAIDs in AD (1, 2), it may be the case that efficient treatment with NSAIDs in AD will yield success when applied to early or preclinical AD dementia cases.

It remains unclear which COX-inhibiting NSAID is the most appropriate candidate for the treatment of AD. Thus further understanding of the role of COX activity (specifically COX-derived PG) in mechanisms leading to Aβ generation is critical to the future development of NSAID therapy for AD. As shown in Scheme 1, our finding showing that COXs may promote Aβ generation via a PG-E₂-mediated pathway and the current evidence suggesting that certain NSAIDs may also directly influence γ-secretase activities support the hypothesis that NSAIDs may bear therapeutic relevance to antiamyloidogenic strategies.

Acknowledgment—We thank Dr. Hanna Reding for constructive discussion of the data.

REFERENCES
1. Zandi, P. P., Anthony, J. C., Hayden, R. K., Mehta, K., Mayer, L., and specimens, K. M. (2001) Am. J. Med. 118, 218–229
2. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
3. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
4. McGeer, P. L., Schulzer, M., and McGeer, E. G. (1996) J. Neurosci. Res. 44, 803–817
5. Zandi, P. P., Anthony, J. C., Hayden, K. M., Mehta, K., Mayer, L., and specimens, K. M. (2001) Am. J. Med. 118, 218–229
6. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
7. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
8. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
9. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
10. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
11. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
12. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
13. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
14. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
15. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926
16. Veld, B. A., Ruitenberg, A., Hofman, A., Launer, L. J., van Duijn, C. M., Stijnen, T., Breteler, M. M., and Stricker, B. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1922–1926