ADAM10 Activation Is Required for Green Tea (−)-Epigallocatechin-3-gallate-induced α-Secretase Cleavage of Amyloid Precursor Protein*

Received for publication, January 20, 2006, and in revised form, April 17, 2006. Published, JBC Papers in Press, April 19, 2006, DOI 10.1074/jbc.M600617200

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Recently, we have shown that green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) exerts a beneficial role on reducing brain Aβ levels, resulting in mitigation of cerebral amyloidosis in a mouse model of Alzheimer disease. EGCG seems to accomplish this by modulating amyloid precursor protein (APP) processing, resulting in enhanced cleavage of the α-COOH-terminal fragment (α-CTF) of APP and corresponding elevation of the NH2-terminal APP product, soluble APP-α (sAPP-α). These beneficial effects were associated with increased α-secretase cleavage activity, but no significant alteration in β- or γ-secretase activities. To gain insight into the molecular mechanism whereby EGCG modulates APP processing, we evaluated the involvement of three candidate α-secretase enzymes, a-disintegrin and metalloprotease (ADAM) 9, 10, or 17, in EGCG-induced non-amyloidogenic APP metabolism. Results show that EGCG treatment of N2a cells stably transfected with “Swedish” mutant human APP (SweAPP N2a cells) leads to markedly elevated active (−60 kDa mature form) ADAM10 protein. Elevation of active ADAM10 correlates with increased α-CTF cleavage, and elevated sAPP-α. To specifically test the contribution of ADAM10 to non-amyloidogenic APP metabolism, small interfering RNA knockdown of ADAM9, -10, or -17 mRNA was accomplished by enzymes known as secretases. Whereas non-amyloidogenic α-secretase cleavage produces the amino-terminal product named soluble APPα (sAPP-α) and the carboxyl-terminal fragment (CTF) α-CTF (also known as C83), the action of amyloidogenic β-secretase on APP results in the amino-terminal product sAPP-β and the carboxyl-terminal product β-CTF (also known as C99). Subsequent γ-secretase complex cleavage of β-CTF yields γ-CTF (also known as C7), and releases Aβ (6–40). Promotion of α-secretase processing leads to both a reduction in Aβ and an increase in sAPP-α, a protein that exhibits neuroprotective properties (11–14). A number of studies have sought to discern the molecular identity of α-secretase, with the hope of targeting such enzyme(s) to modulate Aβ production (15, 16).

A number of reports have implicated members of the a-disintegrin and metalloprotease (ADAM) family, a family of zinc metalloproteases including ADAM9, -10, and -17, as putative α-secretase candidates (15–17). Lammi and colleagues (18) first described the ability of ADAM10 to act as an α-secretase, whereas Buxbaum and co-workers (19) reported that ADAM17 contributes to a-secretase processing of APP. Others have demonstrated the ability of ADAM9 to promote α-secretase cleavage (20). However, Asai and colleagues (17) reported that ADAM9, -10, and -17 all have roles in the processing of APP to sAPP-α in vitro. In cerebrospinal fluid from AD patients, ADAM10 and corresponding sAPP/α-CTFs are decreased (21, 22). Moreover, ADAM10 is also decreased in AD and Down syndrome brains (23). A report by Lopez-Perez and colleagues (24) implicates ADAM10 as a contributor to constitutive sAPP-α production, whereas ADAM17 (also known as tumor necrosis factor-α converting enzyme, TACE) is implicated in a regulated mechanism of sAPP-α production (24). Recently, Postina and colleagues (25) showed that activation of α-secretase significantly reduces AD-like pathology in an animal model of AD. Although individual contributions by putative α-secretases to the AD process still remain unclear, the above mentioned study in mice raises the possibility that a strategy of increasing α-secretase activity may provide a promising therapeutic target for AD.

Much research has focused on the therapeutic potential of compounds that may promote non-amyloidogenic processing of APP (26). One such compound, a polyphenol flavonoid known as (−)-epigallocatechin-3-gallate (EGCG), is found in green tea and is known to enhance ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; TBS, Tris-buffered saline; RT, reverse transcriptase; ANOVA, analysis of variance.

‡ This work was supported in part by grants from the Institute for the Study of Aging (to J.T.) and the Johnnie B. Byrd Senior Alzheimer’s Center and Research Institute (to J.T. and D.S.). 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.

† Supported by a Ruth L. Kirschstein National Institutes of Health/National Research Service Award/National Institute of Aging postdoctoral fellowship and an Alzheimer Association grant.

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** Supported by a Ruth L. Kirschstein National Institutes of Health/National Research Service Award/National Institute of Aging postdoctoral fellowship and an Alzheimer Association grant.

*** The abbreviations used are: APP, amyloid precursor protein; AD, Alzheimer disease; BACE, β-site amyloid precursor protein cleaving enzyme; α-CTF, α-COOH-terminal fragment; s-APP, soluble amyloid precursor protein-α; ADAM, a-disintegrin and metalloprotease; EGCG, (−)-epigallocatechin-3-gallate; siRNA, small interfering RNA;
the processing of APP to sAPP-α in vitro (27, 28). We recently demonstrated that EGCG enhances metabolism of APP to sAPP-α and reduces Aβ levels both in vitro in neuron-like cells and in vivo in the “Swedish” APP transgenic (Tg2576) mouse model of AD (29). To discern the mechanism whereby EGCG promotes non-amyloidogenic APP processing, we have performed Western analysis of ADAM9, -10, and -17 in murine neuron-like N2a cells stably transfected with the Swedish APP mutation (SweAPP N2a). Data show that EGCG treatment results in a dose-dependent increase in the mature form (~60 kDa) of ADAM10, whereas no significant change in ADAM9 or -17 was observed. Small interfering RNA (siRNA) knockdown of ADAM9, -10, or -17 in SweAPP N2a cells reveals the specific requirement of ADAM10 in EGCG-induced modulation of APP metabolism toward the non-amyloidogenic pathway. Both SweAPP N2a cells and primary cultures of neurons from Tg2576 mice show enhanced levels of sAPP-α and mature ADAM10 in response to EGCG treatment. When taken together, these results indicate that ADAM10 is a key effector in EGCG promotion of non-amyloidogenic APP proteolysis. These data provide important mechanistic insight into the potential use of EGCG and/or other stimulators of ADAM10 as therapeutics to oppose cerebral amyloidosis associated with AD.

**EXPERIMENTAL PROCEDURES**

Reagents—Green tea-derived EGCG (95% purity by high-performance liquid chromatography) was purchased from Sigma. Polyclonal antibodies against ADAM10 were obtained from Calbiochem. ADAM17 (TACE) and ADAM9 antibodies were obtained from Sigma. Polyclonal antibody against the carboxyl terminus of APP (369 antibody) was kindly provided by S. Gandy and H. Steiner. Monoclonal antibodies against the amino terminus of APP (22C11) and against actin (1:1,000 dilutions in PBS) were obtained from Signet Laboratories (Dedham, MA).

**ELISA**—Conditioned media were collected and analyzed at a 1:1 dilution using the method as previously described (30) and values were reported as percentage of Aβ1–42 secreted relative to control. Quantitation of total Aβ species was performed according to published methods (31). Briefly, 6E10 (capture antibody) was coated at 2 μg/ml in PBS into 96-well immunoassay plates overnight at 4 °C. The plates were washed with 0.05% Tween 20 in PBS five times and blocked with blocking buffer (PBS with 1% bovine serum albumin, 5% horse serum) for 2 h at room temperature. Conditioned medium or Aβ standards were added to the plates and incubated overnight at 4 °C. Following 3 washes, biotinylated antibody, 4G8 (0.5 μg/ml in PBS with 1% bovine serum albumin), was added to the plates and incubated for 2 h at room temperature. After 5 washes, streptavidin-horseradish peroxidase (1:200 dilutions in PBS with 1% bovine serum albumin) was added to the plates and incubated for 2 h at room temperature. Tetrathymethylbenzidine substrate was added to the plates and incubated for 15 min at room temperature. 50 μl of stop solution (2 N H2SO4) was added to each well of the plates. The optical density of each well was immediately determined by a microplate reader at 450 nm. In addition, Aβ1–40, or Aβ1–42 was separately quantified in these samples using the Aβ1–40 or Aβ1–42 ELISA kits (IBL-America, Minneapolis, MN) in accordance with the manufacturer’s instructions. In all cases, Aβ levels were expressed as a percentage of control (conditioned medium from untreated SweAPP N2a cells).

Western Blot—Cultured cells were lysed in ice-cold lysis buffer as described above, and an aliquot corresponding to 50 μg of total protein was electrophoretically separated using 16.5% Tris-Tricine gels. Electrophoresed proteins were then transferred to polyvinylidene difluoride membranes (Bio-Rad), washed in ddH2O, and blocked for 1 h at ambient temperature in Tris-buffered saline (TBS; Bio-Rad) containing 5% (w/v) nonfat dry milk. After blocking, membranes were hybridized for 1 h at ambient temperature with various primary antibodies. Membranes were then washed 3 times for 5 min each in ddH2O and incubated for 1 h at ambient temperature with the appropriate horseradish peroxidase-conjugated secondary antibody (1:1,000, Pierce Biotechnology). All antibodies were diluted in TBS containing 5% (w/v) nonfat dry milk. Blots were developed using the luminol reagent (Pierce Biotechnology). Densitometric analysis was done using the Fluor-S Multidocument™ with Quantity One™ software (Bio-Rad). For examining aAPP-α, conditioned medium was collected following treatment according to a modified protocol from Chen and Fernandez (32). sAPP-α was extracted using 3K Nanosep centrifugal filters (Pall Life Sciences, Ann Arbor, MI) and protein concentration was prepared for the aforementioned electrophoresis. Antibodies used for Western blot included: antibody 369 (which recognizes the carboxyl terminus of APP; 1:1,500), clone 22C11 (against the amino terminus of APP; 1:1,500), clone 6E10 (against amino acids 1–17 of Aβ; 1:1,500), anti-ADAM9 (1:500), and antibodies against ADAM10 (1:500), ADAM17 (1:500), or actin (1:1,500; as an internal reference control).

To characterize α-CTF detected by antibody 369 in this study, we performed an additional experiment. The blots were first hybridized with an antibody (369) specifically against the carboxyl terminus of APP, were put in stripping solution (62.5 mm Tris-HCl, pH 6.8, 2% SDS, and 100 mm β-mercaptoethanol) and incubated at 50 °C for 30 min in a sealed plastic container in a shaking water bath. After stripping, blots were rinsed with TBS (pH 6.8) and re-blocked with TBSST (TBS + 1% Tween 20) and then re-probed with an antibody that recognizes Aβ1–17 (6E10). Alternatively, membranes with identical samples were probed either with antibody 369 or with antibody 6E10. As expected, the ~11-kDa band was positive for both 369 and 6E10 antibodies probed, thereby confirming its identity as an α-CTF.

Primary Cultures—Breeding pairs of C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Tg2576 mice were provided by the University of South Florida. Primary culture microglial cells were isolated from mouse cerebral cortices and were grown in RPMI 1640 medium supplemented with 5% fetal calf serum, 2 mm glutamine, 100 units/ml penicillin, 0.1 μg/ml streptomycin, and 0.05 mm 2-mercaptoethanol according to previously described methods (32, 33). Briefly, cerebral cortices from newborn mice (~1–2 days old) were isolated under sterile conditions and kept in 75-cm2 flasks, and complete medium was added. Primary cultures were kept for 14 days so that only glial cells remained, and microglial cells were isolated by shaking flasks at 200 rpm at 37 °C in a Lab-Line incubator shaker. More that 98% of these glial cells stained positive for MAC-1 (CD11b/CD18; Roche) confirming their identity as microglia. Mouse primary culture neuronal cells were prepared as previously described (32, 34). Briefly, cerebral cortices were isolated from Tg2576 mouse embryos, between 15 and 17 days in utero, and were mechanically dissociated in trypsin (0.25%) individually after incubation for 15 min at 37 °C. Cells were collected after centrifugation at 1,200 × g, resuspended in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 10% horse serum, uridine (33.6 μg/ml; Sigma), and fluorodeoxyuridine (13.6 μg/ml; Sigma), and seeded in 24-well collagen-coated culture plates at 2.5 × 105 cells per well. When neuronal cells were isolated from Tg2576 mice, to verify the presence of the transgene, PCR genotype analysis was performed as previously described (30) and the human APPtransgene was detected in these cells (data not shown).
Small Interfering RNA-mediated Gene Silencing—SweAPP N2a cells were transfected with siRNA pre-designed to knockdown murine ADAM9, -10, or -17 mRNA (Dharmacon Inc., Lafayette, CO). SweAPP N2a cells were seeded in 24-well plates and cultured until they reached 70% confluence. The cells were then transfected with 50–200 nM anti-ADAM9, -10, or -17 siRNA or anti-green fluorescent protein (non-targeting control; Dharmacon) using Code-Breaker transfection reagent (Promega) and cultured for an additional 18 h in serum-free minimal essential medium. Transfection efficiency was determined to be greater than 80% (data not shown) using no-RISC siGLOW (fluorescently labeled non-functional siRNA; Dharmacon). The cells were allowed to recover for 24 h in complete medium (minimal essential medium, 10% fetal bovine serum) before treatment. The cells were evaluated by Western blot analysis for expression of ADAM9, -10, or -17.

RT-PCR—Analysis of murine ADAM10 was conducted according to previously published methods (35, 36). Briefly, total RNA was isolated from SweAPP N2a cells and subjected to reverse transcription utilizing a commercially available kit (cDNA Cycle® kit; Invitrogen) according to the manufacturer’s instructions on a Bio-Rad iCycler thermocycler. The same machine was used to amplify murine cDNA by PCR using ADAM10 sense
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RESULTS

EGCG Treatment Enhances ADAM10 Activation in Cultured CNS Cells—To determine whether EGCG modulates expression of candidate α-secretases ADAM9, -10, or -17, we treated N2a cells stably transfected with Swedish mutant APP (SweAPP N2a cells) with various doses of EGCG and measured the respective levels of protein expression. Mature ADAM10 (the ∼60-kDa isoform), but not ADAM9 or ADAM17, dose dependently increased in response to EGCG treatment (Fig. 1A). To investigate if EGCG treatment might affect mRNA expression of ADAM10 across the time frame examined above, we isolated total RNA from cells treated in parallel for RT-PCR analysis. Results show no significant between EGCG dose differences on ADAM10 mRNA levels (Fig. 1B). Temporal analysis of the effect of EGCG on ADAMs expression showed significant increases in mature ADAM10 as early as 30 min after treatment with 20 μM EGCG (Fig. 1C), an effect that continued to increase through to 120 min after EGCG challenge. However, no significant effects of EGCG treatment on ADAM9 or -17 were noted (data not shown). Consistent with these findings, EGCG also dose dependently increased ADAM10 maturation in two additional cell types, parental (non-transfected) N2a cells and N9 microglial cells. Relative to N9 microglia, the neuron-like parental N2a cell line demonstrated increased sensitivity to EGCG treatment (Fig. 2A). Similar to N2a and N9 cell lines, primary murine neuronal and microglial cultures also displayed dose-dependent increases in mature ADAM10 in response to EGCG treatment (Fig. 2B), with primary neurons showing increased sensitivity to the lower doses (10 and 20 μM) of EGCG.

EGCG-induced Maturation of ADAM10 Correlates with APP α-Secretase Cleavage—To determine whether the EGCG-mediated dose-dependent increase in mature ADAM10 might result in modulation of APP processing, we subjected SweAPP N2a cells to various doses of EGCG and then analyzed APP metabolism and ADAM10 maturation in parallel. Western analysis revealed dose-dependent increases in α-CTF and sAPP-α with corresponding increases in mature ADAM10 in response to EGCG treatment (Fig. 3, A–C). Moreover, we also observed EGCG dose-dependent reductions in Aβ(1-40) and Aβ(1-42) concentrations after...
FIGURE 3. EGCG-induced ADAM10 activation correlates with APP α-secretase cleavage in vitro. SweAPP N2a cells (A and B) or Tg2576 mouse-derived primary neuronal cells (E and F) were treated with EGCG at various doses for 8 h and subjected to Western blot for APP CTFs and ADAM10. As indicated in panels to the right, densitometry analysis shows the band density ratio of α-CTF to full-length APP (holo-APP) for A and E or mADAM10 to pro-ADAM10 for B and F. One-way ANOVA revealed significant EGCG dose differences on both ratios of α-CTF to holo-APP and mADAM10 to pro-ADAM10 (**, p < 0.001). Conditioned media were collected from SweAPP N2a cells (C and D) or Tg2576 mouse-derived primary neuronal cells (G and H) after EGCG treatment and subjected to Western blot for sAPP-α or Aβ ELISA. Data are represented as % change relative to control (medium from cultured SweAPP N2a cells or primary neuronal cells without any treatment). One-way ANOVA revealed significant EGCG dose differences in both ratios of sAPP-α to actin (**, p < 0.001) and reduction of Aβ1–40 and Aβ1–42 (*, p < 0.05; **, p < 0.001). Similar results were observed in three independent experiments.
EGCG treatment by Aβ ELISA (Fig. 3D), further confirming that 1) EGCG promotes non-amyloidogenic APP processing and 2) that this effect correlates with increased ADAM10 maturation. Accordingly, primary neuronal cells derived from Tg2576 mice were also analyzed for changes in APP metabolism in response to EGCG treatment. Western analysis revealed EGCG promotion of the APP α-secretase cleavage pathway, as quantified by the ratio of α-CTF to holo-APP (Fig. 3E). Similar to data observed in SweAPP N2a cells, APP α-CTF cleavage positively correlated with mature ADAM10 levels (Fig. 3F) and with secreted sAPP-α (Fig. 3G) in these cells. Importantly, we also observed dose-dependent reductions in Aβ1–40 and Aβ1–42 levels following EGCG treatment of primary neurons from Tg2576 mice (Fig. 3H).

ADAM10 Is Required for EGCG-induced APP α-Secretase Cleavage—To directly examine whether ADAM α-secretase activity was required for EGCG promotion of non-amyloidogenic APP cleavage, we conducted siRNA knockdown experiments targeting ADAM9, -10, or -17. First, to confirm siRNA knockdown efficiency, SweAPP N2a cells were treated with ADAM9, -10, or -17 siRNAs and then Western blotted for expression of respective ADAMs. As shown in Fig. 4, A–C, protein expression levels of ADAM9, -10, or -17 were significantly inhibited by respective ADAM-specific siRNAs. In addition, to test the specificity of siRNA against ADAM10 versus ADAM9 or -17, we analyzed expression of ADAM9 and -17 in cell lysates derived from siRNA knockdown cells for ADAM10 using Western blot. Results show that ADAM10 siRNA does not alter the expression of ADAM9 or -17 (Fig. 4D). We next examined α-CTF production in SweAPP N2a cells subjected to siRNA knockdown of ADAMs following treatment with 20 μM EGCG, and observed that only ADAM10 siRNA was able to clearly 1) inhibit expression of ADAM10 as evidenced by decreased band density ratios of pro–ADAM10 to actin and mADAM10 to actin, and 2) block EGCG-induced α-CTF production and sAPP-α secretion (Fig. 5, A and B). This effect of ADAM10 siRNA on blocking EGCG-induced non-amyloidogenic APP processing was further borne out by Aβ ELISA analysis, where only ADAM10 siRNA attenuated EGCG-induced reduction of Aβ1–40 and Aβ1–42 (Fig. 5C). Taken together, these data demonstrate the requirement of ADAM10 for EGCG-mediated promotion of APP α-secretase cleavage.

DISCUSSION

The amyloid cascade hypothesis proposes that cerebral amyloidosis plays a central role in the etiopathogenesis of AD (37). A corollary of this hypothesis is that therapeutics aimed at decreasing Aβ generation/deposition through suppression of amyloidogenic APP processing or promotion of non-amyloidogenic APP processing are probable future treatments for AD (29, 37). α-Secretase processes APP into sAPP-α, γ-secretase-processed soluble fragments, and corresponding CTFs, all of which are not known to contribute directly to amyloid plaques in the brains of AD patients (38). Aside from their abilities to lower Aβ production, α-secretase promoting therapeutics are likely to be indirectly neuroprotective through enhancement of sAPPα formation (11–15). These findings beckon the development of hitherto undiscovered methods for rescuing possibly suppressed α-secretase activity for restoration of the proper balance between APP metabolites: sAPPα and Aβ. In consideration of our previous studies and others, we wondered if particular putative α-secretase molecules might be required for the α-secretase APP processing mediated by the major polyphenol component of green tea: EGCG.

We found in our systems that EGCG treatment primarily induced increases in levels of mature ADAM10 protein and therefore ADAM10 maturation (mature ADAM10/pro–ADAM10 ratio, Fig. 1A). Moreover, this elevation persisted after 18 h (data not shown), thereby differentiating the effect of EGCG on ADAM10 from the more transient (i.e. less than 5 h) effect on ADAM17 that we previously observed under similar conditions (29). This robust increase in mature ADAM10 agrees with studies conducted by Stoeck and colleagues (39), suggesting that ADAM17 displays transient “inducible” activity mediated by protein kinase C activation, whereas ADAM10 achieves inducible and “constitutive” activity for substrate processing (39). Indeed, EGCG induced dramatic increases in mature ADAM10/actin ratios after

FIGURE 4. siRNA knockdown efficiency for ADAM10, -9, or -17 is confirmed by Western blot analysis. Expression of ADAM10 (A), -17 (B), or -9 (C) was analyzed by Western blot in cell lysates from SweAPP N2a cells transfected with siRNA targeting ADAM10, -9, or -17 or at 24 or 48 h after transfection. Densitometry analysis shows the band density ratios of pro-ADAM10, ADAM17, and ADAM9 to actin as indicated in the panels below. One-way ANOVA revealed significant differences between siRNA-transfected cells and control cultures on the ratio of ADAMs to actin (***, p < 0.001). Similar data were obtained in three independent experiments.
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FIGURE 5. ADAM10 is required for EGCG-induced APP α-secretase cleavage. Cell lysates (A) and conditioned media (B and C) were prepared and collected from SweAPP N2a cells transfected with ADAM9, -10, or -17 siRNA or non-targeting siRNA control (siRNA control) for 48 h and then treated with EGCG (20 μM) for 8 h. A, cell lysates were subjected to Western blot for APP CTFs and ADAM10 cleavage analyses. Densitometry analysis shows the band density ratios of α-CTF to holo-APP (upper right panel), pro-ADAM10 to actin (middle right panel), or mADAM10 to actin (lower right panel) as indicated. B, cell culture media were subjected to Western blot for sAPP-α secretion. Densitometry analysis shows the band density ratios of sAPP-α to actin as indicated below. C, cell culture media were subjected to Aβ ELISA. Data are represented as % change relative to control (medium from cultured SweAPP N2a cells without any treatment). A t test revealed a significant difference between ADAM10 siRNA and ADAM9 or ADAM17 siRNA or siRNA control (**) on the ratios of α-CTF to holo-APP, pro-ADAM10 to actin, or mADAM10 to actin, and reduction of sAPP-α and Aβ species as indicated. However, there were no significant differences between ADAM9 or ADAM17 siRNA and siRNA control by a t test (p > 0.05). Data are representative of three independent experiments.

only 30 min and through 120 min, at which time we observed a drop off in pro-ADAM10/actin ratios (Fig. 1C). These data in combination with semiquantitative RT-PCR analysis of ADAM10 RNA expression (Fig. 1B) suggest that EGCG activates ADAM10 predominantly at the post-translational level possibly through endoproteolysis of the zymogen pro-form. Curiously, whereas the ~75-kDa form of ADAM10 is reduced in N9 microglia cells (Fig. 2A) and nearly absent from primary cells (Fig. 2B), it does not significantly change after EGCG treatment and could repre-
sent a previously reported ADAM10 non-glycosylated isoform (40–42).

Mechanisms governing ADAM10 activation are currently not fully understood, yet studies implicate proprotein convertase family members, particularly furin and PC7 (43, 44), as mediators of endoproteolytic activation of ADAM10. Interestingly, furin is also involved in endoproteolysis of the N-glycosylated zymogen/pro-form of β-site amyloid precursor protein cleaving enzyme (BACE), thereby enabling β-secretase cleavage of APP in the trans-Golgi network (43, 44). Additionally, overexpression of full-length BACE reduces SAPPa while driving β-CTF production, and only moderately increases Aβ formation; whereas overexpression of mature BACE results in dramatic increases in Aβ production (45, 46). Altogether, these data suggest an attractive notion whereby furin may be rate-limiting for the endoproteolytic activation of both BACE and α-secretases like ADAM10. Imbalances in substrate processing by furin could represent a mechanism of disease. In addition, recent studies further suggested that BACE and α-secretases compete for APP processing with β-secretase cleavage precluding APP processing by α-secretases and vice versa (47, 48).

Our initial data, and reports by others (17, 38) suggesting that one or multiple ADAM family members play(s) dominant roles in inducible and/or constitutive methods of substrate processing, also led us to test which of putative secretases analyzed here were required and whether intra-α-secretase modulation (49) was involved in EGCG-mediated α-secretase processing of APP. As expected, we found that siRNA knockdown of ADAM10 primarily blocked EGCG induction of APP α-secretase metabolism (Fig. 5, A and B). In addition, siRNA knockdown of ADAM17 also slightly inhibited EGCG modulation of APP metabolism (Fig. 5A), in accordance with our previous findings that ADAM17 may transiently conduct α-secretase proteolysis following EGCG treatment (29). Importantly, we also confirmed that anti-ADAM9, -10, and -17 siRNA efficiently reduced their specific targets (Fig. 4, A–C). These observations, coupled with our findings that anti-ADAM10 siRNA failed to affect ADAM9 or -17 protein levels (Fig. 4D), greatly diminishes the probability of the involvement of intramolecular regulation between ADAMs as a contributing factor in EGCG-mediated APP metabolism. Thus, whereas ADAM9 and/or -17 may play minor or transient roles in EGCG modulation of APP metabolism, ADAM10 appears to be the major downstream effector.

Direct and/or indirect molecular interactions between EGCG and ADAM10 remain to be determined. As EGCG is an amphipatic small molecule, it is very likely that it reaches its molecular target by passive diffusion through cell membranes. This means of cellular uptake may explain the rapid onset of ADAM10 maturation as we observed following EGCG treatment (Fig. 1C) and suggest an intracellular molecular target. On the other hand, cell surface receptor interactions have also been suggested (50, 51). Furthermore, some groups studying regulation of cancer cell proliferation by EGCG have discovered that the tyrosine phosphorylation status of important signal transduction proteins may be altered and account for the mitigation of cancer cell growth by EGCG (52–55). Because ADAM10 activation induced by EGCG treatment may be regulated by such tyrosine phosphorylation, whether induced directly or receptor mediated, we treated SweAPP N2a cells with the tyrosine phosphatase inhibitor phen (potassium bisperoxo(1,10-phenanthroline)oxovanadate) in the presence of EGCG. We found that phen dramatically reduced mature ADAM10 and maturation of ADAM10 mediated by EGCG as well as reduced α-secretase processing of APP in the presence of EGCG (data not shown). Co-treatment of these cells with okadaic acid and EGCG failed to result in significant alteration of APP α-secretase processing or ADAM10 maturation, suggesting that serine/threonine phosphatases may not be required for EGCG enhancement of ADAM10 maturation and subsequent APP α-secretase cleavage (data not shown).

In summary, we have demonstrated that EGCG induces ADAM10 maturation and promotes non-amyloidogenic α-secretase processing of APP in CNS cells. We have further suggested that α-secretase processing of APP is primarily mediated by ADAM10 and not ADAM9 or -17. These findings provide mechanistic insight into the use of EGCG as a therapeutic strategy for AD, and suggest that this and other pharmacotherapeutic strategies that promote ADAM10 activity may be beneficial in reducing amyloidogenic processing of APP and consequent cerebral amyloidosis associated with AD. However, future studies are needed to determine the molecular mechanism(s) whereby proprotein convertases and/or tyrosine phosphorylation may regulate EGCG activation of ADAM10 and enhancement of α-secretase APP processing.

Acknowledgments—We thank S. Gandy (Thomas Jefferson University) and Heiner (Adolf Butenandt-Institute, Ludwig-Maximilians University) for providing antibodies against the carboxyl terminus of APP (antibody 369) and S. Gandy for providing the N2a cell lines that stably overexpress human Swedish-mutant APP-695 or wild-type APP-695. We extend our gratitude to Dr. Paola Ricciardi-Castagnoli (Cellular Pharmacology Center, Milano, Italy) for providing the murine microglial cell line (N9).

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