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Cytosolic Prostaglandin E Synthase: Expression Patterns in Control and Alzheimer's Disease Brains

Uzma A. Chaudhry, MD and Sylvain Doré, PhD

Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Abstract

Anti-inflammatory drugs reduce the risk of Alzheimer's disease but fail to slow its progression. Studying the expression of prostaglandin E₂ synthases downstream of cyclooxygenase-2 is important. Here, the expression patterns of cytosolic prostaglandin E₂ synthases, an immediate prostaglandin E₂ source was investigated. Sections taken from the middle frontal gyrus of brains of 10 patients with Alzheimer's and 5 age-matched controls were examined by immunostaining for the presence of the synthases. Immunofluorescence analysis of control brains showed that cytosolic prostaglandin E₂ synthases co-localize with microglia, neurons, and endothelium markers, but not with astrocytes or smooth muscle cells. Immunohistochemical staining for the synthases was positive in the pyramidal neurons of controls but barely detectable in the brain of Alzheimer's patients. These findings revealed that cytosolic prostaglandin E₂ synthases is found in microglia, neurons, and endothelium of control human middle frontal gyrus and that its levels decrease in pyramidal cells of Alzheimer's disease brains.

Keywords

cyclooxygenase; drug development; eicosanoid; human; inflammation; prostaglandin E₂

Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative disease characterized by dementia and a progressive decline of cognitive abilities, such as learning and memory. Clinicopathologic features of this disease include loss of cholinergic neurons; accumulation of the neurotoxic peptide beta amyloid (A β), which forms amyloid plaques; and abnormally phosphorylated tau protein, which creates neurofibrillary tangles.^{1,2}

Prostaglandin (PG) E₂ is of interest in AD because it is involved in inflammation and has been shown to be neuroprotective in the presence of A β .³ It is derived from the stimulus-induced liberation of arachidonic acid from glycerophospholipid cell membranes via phospholipase A₂ (PLA₂). Subsequently, arachidonic acid is converted by cyclooxygenase (COX)-1 or COX-2 to prostaglandin G₂ (PGG₂), and then to prostaglandin H₂ (PGH₂), which is converted to various prostanoids via their respective terminal prostanoid synthases. Three types of terminal synthases for the production of prostaglandin E₂ (PGE₂) have been characterized: (1) microsomal prostaglandin E synthase-1 (mPGES-1), an inducible glutathione (GSH)-dependent perinuclear enzyme that is preferentially functionally coupled

to COX-2; (2) mPGES-2, a constitutively expressed, GSH-independent enzyme found both perinuclearly and cytoplasmically that is functionally coupled to COX-1 and COX-2; and (3) cytosolic prostaglandin E synthase (cPGES), a ubiquitously and constitutively expressed cytoplasmic GSH-dependent enzyme that is functionally coupled to COX-1.⁴ Cytosolic prostaglandin E synthase is also found in a perinuclear form that is preferentially functionally coupled to COX-2.⁵ The *cPGES* gene has many acronyms, but the official gene name is *PTGES3* (prostaglandin E synthase-3).⁶ The purpose of this study was to investigate the cPGES expression patterns in the brains of control humans and those of individuals with AD. To our knowledge, this study is the first to describe the expression patterns of cPGES in control human and AD brain tissue.

Participants and Methods

Middle frontal gyrus (MFG) brain tissue of 5 controls and 10 patients with AD was obtained from the Johns Hopkins Brain Resource center. Informed consent was granted by patients before the time of death.⁷ Table 1 shows the demographic and pathological information on each tissue specimen. Alzheimer's disease diagnosis was established by the Consortium to Establish a Registry of AD (CERAD) criteria.⁸ Staging was evaluated neuropathologically via Braak scoring.⁹ Frozen brain sections were first blocked in 10% normal goat serum (NGS) and then incubated overnight at 4° C in polyclonal anti-rabbit cPGES antibody (1:1500; Cayman Chemical, Ann Arbor, Mich) paired with antibodies to microglia (monoclonal anti-rat CD11b [1:1000; Serotec, Kidlington, Oxford, UK]), astrocytes (monoclonal anti-rat glial fibrillary protein [GFAP; 1:500; Zymed, San Francisco, Calif]), neurons (monoclonal anti-mouse SMI-32 [1:1000; Sternberger Monoclonals, Lutherville, Md]), endothelium (monoclonal anti-mouse von Willebrand factor [vWb; 1:200; Vector Laboratories, Burlingame, Calif]), and smooth muscle cell (monoclonal anti-mouse SMC [1:200; Zymed, San Francisco, Calif]). Sections were subsequently incubated at room temperature for 4 hours with the corresponding secondary antibodies: goat anti-rat immunoglobulin G (IgG) or red Cy3-conjugated affinipure goat anti-mouse IgG (1:200; Jackson IR Laboratories, Bar Harbor, Me) and green Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:500; Molecular Probes, Carlsbad, Calif). Paraffinized sections were deparaffinized and hydrated. Antigen retrieval was achieved by microwaving slides in boiling 1 M citric acid (pH 6). Next, endogenous peroxides were removed by immersing slides in 0.3% H₂O₂ and 1% methanol in tris-buffered saline (TBS) for 30 minutes. The sections were first incubated with 0.3% Triton X-100 and 10% NGS in TBS, then incubated overnight at 4° C in primary antibody against cPGES (1:1500), and then for 1 hour in secondary antibody (goat anti-rabbit IgG, Vector Laboratories). Subsequently, the sections were incubated for 1 hour in VectaElite ABC complex (Vector Laboratories) and then for 5 minutes in diaminobenzadine tetrahydrochloride (Vector Laboratories). Nonspecific nuclei were stained with Mayer's hematoxylin to distinguish the background from cells, as previously described.⁷ All antibodies used were cross-reactive with human tissue.

Results

Immunofluorescence revealed that in control brain sections, cPGES co-localized with markers for neurons (Figure 1A-C), microglia (Figure 1D-F), and endothelial cells (Figure 1G-I) but not astrocytes (Figure 1J-L) or smooth muscle cells (Figure 1M-O). Negative control brain sections showed red and green nonspecific and/or autofluorescence (data not shown), but it was distinguishable from that of the immunostained specimens.

Immunostaining for cPGES in fixed tissue was positive in neurons from 4 of 5 control brains (Figure 1Q1, 1Q2, 1S, and 1U). However, staining was barely detectable in the neurons of familial AD (Figure 1S) and sporadic AD (Figure 1U) brains (Table 1).

Discussion

Cytosolic PGES is widespread throughout the nervous system and potentially provides a more immediate source of PGE₂ than the COX-2/mPGES-1 pathway.¹⁰ To our knowledge, this qualitative study is the first to describe the expression patterns of cPGES in control human and AD brain tissue. Other studies regarding COX-1 and COX-2 that are similar and complementary to ours also have attempted to find patterns of expression using immunohistochemistry.¹¹ In our immunofluorescence study, we showed that in control aged brains, cPGES co-localizes with markers for microglia, neurons, and endothelium but not with those for astrocytes or smooth muscle cells. In our immunohistochemical study, cPGES immunoreactivity was weak in the pyramidal neurons of brains of patients with AD compared to that in normal controls, suggesting diminished cPGES levels. The immunoreactivity of other cells is unknown because we were able to identify only neurons by shape and did not stain for specific cells. Similar reductions in COX-2 expression have been reported in hippocampal subfields of patients with end-stage AD; however, the tissue we used was from a different brain region.¹² Furthermore, we do not know whether cPGES levels diminish throughout the progression of the disease because we used only patients with end-stage AD. However, reduced cPGES levels in AD could result in the brain having a slower and less efficient regulation of PGE₂, particularly in maintenance functions.

Cytosolic PGES is distributed throughout most of the brain in mice. According to the Allen Brain Atlas,¹³ gene expression density of cPGES is 100% in the cortex, hypothalamus, medulla, olfactory areas, pallidum, striatum-like amygdalar nuclei, and the retrohippocampal region but is lower in the rest of the brain, with the least being 42% in the cerebellum. The levels of cPGES protein are 100% in the cortex, hippocampus, hippocampal formation, and medulla, with less in the remainder of the brain, the least being 32% in the striatum dorsal region (Table 2). In normal humans, cPGES has been found to be present in the frontal lobe on Western blot.¹⁴ In our study, we specifically used the MFG, which is a part of the frontal lobe that is affected in end-stage AD.

Previously, we have shown that mPGES-1 is elevated in the MFG of patients with AD⁷; however, these increases may not produce enough PGE₂ to compensate for the apparent end-stage loss of cPGES. A previous study reported that unlike mPGES-1 and mPGES-2, cPGES is not present in dendritic spines of mice,¹⁵ indicating that loss of cPGES might not affect synaptic activity. However, the reduction of cPGES in rat spinal cord diminished early responses in nociceptive processing.¹⁶ If cPGES also is downregulated in the spinal neurons of patients with AD, nociception in these patients could be affected, as some clinical studies have suggested.^{17,18}

Another study using immunohistochemistry and Western blot analysis of postmortem brains showed that cPGES was significantly reduced in the frontal cortex of patients with bipolar disorder, major depression, and schizophrenia, although COX-1 and COX-2 were left unaffected.¹⁴ The authors also reported that postmortem patients that had been on mood stabilizers during life had near-normal levels of cPGES.¹⁴ Those results could indicate that decreased cPGES can also be found in other neurological disorders and that conventional drug treatments might help slow the progression of AD. Additionally, cPGES knockout mice are not viable, emphasizing its importance to life.¹⁹ Interestingly, PGE₂ has been found to be inexplicably diminished in the cerebrospinal fluid late in AD progression,²⁰ a finding that further supports the decrease in cPGES as shown in our study.

Cytosolic PGES activity depends on GSH and association with hsp90 and casein kinase II (CKII), forming the cPGES-CKII-hsp90 complex.²¹ CKII has also been found to preferentially phosphorylate amino-terminal inserts of human tau isoforms.²² In AD, tangle-

bearing hippocampal neurons with strong anti-tau immunoreactivity showed a 22% increase in anti-CKII immunoreactivity compared to control.²³ Casein kinase II may become more involved in tau hyperphosphorylation, making ATP and CKII less available to bind to cPGES for maximal PGE₂ formation.²⁴ Additionally, NMDA (N-methyl-D-aspartic acid), which is believed to be elevated in AD, can diminish phosphorylation in aged but not young rats.²⁵ This activity may further diminish amounts needed for full cPGES activation.

Finally, it is possible that during chronic neuroinflammatory states such as AD, elevation of mPGES-2, mPGES-1, or the PGE₂ product itself⁷ might trigger a reduction in cPGES levels. For the most part, COX-2, but not COX-1, is believed to be present in neurons of the temporal and frontal cortex and especially elevated in AD.¹¹ Therefore, mPGES-2 might take over the role of cPGES in producing constitutive levels of PGE₂, as cPGES is primarily functionally coupled to COX-1.

In conclusion, we have demonstrated a pattern of little to no cPGES immunoreactivity in AD brains in comparison to normal age-matched control brains, which stained strongly positive for cPGES. Identifying the patterns of cPGES expression in the end and earlier stages of AD could lead to novel pharmacotherapeutics to preserve neurons in AD. The ability to preserve neuronal health will ultimately be important for slowing AD progression and preserving the quality of life.

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Figure 1.

Localization of cytosolic prostaglandin E synthase (cPGES) in control and Alzheimer's disease (AD) brain tissue. Immunofluorescence was used to determine cPGES (green) co-localization with various cell types (red) in control brains. Yellow areas indicate areas of co-localization. Cytosolic prostaglandin E synthase-1 co-localized with the neuronal marker SMI32 (A-C), the microglial marker CD11b (D-F), and the endothelial cell marker vWB (G-I; $\times 40$). Cytosolic prostaglandin E synthase (cPGES) did not appear to co-localize with the astrocytic marker (GFAP; J-L; $\times 40$) or the smooth muscle cell marker (SMC; M-O; $\times 40$). All scale bars represent 50 μ m. Images in A-F and J-O were obtained with a SPOT camera, whereas the images in G-I were obtained with a Nikon camera.

Immunohistochemical analysis of control brain sections used as negative controls show negligible staining (P1-P2). Control brain sections showing positive staining with cPGES antibody (Q1-Q2; $\times 40$). Neurons, but not astrocytes or microglia, are visible. Negative control section from a familial AD brain (R). Brain section from a familial AD participant is positively stained by cPGES antibody (S). The staining is primarily in pyramidal-shaped neurons and appears to be less intense than that seen in the control section ($\times 40$). Negative control section from a sporadic AD brain (T). A brain section from a participant with sporadic AD participant was immunostained with cPGES antibody (U); the intensity does not appear to be different from that of the negative control ($\times 40$). GFAP indicates glial fibrillary acidic protein. The scale bar represents 100 μm .

Table 1

Case Demographics and Neuronal cPGES Intensity

Diagnosis	Age (Years)	Sex	PMT (hours)	CERAD	Braak Stage	cPGES Staining
SAD	62	F	11.0	C	6	+
SAD	72	F	19.0	C	6	-
SAD	80	F	12.0	C	5	-
SAD	82	F	6.0	C	6	-
SAD	89	M	9.5	C	6	-
FAD	63	M	9.0	C	6	+
FAD	74	F	5.5	C	6	+
FAD	78	M	4.0	C	6	+
FAD	92	F	8.0	C	6	+
FAD	94	F	4.0	C	6	+
Control	68	M	10.0	-	2	++
Control	71	F	16.0	-	0	+++
Control	74	M	4.0	-	2	++
Control	91	F	8.0	-	1	++
Control	80	F	8.0	-	0	++

Abbreviations: cPGES, cytosolic prostaglandin E synthase; PMT, postmortem time; CERAD, consortium to establish a registry of AD; AD, Alzheimer's Disease; SAD, sporadic AD; FAD, familial AD; -, no staining; +, weak staining; ++, moderate staining; +++, strong staining.

Table 2

Percent Expression Density and Level Values for *PTGES3* from the Allen Brain Atlas¹³

Area	CB	CTX	HIP	HPF	HY	LSX	MB	MY	OLF	P	PAL	sAMY	STR	STRd	STRv	TH	RHP
Density (%)	42	100	75	90	100	100	94	100	100	91	100	100	73	61	79	88	100
Level (%)	96	100	100	100	75	62	75	100	77	90	89	82	40	32	42	63	87

Abbreviations: CB, cerebellum; CTX, cerebral cortex; HIP, hippocampus; HPF, hippocampal formation; HY, hypothalamus; LSX, lateral septal complex; MB, midbrain; MY, medulla; OLF, olfactory areas; P, pons; PAL, pallidum; sAMY, striatum-like amygdalar nuclei; STR, striatum; STRd, striatum dorsal region; STRv, striatum ventral region; TH, thalamus; RHP, retrohippocampal region.