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Prostaglandin E_2 regulates amyloid precursor protein expression via the EP2 receptor in cultured rat microglia

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Abstract

We investigated the effects of prostaglandin E_2 (PGE_2) on amyloid precursor protein (APP) expression in cultured rat microglia. PGE_2 treatment significantly increased the expression of APP holoprotein and was associated with an elevation in cyclic AMP (cAMP). Direct activation of adenylate cyclase with forskolin also increased APP expression. Co-treatment of microglia with PGE_2 and the PKA inhibitor H-89 suppressed the overexpression of APP caused by PGE_2 alone. The prostaglandin EP2 receptor is known to be positively coupled to cAMP production. Stimulation of the EP2 receptor with butaprost increased APP holoprotein, whereas co-incubation of the cells with PGE_2 and the EP2 receptor antagonist AH-6809 blocked the effect of PGE_2 on APP expression. These data suggest that PGE_2 is able to regulate the expression of APP, and that this effect may be mediated by the EP2 receptor and the cAMP signaling cascade.
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The neurodegenerative disorder Alzheimer's disease (AD) is characterized by cognitive decline and memory loss. The accumulation of extracellular amyloid plaques is a hallmark of AD. Amyloid β (Aβ), the principal component of such plaques, is derived by proteolytic cleavage of the amyloid precursor protein (APP) by the β- and γ-secretases. APP may alternatively undergo a single cleavage by the α-secretase within the Aβ region, causing the formation of the non-amyloidogenic soluble APP fragment and thereby preventing the formation of Aβ [3,18].

Brain injury, a major risk factor for AD [4,12,22], is associated with APP overexpression [19] and an increase in cerebrospinal fluid (CSF) Aβ levels [15]. Trauma to the brain can activate an inflammatory pathway that involves the release of arachidonic acid from cellular phospholipids by cytosolic phospholipase A_2 (PLA_2) [17], and its subsequent cyclooxygenation to form prostaglandin E_2 (PGE_2). In brains of AD patients, the activation of such components of this pathway as PLA_2 [21] and cyclooxygenase-2 (COX-2), the inducible enzyme responsible for prostaglandin production in response to injury or inflammation, is enhanced [14]. Moreover, PGE_2 levels are elevated in the CSF of patients thought to have AD [11].

In rat astrocytes, PGE_2 stimulates the overexpression of APP. This effect is probably mediated by the cyclic AMP (cAMP) signaling pathway [8]. Moreover, studies have shown that treatments that increase cAMP levels may enhance the formation of amyloidogenic Aβ [6,9]. PGE_2 can interact with the cAMP pathway by stimulating the G protein-coupled EP2 receptor, which activates adenylate cyclase and increases cAMP production [1]. EP2 receptors are expressed by microglia [2], the primary mediators of brain inflammation. Microglia are activated by neuronal damage [7,13] and therefore may be involved in the overexpression of APP observed following brain trauma. In the present study, we examined the relationship of the inflammatory mediator PGE_2 to production of APP in rat microglia.

Microglial cells were prepared by agitating primary cultured astrocytes and replating the detached microglia onto fresh, uncoated culture dishes using procedures similar to those described by Giulian and Baker [5]. Briefly, dissociated astrocytes were cultured from cortices of rat pups (postnatal day 1–2) as previously described [10]. Cells

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from dissociated cortices were plated onto poly-L-lysine-coated 35 mm culture dishes. The initial culture medium, Minimal Essential Medium (MEM, Invitrogen, Carlsbad, CA) containing 5% horse serum (Cambrex, Rockland, ME), was aspirated 2–5 h after plating to remove unattached cells and debris, and replaced with MEM containing 5% fetal bovine serum (FBS, Cambrex, Rockland, ME). Half the medium was replaced with MEM/10% FBS twice weekly. The cells were kept at 37 °C in a humidified 5%CO_2/95% air incubator for 10–14 days, by which time the cultures were confluent.

Floating microglia were harvested from the underlying monolayer of astrocytes by gentle shaking of the cell culture dishes, seeded onto uncoated 75 cm^2 culture flasks and maintained in MEM/5% FBS for 2 weeks. When the microglia were 60–90% confluent, they were detached by trypsin and harvested. Cells were then plated onto six-well plates and maintained in MEM/5% FBS for approximately 2 weeks; by this time the cultures were confluent and ready for use in experiments.

The following drugs were used: PGE_2, cycloheximide and forskolin (Calbiochem, San Diego, CA); H-89 dihydrochloride, Sp-cAMP triethylamine, butaprost and AH6809 (Sigma, St. Louis, MO). Frozen aliquots of these drugs were diluted in serum-free MEM (37 °C) to appropriate concentrations. Experiments were conducted in triplicate unless otherwise stated.

Cell-associated APP levels were measured in cultured microglia grown on six-well plates. The medium was aspirated, and microglia were scraped in 100 μl lysis buffer (60 mM Tris–HCl, 4% SDS, 20% glycerol, 1 mM dithiothreitol, 1 mM AEBSF, 8 μM aprotinin, 500 μM bestatin, 15 μM E64, 200 μM leupeptin, 10 μM pepstatin A). The total amount of protein in each sample, estimated by the bicinchoninic acid assay (Sigma, St. Louis, MO), was not altered by pharmacological treatments. Prior to gel electrophoresis, bromphenol blue solution (0.07%) was added to each sample. The amount of cell protein loaded for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4–20% SDS PAGE; Bio-Rad, Hercules, CA) was normalized to the amount of protein per sample. Proteins (equivalent to approximately 50 μg cell protein/lane) were separated by electrophoresis, electroblotted onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Billerica, MA), and blocked in bovine serum albumin for 1 h. After 3 × 10 min rinses in Tris-buffered saline (TBST), the membranes were incubated in TBST containing an appropriate antibody. Monoclonal antibody 22C11 (Roche, Indianapolis, IN) was used to detect the N-terminus of APP. Antibodies against glial fibrillary acid protein (GFAP; Roche, Indianapolis, IN) and CD11b/membrane attack complex 1 (MAC-1; Serotec, Raleigh, NC) were used to confirm that the cells were microglial. Cells used in this study did not express the astrocytic marker GFAP, but did express, when activated by PGE_2 or lipopolysaccharide, the microglial marker CD11b/MAC-1 (data not shown). Protein–antibody complexes were detected and visualized using the ECL system (Amersham, Piscataway, NJ) and Kodak X-AR film, respectively, as suggested by the manufacturer. Films were digitized using a Supervista S-12 scanner with a transparency adapter (UMAX Technologies, Freemont, CA). Analysis was performed using the public domain NIH Image program (developed at the U.S. National Institute of Health), available on the internet at http://rsb.info.NIH.gov/NIH-IMAGE/. Levels of cAMP were measured in rat microglia grown on 35 mm dishes using an [8-^{3}H]cAMP assay kit (Amersham, Piscataway, NJ) according to the manufacturer's instructions. Data were analyzed using analysis of variance (ANOVA), and the Newman–Keuls test was used to evaluate differences between groups (significance level, $P < 0.05$), with drug treatments as the independent variable. Data are presented as means ± SEM.

Microglia were treated for 24 h with 0.1, 1 or 10 μM PGE_2, which produced dose-dependent and significant increases in levels of APP holoprotein, relative to those in controls (Fig. 1a,b). Cycloheximide (1 μM), a protein synthesis inhibitor, suppressed the increase in APP holoprotein stimulated by PGE_2 (data not shown). In order to determine how PGE_2 affects APP production, we next assessed the effect of PGE_2 on the second messenger cAMP.

PGE_2 treatment (1 or 10 μM) significantly increased cellular cAMP levels in the cultured microglia (Fig. 2), suggesting that PGE_2 may exert its effect on APP via the cAMP signaling pathway. The effect of PGE_2 on APP was mimicked by direct activation of the cAMP pathway: forskolin (1 or 10 μM), an activator of adenylate cyclase, significantly increased APP expression in the microglia (Fig. 3). Activation of protein kinase A (PKA) by sp-cAMP triethylamine also significantly increased APP levels relative to those in control cells (data not shown). Furthermore, the increase in APP levels caused by PGE_2

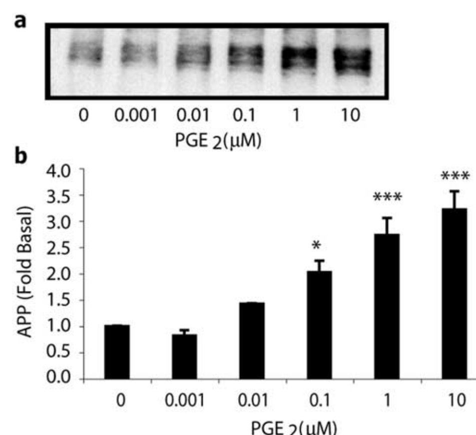

Fig. 1. PGE_2 treatment increases cellular APP levels in rat microglia. (a) APP expression was determined by Western blotting using the antibody 22C11. (b) Treatment with 0.1, 1 and 10 μM PGE_2 for 24 h stimulated significant increases in levels of cellular APP holoprotein. Values represent means ± SEM. *$P < 0.05$, ***$P < 0.001$ vs. control.

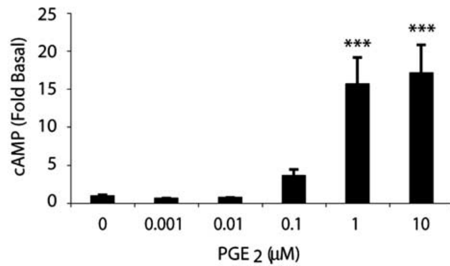

Fig. 2. PGE_2 treatment increases cAMP production in cultured rat microglia. Intracellular cAMP levels were significantly enhanced after 1 h treatment with 1 and 10 µM PGE_2. Values represent means ± SEM. ***$P < 0.001$ vs. control.

treatment was significantly inhibited by co-treatment with the PKA inhibitor H-89 (10 µM); treatment with H-89 alone had no effect on basal APP levels (data not shown). Taken together, these results suggest that PGE_2 may increase APP production by stimulating the cAMP signaling pathway. To determine how PGE_2 may be affecting cAMP levels, we examined the role of prostaglandin EP2 receptors, which are known to be positively coupled to cAMP formation [13].

We found that stimulation of microglial EP2 receptors for 24 h by the EP2 receptor-specific agonist butaprost significantly and dose-dependently increased APP expression (Fig. 4a). Moreover, co-incubation of microglial cells with PGE_2 and the EP2 receptor antagonist AH6809 dose-dependently suppressed the effect of PGE_2 on APP overexpression (Fig. 4b). Both of these findings support the hypothesis that EP2 receptors may mediate PGE_2-induced APP overexpression.

These data show that the inflammatory mediator PGE_2 increases APP production in primary cultured rat microglia possibly via activation of the cAMP signaling pathway. The stimulatory effect of PGE_2 on APP overexpression was suppressed by the PKA inhibitor H-89. Since H-89 did not fully inhibit the effect of PGE_2, it is possible that the cAMP produced following PGE_2 stimulation may also increase the expression of APP via a PKA-independent mechanism [20]. One such pathway could involve cAMP binding to, and activation of, the guanine nucleotide exchange factor Epac [16]. The unavailability of selective Epac inhibitors at this time makes exploring this possibility difficult.

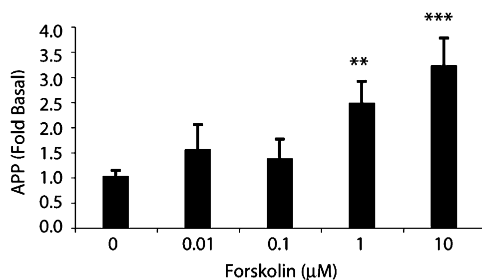

Fig. 3. APP expression in cultured rat microglia is increased following treatment with the protein kinase A activator forskolin. Treatment with 1 and 10 µM forskolin for 24 h stimulated significant increases in intracellular APP levels. Values represent means ± SEM. **$P < 0.01$, ***$P < 0.001$ vs. control.

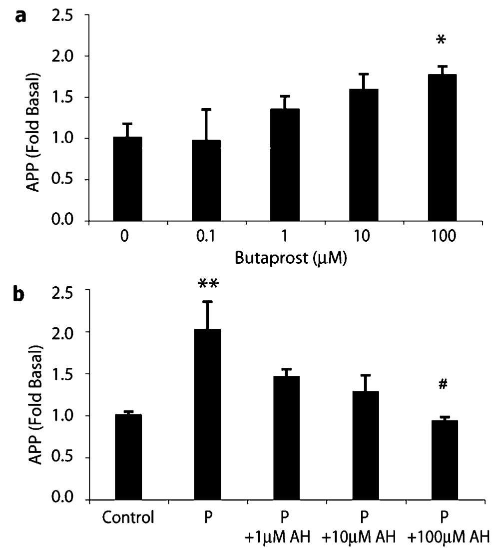

Fig. 4. The EP2 receptor can modulate APP expression. (a) Cells were treated for 24 h with the EP2 receptor agonist butaprost. Treatment with 100 µM butaprost significantly increased APP expression. (b) Co-treatment of the cells with PGE_2 (P) plus the EP2 receptor antagonist AH6809 (AH) inhibited the overexpression of APP caused by PGE_2 treatment alone. Values represent means ± SEM. *$P < 0.05$, **$P < 0.01$ vs. control; $^{\#}P < 0.05$ vs. PGE_2 treated.

These data suggest that the effect of PGE_2 on APP production involves the cAMP signaling pathway, at least in part, and that the prostaglandin receptor EP2, which is coupled to cAMP activation, might mediate the effect of PGE_2 on APP expression. Treatment of microglia with butaprost, an EP2 receptor agonist, increased APP levels, which suggests that stimulation of the EP2 receptor is sufficient to affect APP expression. Incubation of the cells with AH6809, an EP2 receptor antagonist, fully blocked the APP overexpression caused by PGE_2. These findings provide the first evidence that the stimulatory effect of PGE_2 on APP levels may involve activation of the cAMP signaling cascade by the binding of PGE_2 to the prostaglandin receptor EP2. Our results are consistent with the idea that microglia may contribute to the neuropathology of AD, possibly by increasing APP expression in response to inflammatory prostaglandins acting at the microglial EP2 receptor.

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References

[1] S. An, J. Yang, M. Xia, E.J. Goetzl, Cloning and expression of the EP2 subtype of human receptors for prostaglandin E2, Biochem. Biophys. Res. Commun. 197 (1993) 263–270.

[2] A.O. Caggiano, R.P. Kraig, Prostaglandin E receptor subtypes in cultured rat microglia and their role in reducing lipopolysaccharide-induced interleukin-1beta production, J. Neurochem. 72 (1999) 565–575.

[3] F.S. Esch, P.S. Keim, E.C. Beattie, R.W. Blacher, A.R. Culwell, T. Oltersdorf, D. McClure, P.J. Ward, Cleavage of amyloid beta peptide during constitutive processing of its precursor, Science 248 (1990) 1122–1124.

[4] S. Fleminger, D.L. Oliver, S. Lovestone, S. Rabe-Hesketh, A. Giora, Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication, J. Neurol. Neurosurg. Psychiatry 74 (2003) 857–862.

[5] D. Giulian, T.J. Baker, Characterization of ameboid microglia isolated from developing mammalian brain, J. Neurosci. 6 (1986) 2163–2178.

[6] A. Kumar, F.G. La Rosa, A.R. Hovland, W.C. Cole, J. Edwards-Prasad, K.N. Prasad, Adenosine $3',5'$-cyclic monophosphate increases processing of amyloid precursor protein (APP) to beta-amyloid in neuroblastoma cells without changing APP levels or expression of APP mRNA, Neurochem. Res. 24 (1999) 1209–1215.

[7] N. Laflamme, S. Rivest, Effects of systemic immunogenic insults and circulating proinflammatory cytokines on the transcription of the inhibitory factor kappaB alpha within specific cellular populations of the rat brain, J. Neurochem. 73 (1999) 309–321.

[8] R.K. Lee, S. Knapp, R.J. Wurtman, Prostaglandin E2 stimulates amyloid precursor protein gene expression: inhibition by immunosuppressants, J. Neurosci. 19 (1999) 940–947.

[9] P. Marambaud, N. Chevallier, K. Ancolio, F. Checler, Post-transcriptional contribution of a cAMP-dependent pathway to the formation of alpha- and beta/gamma-secretases-derived products of beta APP maturation in human cells expressing wild-type and Swedish mutated beta APP, Mol. Med. 4 (1998) 715–723.

[10] K.D. McCarthy, J. de Vellis, Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue, J. Cell Biol. 85 (1980) 890–902.

[11] T.J. Montine, K.R. Sidell, B.C. Crews, W.R. Markesbery, L.J. Marnett, L.J. Roberts 2nd, J.D. Morrow, Elevated CSF prostaglandin E2 levels in patients with probable AD, Neurology 53 (1999) 1495–1498.

[12] J.A. Nicoll, G.W. Roberts, D.I. Graham, Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury, Nat. Med. 1 (1995) 135–137.

[13] M. Overmyer, S. Helisalmi, H. Soininen, M. Laakso, P. Riekkinen Sr., I. Alafuzoff, Reactive microglia in aging and dementia: an immunohistochemical study of postmortem human brain tissue, Acta Neuropathol. 97 (1999) 383–392.

[14] G.M. Pasinetti, P.S. Aisen, Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain, Neuroscience 87 (1998) 319–324.

[15] C.A. Raby, M.C. Morganti-Kossmann, T. Kossmann, P.F. Stahel, M.D. Watson, L.M. Evans, P.D. Mehta, K. Spiegel, Y.M. Kuo, A.E. Roher, M.R. Emmerling, Traumatic brain injury increases beta-amyloid peptide 1-42 in cerebrospinal fluid, J. Neurochem. 71 (1998) 2505–2509.

[16] H. Rehmann, F. Schwede, S.O. Doskeland, A. Wittinghofer, J.L. Bos, Ligand-mediated activation of the cAMP-responsive guanine nucleotide exchange factor Epac, J. Biol. Chem. 278 (2003) 38548–38556.

[17] E. Shohami, Y. Shapira, G. Yadid, N. Reisfeld, S. Yedgar, Brain phospholipase A2 is activated after experimental closed head injury in the rat, J. Neurochem. 53 (1989) 1541–1546.

[18] S.S. Sisodia, E.H. Koo, K. Beyreuther, A. Unterbeck, D.L. Price, Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing, Science 248 (1990) 492–495.

[19] C. Sola, F.J. Garcia-Ladona, G. Mengod, A. Probst, P. Frey, J.M. Palacios, Increased levels of the Kunitz protease inhibitor-containing beta APP mRNAs in rat brain following neurotoxic damage, Brain Res. Mol. Brain Res. 1–2 (1993) 41–52.

[20] K.J. Staples, M. Bergmann, K. Tomita, M.D. Houslay, I. McPhee, P.J. Barnes, M.A. Giembycz, R. Newton, Adenosine $3',5'$-cyclic monophosphate (cAMP)-dependent inhibition of IL-5 from human T lymphocytes is not mediated by the cAMP-dependent protein kinase A, J. Immunol. 167 (2001) 2074–2080.

[21] D.T. Stephenson, C.A. Lemere, D.J. Selkoe, J.A. Clemens, Cytosolic phospholipase A2 (cPLA2) immunoreactivity is elevated in Alzheimer's disease brain, Neurobiol. Dis. 3 (1996) 51–63.

[22] K. Uryu, H. Laurer, T. McIntosh, D. Praticò, D. Martinez, S. Leight, V.M.-Y. Lee, J.Q. Trojanowski, Repetitive mild brain trauma accelerates $A\beta$ deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis, J. Neurosci. 22 (2002) 446–454.