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PGE₂ EP1 receptor exacerbated neurotoxicity in mouse model of cerebral ischemia and Alzheimer's disease

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Abstract

Stroke and Alzheimer's disease (AD) are major age-related neurodegenerative diseases that may worsen the prognosis of each other. Our study was designed to delineate the prostaglandin E₂ EP1 receptor role in AD and in the setting of cerebral ischemia. Genetic deletion of the prostaglandin EP1 receptor significantly attenuated the more severe neuronal damage ($38.5 \pm 10.6\%$) and memory loss induced by ischemic insult that observed in AD transgenic mice (percentage of viable hippocampal CA1 neurons: $11.2 \pm 2.9\%$) when compared to wildtype mice ($45.1 \pm 9.1\%$). In addition, we found that the amyloid plaques were reduced in EP1 deleted AD mice. A β -induced toxicity ($18.0 \pm 7.1\%$) and Ca²⁺ response ($91.8 \pm 12.9\%$) were also reduced in EP1^{-/-} neurons compared to control neurons in *in vitro*. Hence, EP1 might mediate most of the toxicity associated with COX-2 and contribute substantially to the cell death pathways in AD and stroke. Exploring potential therapeutic agent targeting EP1 receptor could potentially benefit treatments for stroke and AD patients.

Keywords

Beta-amyloid; Cyclooxygenase; Neuroinflammation; Prostaglandin E₂; Stroke

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1. Introduction

Accumulating evidence linking stroke and Alzheimer's disease (AD) indicates that each exacerbates the severity of the other (Jendroska et al., 1995; Koistinaho and Koistinaho, 2005). Because there are few effective treatments for AD, especially when stroke supervenes in these patients, finding a common pathway that exacerbates neuronal loss and brain damage might provide a potential therapeutic target. Over the years, prostaglandin E_2 (PGE₂) and cyclooxygenase-2 (COX-2), the rate-limiting enzyme responsible for prostaglandin production, have become largely discussed targets for exploration in therapy for AD and stroke patients (Ahmad et al., 2008; Bazan et al., 2002; Santovito et al., 2009). Clinical use of non-steroidal anti-inflammatory drugs or selective COX inhibitors is associated with various concerning side effects (Donnelly and Hawkey, 1997; Ray et al., 2004); thus, studying the downstream pathway may provide a unique therapeutic target which would allowed to be more selective and at the same time not simply blocking synthesis of all prostaglandins. Previously, the EP1 receptor expression in CNS neurons has been documented in the literature; for example, signals for EP1 receptor mRNA have been exposed in C57BL/6 mouse brain e.g. www.brain-map.org, and the EP1 receptor has also be documented by various independent teams at the protein level in the CNS and on neuronal cells (Kawano et al., 2006; Carlson et al., 2009). Additionally, previous studies by others and by us have provided physiological evidence that deletion of EP1 receptor significantly attenuates focal ischemic and excitotoxic brain damages and that treatment with selective receptor ligands provided significant anatomical and functional biological outcomes (Ahmad et al., 2006; Kawano et al., 2006). Thus, our study focuses on delineating the role of PGE₂ EP1 receptor in AD model in the setting of cerebral ischemia. Based on this team effort, we propose that the EP1 receptor may be critical in the inflammatory and cell death pathways that are both hallmarks in the neurodegenerative conditions of AD and stroke.

We used the APP^{swe}/PS1 Δ E9 double gene mutation (APP/PS1) AD mouse model and crossed them with EP1 gene knockout (EP1^{-/-}) mice, and explored the potential EP1 receptor role in stroke and AD-like conditions. We compared β -amyloid (A β) plaques, A β 40 and A β 42 levels, and behavioral outcomes of ischemia in APP/PS1xEP1^{-/-} and APP/PS1 mice in together with their none-AD control mice. Furthermore, to explore the potential mechanism of the neuroprotective effect of genetic knocking out the EP1 receptor, we investigated intracellular Ca²⁺ response in an A β -enriched environment in cultured neurons.

2. Materials and Methods

2.1. Animals

All animal protocols were approved by the Animal Care and Use Committee of Johns Hopkins University. Male APP/PS1 mice, APP/PS1xEP1^{-/-} mice, and their age-matched WT littermates were obtained from Dr. David Borchelt.

2.2. Cell Cultures, Ca²⁺ Imaging, A β toxicity, and Cell Survival

Neuronal cell cultures were prepared with the protocol described previously (Shah et al., 2010). The [Ca²⁺]_i was measured in neuronal cultures by using Fura-2 AM (Kim et al., 2007). After treatment with freshly prepared 2.5 μ M A β 42 for 16 h, neuronal cell survival was monitored.

2.3. Induction of Global Cerebral Ischemia, Assessment of Intracranial Vascular Structure, and Neurobehavioral Tests

Global ischemia and subsequent analysis of hippocampal damage were conducted as that previously published (Zhen and Dore, 2007). Every fifth section throughout the

hippocampus was sampled, and viable hippocampal CA1 neurons were counted in three serial hematoxylin and eosin-stained slides of the dorsal hippocampus (1.73 ± 0.15 mm posterior of the bregma) in each animal. The GEMINI apparatus (San Diego Instruments, San Diego, CA) was used to assess learning and memory via the step-through passive avoidance task.

2.4. Statistical Analysis

Based on the existence of PcomA, zero to two hemispheres per mouse were included in the statistical analysis. Data are expressed as mean $\pm$ S.E.M. Statistical analysis was performed by one-way ANOVA followed by Bonferroni post hoc test or Student's *t*-test. Significance was set at $P < 0.05$.

2.5. Additional Methodology

Detailed methods for Oxygen-glucose deprivation (OGD), intracellular Ca^{2+} imaging, the MTT assay, global cerebral ischemia, assessment of hippocampal injury, neurobehavioral testing, and immunohistochemistry are described in the Supplementary Methods.

3. Results

First, we compared A β plaque number, A β concentration and neurobehavior functions between naïve APP/PS1 and APP/PS1xEP1^{-/-} mice. Significantly lower A β burden in APP/PS1xEP1^{-/-} mice was evidenced by fewer plaques in the hippocampal area and a lower concentration of soluble A β 42 and A β 40 (Fig 1A). The reduced plaque load and A β levels may explain the benefits seen in memory function in APP/PS1xEP1^{-/-} mice (Fig. 1A). To ascertain whether the A β -mediated cell death mechanism is different in the absence of EP1, we exposed hippocampal slice cultures and neuronal cell cultures to A β 42. The CA1 regions in slice cultures and the cultured neurons from EP1^{-/-} mice each were significantly more resistant to A β 42 toxicity than were the corresponding WT controls (Fig. 1B). We then performed Ca^{2+} imaging in neuronal cell cultures to explore the potential mechanism by which EP1 receptor inhibition improves neuronal survival during A β -induced toxicity. The $[\text{Ca}^{2+}]_i$ and Ca^{2+} transients were increased after A β 42 treatment in WT controls; this increase was prevented by pretreatment with an EP1 receptor antagonist (SC51089) and blocked in EP1^{-/-} neurons (Fig. 1C).

We then asked whether the reduced A β burden in APP/PS1xEP1^{-/-} mice led to reduced injury development after cerebral ischemia. We approached this question *in vitro* by exposing cultured hippocampal slices from each of the mouse genotypes to OGD. Cell death was significantly decreased in EP1^{-/-} tissue ($P < 0.001$) and increased in the APP/PS1 tissue compared to WT controls ($P < 0.01$) but was significantly attenuated in the APP/PS1xEP1^{-/-} tissue compared to APP/PS1 tissue ($P < 0.001$; Fig. 2A).

To correlate our results with *in vivo* experiments, we assessed delayed hippocampal neuronal damage by subjecting mice to global forebrain ischemia. Similar to the *in vitro* findings, CA1 neuronal loss was much greater in the APP/PS1 than in the WT mice and was significantly attenuated in the APP/PS1xEP1^{-/-} mice compared to the APP/PS1 mice (Fig. 2B). Hippocampal damage caused by ischemic insult can lead to impaired memory retention, reflected in shortened response latency in the passive avoidance task (Sakurai et al., 2008). Correlating with the anatomical differences, APP/PS1 mice had impaired memory retention after ischemic insult compared to WT mice subjected to ischemia and sham-operated APP/PS1 mice. There was no statistical difference between the APP/PS1xEP1^{-/-} and APP/PS1 ischemia groups, but the APP/PS1xEP1^{-/-} mice tended to show greater memory retention (Fig. 2C). We also questioned whether A β burden increases after

ischemic insult in APP/PS1 mice and if so, whether this increase is attenuated in the absence of the EP1 receptor. We observed less intense A β immunostaining in the CA1 neurons of APP/PS1xEP1^{-/-} mice after ischemia compared to that of APP/PS1 mice (Fig. 2D). The ELISA assay revealed that the insoluble A β 42 concentration rose significantly after ischemia in APP/PS1 mice but remained unchanged in APP/PS1xEP1^{-/-} mice. Both the soluble and insoluble A β concentrations were higher in APP/PS1 mice than in APP/PS1xEP1^{-/-} mice post-ischemia (Fig. 2E). The increase of insoluble A β in APP/PS1 mice suggests a tendency for soluble A β to accumulate and potentially fibrillate after ischemia. Deletion of EP1 appeared to attenuate the increase of A β generation and accumulation.

4. Discussion

We observed that following transient global forebrain ischemia, APP/PS1 double mutant mice sustain more severe neuronal and functional damage than WT controls. This suggests that the A β burden is at least partially responsible for such outcomes. Knowing that brain levels of both COX-2 and PGE₂ are increased in AD and in stroke, we questioned whether the deletion of the EP1 receptor—which has been proposed to be a key modulator of COX-2/PGE₂-related neurotoxicity—would be sufficient to alleviate most of the negative outcomes. In our study, we provide the first evidence that genetic deletion of EP1 is sufficient to decrease basal and post-ischemic levels of A β in APP/PS1 mice. We discovered that hippocampal neuron cell death and neurobehavioral deficits after ischemic insult are attenuated in the APP/PS1^{-/-}xEP1 as compared to APP/PS1 mice. Isolated neurons and brain slice cultures revealed that neurons derived from EP1^{-/-} mice were more resistant to A β toxicity than those from WT mice. Neuronal Ca²⁺ signaling and Ca²⁺ homeostasis regulate multiple neuronal functions, including synaptic transmission, plasticity, and cell survival (Wojda et al., 2008). EP1 receptor inhibition prevented induction of Ca²⁺ signaling initiated by A β exposure by decreasing the frequency of Ca²⁺ transients and mitigating the increase in baseline Ca²⁺ levels. Prevention of A β -associated Ca²⁺ signaling in neurons by EP1 receptor inhibition is likely an additional pathway for the neuroprotective effects seen. Our study provides the rationale to further explore potential therapeutic agents targeting EP1 receptor in stroke and AD-like neuropathologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Effect of EP1 receptor on Aβ burden, Aβ42-induced toxicity and Ca²⁺ response in neurons. (A) The upper panel, from left to right: Representative images show Aβ staining in brain sections from APP/PS1 and APP/PS1xEP1^{-/-} mice; quantification of Aβ plaques in hippocampus (**P < 0.001); test latency in passive avoidance test (n = 12–19, *P < 0.05 vs. WT, #P < 0.05 vs. APP/PS1). The lower panel: soluble Aβ42 and Aβ40 concentrations by ELISA assay in APP/PS1xEP1^{-/-} and APP/PS1 mice (*P < 0.05, ***P < 0.001 vs. APP/PS1 soluble). (B) Fluorescence images of dead cells in cultured hippocampal slices. The left bar graph represents CA1 neuronal damage measured by PI fluorescence intensity in hippocampal slices culture (n = 20 slices per group, **P < 0.01). The right bar graph

represents the viability of neuronal cell cultures after exposure to A β 42 (* P < 0.05). (C) [Ca²⁺]_i changes after A β 42 treatment in WT cells (left trace), WT cells pretreated with EP1 receptor antagonist [SC51089 (WT+SC), center trace], and cells from EP1^{-/-} mice (right trace). The bar graph shows amplitudes of A β 42-induced [Ca²⁺]_i increases in each group (number of cells = 22–25, *** P < 0.001).

Fig. 2.

The effect of EP1 receptor on neuronal damage and A β changes after ischemia in an AD model. (A) Quantification of CA1 neuronal damage measured after OGD (** $P < 0.01$, *** $P < 0.001$ compared with WT group, ### $P < 0.001$ compared with APP/PS1 group, $n = 13-16$). (B) Quantification of neuronal viability in brain sections from post-ischemic mice. The proportion of viable neurons was derived by dividing the number of viable neurons of the ischemic groups by the mean value of their gene-matched, sham-operated controls. WT sham, $n = 20$ hemispheres; WT ischemia, $n = 17$; EP1^{-/-} sham, $n = 12$; EP1^{-/-} ischemia, $n = 12$; APP/PS1 sham, $n = 23$; APP/PS1 ischemia, $n = 14$; APP/PS1xEP1^{-/-} sham, $n = 6$; APP/PS1xEP1^{-/-} ischemia, $n = 11$. ** $P < 0.01$ compared with WT group; # $P < 0.05$ compared

with APP/PS1 group; *ns*, not significant. (C) Memory latency in the passive avoidance task. WT sham, *n* = 9 mice; WT ischemia, *n* = 11; APP/PS1 sham, *n* = 5; APP/PS1 ischemia, *n* = 6; EP1^{-/-} sham, *n* = 6; EP1^{-/-} ischemia, *n* = 8; APP/PS1xEP1^{-/-} sham, *n* = 3; APP/PS1xEP1^{-/-} ischemia, *n* = 7. **P* < 0.05 compared with WT ischemia group. (D) Representative images of hippocampal sections immunostained for Aβ around the neurons in the CA1 subfield. Aβ appears brown. (E) Aβ concentration in brain homogenate. The left graph shows concentration of Aβ42, and the right graph shows concentration of Aβ40. *n* = 3 mice per group, **P* < 0.05, ***P* < 0.01.