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Peroxisome proliferator-activated receptor γ is expressed in hippocampal neurons and its activation prevents β-amyloid neurodegeneration: role of Wnt signaling

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Abstract

The molecular pathogenesis of Alzheimer's disease (AD) involves the participation of the amyloid-β-peptide (Aβ), which plays a critical role in the neurodegeneration that triggers the disease. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, which are members of the nuclear receptor family. We report here that (1) PPARγ is present in rat hippocampal neurons in culture. (2) Activation of PPARγ by troglitazone and rosiglitazone protects rat hippocampal neurons against Aβ-induced neurodegeneration, as shown by the 3-[4,5 -2yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay, immunofluorescence using an anti-heavy neurofilament antibody, and quantitative electron microscopy. (3) Hippocampal neurons treated with several PPARγ agonists, including troglitazone, rosiglitazone, and ciglitazone, prevent the excitotoxic Aβ-induced rise in bulk-free Ca^{2+}. (4) PPARγ activation results in the modulation of Wnt signaling components, including the inhibition of glycogen synthase kinase-3β (GSK-3β) and an increase of the cytoplasmic and nuclear β-catenin levels. We conclude that the activation of PPARγ prevents Aβ-induced neurodegeneration by a mechanism that may involve a cross talk between neuronal PPARγ and the Wnt signaling pathway. More important, the fact that the activation of PPARγ attenuated Aβ-dependent neurodegeneration opens the possibility to fight AD from a new therapeutic perspective.
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Keywords: Alzheimer's disease; Aβ peptide; PPARγ; Anti-diabetic drugs; Cell survival; Wnt signaling

Introduction

Progressive dysfunction of neurons in the limbic and association cortices underlies the decline of cognitive functions and psychomotor abilities of individuals with Alzheimer's disease (AD) [1]. This cellular dysfunction is preceded by the intracellular and extracellular accumulation of the amyloid-β-peptide (Aβ) as amyloid deposits that trigger the appearance of neurofibrillary tangles [2]. The mechanism of β-amyloid neurotoxicity and its precise cellular locus of action are unsettled, but aggregated forms of Aβ interact with several different neuronal cell surface receptors that may activate signal transduction cascades that trigger caspase activation, free-radical generation, and alteration of Ca^{2+} homeostasis [2–5]. An increased activation of glycogen synthase kinase-3β (GSK-3β), the hyperphosphorylation of tau proteins, and the loss of the microtubule network have all been observed in primary cultures of hippocampal and cortical neurons exposed to β-amyloid [6,7]. A loss of function of the Wnt signaling pathway has been also found to play a role during β-amyloid neurotoxicity [8–10], and key components of such pathway are affected in AD, that is, β-catenin is reduced [11] and GSK-3β is activated in preneurofibrillary lesions

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[12]. The Wnt signaling pathway is one of the best-studied signaling cascades implicated in neural development and maintenance of the central nervous system [13].

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor family of ligand-activated transcription factors that include receptors for steroids, thyroid hormone, vitamin D, and retinoic acid [14]. To date, three mammalian PPAR subtypes have been isolated and termed PPARα, PPARβ/δ, and PPARγ [15]. PPARα is highly expressed in several tissues. PPARβ/δ is an APC-regulated target of nonsteroidal anti-inflammatory drugs [16], and PPARγ participates in biological pathways of intense basic and clinical interest, such as differentiation, insulin sensitivity, type 2 Diabetes, arteriosclerosis, and cancer [14,15]. In particular, PPARγ is a critical regulator of adipocyte differentiation [14], a process also dependent of the Wnt signaling pathway [17]. In this context, it is interesting to mention that thiazolidinedione drugs have a potent insulin-sensitizing action mediated by PPARγ [18], and recent studies suggest that treatment of insulin resistance with a PPARγ agonist retards the development of AD [19]. Although PPARγ function has been restricted to tissues other than the brain [14], the presence of PPARγ mRNA has been detected at low levels in the whole brain by Northern blotting [20]. Recent studies, using a very sensitive immunocytochemical method, showed the presence of PPARγ at the neuronal level in the hippocampus and cortex in vivo [21]. In this context, it is interesting to mention that PPARγ may change in the temporal cortex of AD brains, suggesting that it might be involved in AD pathophysiology [22].

In this study, we found that PPARγ both at its mRNA and protein level is present in rat hippocampal neurons in culture. We also demonstrated that PPARγ agonists and anti-diabetic thiazolidinedione drugs, troglitazone and rosiglitazone, but not the PPARγ antagonist GW-9662, prevent Aβ-dependent neurotoxicity. This effect occurs concomitantly with an increase of cytoplasmic β-catenin and an inhibition of GSK-3β activity. Besides, treatment with several PPARγ agonists prevents the cytosolic calcium increase induced by Aβ in hippocampal neurons loaded with Fluo 3 AM.

Materials and methods

Materials

Synthetic $A\beta_{1-40}$ peptide corresponding to the human Aβ wild-type sequence was obtained from Chiron Corp. Inc. (Emeryville, CA) and Bachem Inc. (Torrance, CA). Chemicals, culture media, and serum were obtained from Sigma (St. Louis, MO), Roche (Alameda, CA), Merck (Darmstadt, Germany), Gibco BRL (Paisley, UK), and Fluo3 AM from Molecular Probes (Leiden, The Netherlands). Antibody anti-neurofilament, anti-β-catenin, anti-c-*Jun*, and anti-GSK-3β from Santa Cruz Biotechnology, San Diego, CA, and GS-2 substrate peptide from Upstate, Biotechnology, Lake Placid, NY.

Culture of rat neurons and preparation of Aβ

Hippocampi from Sprague–Dawley rats at embryonic day 18 were dissected and primary rat hippocampal cultures were prepared as described previously [8,9,23]. Hippocampal cells were seeded in polylysine-coated wells on day 3 of culture; cells were treated with 2 μM 1-β-D-arabinofuranosylcytosine for 24 h to reduce almost all the glial cells present in the culture. Seven days later, cultured hippocampal neurons were used for various experiments. The average number of neurons in each experiment corresponded approximately to 98% of total cells present in the cultures. The human $A\beta_{1-40}$ peptide used in the present studies was subjected to stirring aggregation as described previously [24].

Cell survival assay

Cell viability was measured by the modified 3-[4,5 -2yl]-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [25].

Cell fractionation and Western blot analysis

Neurons were homogenized and subjected to centrifugation at 100,000 × *g* for 1 h at 4°C, the supernatants were collected and analyzed by 10% SDS-PAGE, the protein bands were transferred to nitrocellulose membranes and incubated with appropriate primary antibodies [8,9].

Immunofluorescence studies

Hippocampal neurons plated on polylysine-coated covers (25,000 cells/cover) were immunostained using monoclonal anti-β-catenin antibody (1:200) (Santa Cruz Biotechnology), polyclonal anti-PPARγ antibody (1:300) (Santa Cruz Biotechnology), and neurofilament NF-200 (Sigma). Covers were mounted and analyzed using a Zeiss confocal microscope.

Morphometric analysis

The number, length, and area of neurites were quantified using an Image-Pro plus software as described previously [26].

Electron microscopy

Hippocampal neurons were fixed directly in polylysine-coated wells after 7 days of culture. The fixative contained 3% glutaraldehyde in 50 mM sodium cacodylate buffer pH 7.2. Samples were fixed for 4 h at room temperature (25°C). They were treated with 1% osmium tetroxide in cacodylate

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