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Prostaglandin J2 promotes *O*-GlcNAcylation raising APP processing by α- and β-secretases: relevance to Alzheimer's disease

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ABSTRACT

Regulation of the amyloid precursor protein (APP) processing by α- and β-secretases is of special interest to Alzheimer's disease (AD), as these proteases prevent or mediate amyloid beta formation, respectively. Neuroinflammation is also implicated in AD. Our data demonstrate that the endogenous mediator of inflammation prostaglandin J2 (PGJ2) promotes full-length APP (FL-APP) processing by α- and β-secretases. The decrease in FL-APP was independent of proteasomal, lysosomal, calpain, caspase, and γ-secretase activities. Moreover, PGJ2-treatment promoted cleavage of secreted APP, specifically sAPPα and sAPPβ, generated by α and β-secretase, respectively. Notably, PGJ2-treatment induced caspase-dependent cleavage of sAPPβ. Mechanistically, PGJ2-treatment selectively diminished mature (*O*- and *N*-glycosylated) but not immature (*N*-glycosylated only) FL-APP. PGJ2-treatment also increased the overall levels of protein *O*-GlcNAcylation, which occurs within the nucleocytoplasmic compartment. It is known that APP undergoes *O*-GlcNAcylation and that the latter protects proteins from proteasomal degradation. Our results suggest that by increasing protein *O*-GlcNAcylation levels, PGJ2 renders mature APP less prone to proteasomal degradation, thus shunting APP toward processing by α- and β-secretases.

1. Introduction

Neuroinflammation is involved in the pathogenesis and progression of AD (Glass et al., 2010; Heneka et al., 2015). Cyclooxygenases play a central role in neuroinflammation by converting arachidonic acid into prostaglandins (Aoki and Narumiya, 2012). Cyclooxygenase-2 (COX-2) induction correlates with AD severity and neurodegeneration (Liang et al., 2007; Wyss-Coray and Rogers, 2012). COX-1 is also involved in AD pathology (Choi et al., 2013). In the brain, cyclooxygenases mediate the production of prostaglandins D2, E2, and J2 (Liang et al., 2007; Ricciotti and FitzGerald, 2011). PGD2 is the most abundant prostaglandin in the brain and increases the most under pathologic conditions (Hertting and Seregi, 1989; Ricciotti and FitzGerald, 2011). The levels and capacity to form PGD2 are significantly higher in the frontal cortex of AD brains than those in controls (Iwamoto et al., 1989), supporting a role for PGD2 in AD.

PGD2 has a half-life of approximately 1.1 minutes in the brain and is converted to PGJ2 by spontaneous dehydration (Suzuki et al., 1986). PGJ2 is highly neurotoxic (Li et al., 2004b) and mediates PGD2 neurotoxicity (Liu et al., 2013b). PGJ2 treatment upregulates COX-2, initiating a positive feedback loop with potential long-term effects (Aoki and Narumiya, 2012; Li et al., 2004a). PGJ2 binds covalently to proteins through its α,β-unsaturated carbonyl group (Uchida and Shibata, 2008). Thus, local concentrations of PGJ2 are potentially higher (Liu et al., 2013b). PGJ2 is also upregulated in motor neurons of ALS patients (Kondo et al., 2002; Zhang et al., 2010).

Prostaglandins are present in body fluids in the pico to nanomolar range reaching low micromolar levels at local sites of acute inflammation (Hertting and Seregi, 1989; Offenbacher et al., 1986). In particular, plasma levels of 15d-PGJ2 increased 12-fold and 23-fold in patients following acute stroke or with vascular risk factors and atherothrombotic infarcts, respectively (Blanco et al., 2005). Like its PGD2 precursor, PGJ2 and its metabolites can be considered some of the most abundant prostaglandins in the brain (Katura et al., 2010). In rodents, upon stroke and traumatic brain injury, the in vivo concentration of free PGJ2 in the brain increases from almost undetectable to the 100 nM range (Liu et al., 2011, 2013a). However, PGJ2 binds covalently to proteins through its α,β-unsaturated carbonyl group (Uchida and Shibata, 2008); therefore, reported levels of free PGJ2 do not represent its total amounts. In addition, PGJ2 is uptaken by cells via a carrier-mediated active transport,

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ending up in the cytoplasm and nucleus (Narumiya and Fukushima, 1986). In contrast, PGD2 and PGE2 are not taken up by cells (Narumiya and Fukushima, 1986). In conclusion, the reported in vivo PGJ2 levels represent average brain concentrations, but it is predicted that local cellular and intracellular concentrations of PGJ2 and its metabolites are much higher (Liu et al., 2013b).

PGJ2 has a short half-life; therefore, the extent of its levels in AD is unclear. However, PGJ2 induces neuronal dysfunction (reviewed in Figueiredo-Pereira et al., 2015; Yagami et al., 2017). In relation, PGJ2 induces caspase activation and caspase-mediated cleavage of tau, generating aggregation prone Δtau in neuronal cells (Arnaud et al., 2009; Metcalfe et al., 2012). The latter seeds tau aggregation before neurofibrillary tangle formation (de Calignon et al., 2010; Gamblin et al., 2003). These data suggest that inflammation mediated by PGJ2 contributes to some aspects of AD pathology, in particular neuronal apoptosis and tau aggregation. In the current studies, we investigated whether PGJ2 affects APP processing.

APP is a type I transmembrane glycoprotein with most of its full-length present in the exoplasmic space. APP has 3 main isoforms, comprising 695, 751, or 770 residues (Zhang et al., 2011). APP695 is predominantly expressed in neurons (Zheng and Koo, 2006). APP is primarily processed (~90%) by the non-amyloidogenic α-secretase and to a lesser extent by the amyloidogenic β-secretase (Placido et al., 2014). APP processing within the amyloid beta (Aβ) domain by α-secretase releases a free sAPPα ectodomain excluding Aβ formation (Zhang et al., 2011). APP processing by β-secretase releases sAPPβ and generates a C99 fragment that is additionally cleaved by γ-secretase to generate Aβ (Zhang et al., 2012).

APP plays a critical role in AD pathology as it is the precursor of Aβ that accumulates in senile plaques and cerebrovascular amyloid deposits, which are hallmarks of the disease (Ghiso et al., 2014). Since Aβ accumulation correlates with increased inflammation in AD brains (Kotilinek et al., 2008; Niranjan, 2013), we investigated how prostaglandins D2, J2, and E2 alter APP processing in rat primary neuronal cultures and in SY5Y neuroblastoma cells overexpressing human APP. From the 3 prostaglandins, PGJ2 induced the most significant changes by promoting APP cleavage by α and β-secretases. Our studies identify potential APP-processing mechanisms that link neuroinflammation mediated by PGJ2 to AD pathophysiology.

2. Materials and methods

2.1. Reagents and antibodies

Tissue cultures supplies (Life Technologies, Carlsbad, CA, USA). Prostaglandins D2, E2, J2, and the J2 analog CAY10410 (Cayman Chemical, Ann Arbor, MI, USA). Deglycosylation *O*-Glycosidase and Neuraminidase Bundle (New England Biolabs, Ipswich, MA, USA).

2.1.1. Inhibitors

Epoxomicin (Peptides International Inc, Louisville, KY, USA); calpeptin (Z-Leu-Nle-CHO), pan caspase inhibitor (Z-VAD-FMK), β-secretase inhibitor 2 (Z-VLL-CHO, BACE1-2) and 4 (BACE1-4), and tunicamycin (Calbiochem/EMD Bioscience, Gibbstown, NJ, USA); chloroquine and brefeldin A (Sigma, St. Louis, MO, USA); and α-secretase inhibitor TAPI-2 and γ-secretase inhibitor BMS 299897 (Santa Cruz Biotechnology, Dallas, TX, USA).

2.1.2. Primary antibodies

Mouse monoclonal anti-APP (clone 22C11, 1:1000, cat# MAB348, epitope a.a. 66-81) (Millipore, Billerica, MA); mouse monoclonal anti-CTF of APP (1:500, cat# 802801, epitope a.a. 676-695 of APP695), anti-β-tubulin (1:10,000, cat# MMS-435P), anti-*O*-GlcNAc (1:500, cat# 838004) and rabbit polyclonal anti-sAPPβ (1:1000, cat# 813401), anti-sAPPα (1:1000, cat# 813501), and anti-βIII-tubulin (1:1000, cat# 802001, for immunofluorescence) (BioLegend, San Diego, CA); rabbit monoclonal anti-BACE1 (clone D10E5, 1:1000, cat# 5606), anti-calreticulin (endoplasmic reticulum (ER) maker, 1:500, cat# 12,238), and rabbit polyclonal anti-APP (1:1000, cat # 2452) (Cell Signaling Technology, Danvers, MA); mouse monoclonal anti-β-actin (1:50,000, cat# A-2228), and rabbit polyclonal anti-β-actin (1:10,000, cat# A-2066) (Sigma, St. Louis, MO); and mouse monoclonal anti-TGN38 (Golgi marker, 1:1000, cat# MA3-063, ThermoScientific, Bridgewater, NJ).

2.1.3. Secondary antibodies

Secondary antibodies with HRP conjugate (1:10,000) (Bio-Rad Laboratories, Hercules, CA, USA).

2.2. Cell cultures

2.2.1. Primary cerebral cortical cultures

Dissociated cultures from Sprague Dawley rat embryonic (E18, both sexes) cerebral cortical neurons were prepared as in the study by Kiprowska et al., 2017 and plated (6×10^6 cells/10 cm dish, or 2.5×10^5 cells/well on 24-well plates, cell viability only). Cultures were maintained in Neurobasal media supplemented with 2% B27 and 0.5 mM L-Glutamax. Half of the medium was changed every 4 days. Experiments were performed at 7 DIV. Neurobasal medium contains several proprietary factors that ensure a mostly pure (>95%) neuronal culture; glial growth is inhibited without a need for the anti-mitotic agent arabinofuranosyl cytidine (Brewer et al., 1993; Nam et al., 2007).

2.2.2. APP-SY5Y cells

SH-SY5Y cells are human-derived neuroblastoma cells with neuron-like properties. The APP-SY5Y cells (obtained from Dr N. Robakis) were stably transfected to overexpress wild-type human APP695 (Pangalos et al., 1995). Cultures were maintained in a 1:1 ratio American Tissue Culture Collection-formulated minimum essential media (MEM) growth media (Life Technologies, cat#11095-080) and F12 (Life Technologies, cat# 11765-054) supplemented with 1x nonessential amino acids (Corning Cellgro, cat# 25-025-Cl), 1-mM sodium pyruvate (Corning Cellgro, cat# 25-000-Cl), 1.5-g/L sodium bicarbonate (Corning, cat# 25-035-Cl), 5% FBS (RMBI), and 1% pen/strep (Invitrogen, cat#15140-122). All cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Culture treatments

Cortical neurons were treated acutely (4, 8, 16, or 24 hours) by adding dimethyl sulfoxide (DMSO) (vehicle control) or different drugs (dissolved in DMSO) directly to DMEM supplemented with 0.5 mM L-Glutamax and 1 mM sodium pyruvate (all from Invitrogen) in the absence of serum. APP-SY5Y cells were treated similarly, except that the drugs were added directly into their respective growth medium with all the components excluding 5% FBS. The final DMSO concentration in the medium was 0.5% for all cells.

2.4. Western blotting

After treatment, cells were harvested as in the study by Kiprowska et al., 2017. Following lysis (overnight at −80 °C), cell extracts were centrifuged (19,000 × *g* for 10 minutes) at 4 °C. Protein concentration (NP40-soluble supernatants) were determined with the BCA assay. Western blot analysis was carried out following SDS-PAGE as described in Kiprowska et al., 2017, with