Biochimica et Biophysica Acta 1862 (2016) 213–222

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Broad neutralization of calcium-permeable amyloid pore channels with a chimeric Alzheimer/Parkinson peptide targeting brain gangliosides

Coralie Di Scala [a], Nouara Yahi [a], Alessandra Flores [a], Sonia Boutemeur [a], Nazim Kourdougli [b], Henri Chahinian [a], Jacques Fantini [a,*]

[a] Aix-Marseille Université, PPSN-EA4674, Faculté des Sciences, Marseille, France
[b] INSERM, INMED, Parc Scientifique de Luminy, 13009 Marseille, France

ARTICLE INFO

Article history:
Received 10 September 2015
Received in revised form 27 October 2015
Accepted 30 November 2015
Available online 2 December 2015

Keywords:
Alzheimer
Parkinson
β-amyloid peptide
α-Synuclein
Ganglioside
Amyloid pore
Ion channel
Calcium

ABSTRACT

Growing evidence supports a role for brain gangliosides in the pathogenesis of neurodegenerative diseases including Alzheimer's and Parkinson's. Recently we deciphered the ganglioside-recognition code controlling specific ganglioside binding to Alzheimer's β-amyloid (Aβ1-42) peptide and Parkinson's disease-associated protein α-synuclein. Cracking this code allowed us to engineer a short chimeric Aβ/α-synuclein peptide that recognizes all brain gangliosides. Here we show that ganglioside-deprived neural cells do no longer sustain the formation of zinc-sensitive amyloid pore channels induced by either Aβ1-42 or α-synuclein, as assessed by single-cell Ca^{2+} fluorescence microscopy. Thus, amyloid channel formation, now considered a key step in neurodegeneration, is a ganglioside-dependent process. Nanomolar concentrations of chimeric peptide competitively inhibited amyloid pore formation induced by Aβ1-42 or α-synuclein in cultured neural cells. Moreover, this peptide abrogated the intracellular calcium increases induced by Parkinson's-associated mutant forms of α-synuclein (A30P, E46K and A53T). The chimeric peptide also prevented the deleterious effects of Aβ1-42 on synaptic vesicle trafficking and decreased the Aβ1-42-induced impairment of spontaneous activity in rat hippocampal slices. Taken together, these data show that the chimeric peptide has broad anti-amyloid pore activity, suggesting that a common therapeutic strategy based on the prevention of amyloid-ganglioside interactions is a reachable goal for both Alzheimer's and Parkinson's diseases.

1. Introduction

Alzheimer's and Parkinson's diseases are the most prevalent neurodegenerative diseases in humans, with millions of people affected worldwide [1,2]. Unfortunately, despite decades of research efforts we are still seeking a cure for these fatal diseases [3]. A category of proteins with exceptional conformational plasticity and aggregation properties have been identified as potential therapeutic targets. These proteins, collectively referred to as amyloid proteins [4], have been found in various deposits such as fibrils and plaques [5] that were initially suspected to be the causative agents of the diseases [6,7]. This concept has logically led to therapeutic strategies aimed at disrupting amyloid plaques, e.g. through immunization protocols [8,9]. Unfortunately, these attempts have not been successful and in some cases the trials had to be prematurely stopped due to severe toxic effects [10,11]. Moreover, it appeared that the presence of amyloid plaques in the brain of aged subjects was not always correlated with Alzheimer's disease or cognitive defect [12,13].

Overall, these data indicate that amyloid plaques can no longer be considered as responsible for neurodegenerative symptoms in Alzheimer's disease [12]. Instead, it is now considered that small oligomers are the main neurotoxic forms of Alzheimer's β-amyloid (Aβ1-42) peptide [14–16]. Several types of oligomers have been detected in the brain of patients with Alzheimer's disease [17,18]. In the plasma membrane of brain cells, these oligomers can form pore-like structures (the so-called 'amyloid pores') that behave as Ca^{2+}-selective ion channels [19–22]. The massive entry of Ca^{2+} through amyloid pores is believed to be one of the earliest steps in Alzheimer's, Parkinson's, and other neurodegenerative diseases [23,24]. For this reason, it is urgent to find compounds able to prevent amyloid pore formation in the plasma membrane of brain cells [3,25].

One possibility could be to inhibit the initial binding of amyloid proteins to the surface of brain cells. In this respect, one should exploit the common property of amyloid proteins to specifically interact with gangliosides in lipid raft domains [26,27]. However, this is not an easy task because Alzheimer's peptides Aβ1-42 and/or Aβ1-40 bind to several gangliosides including the main brain species GT1b, GD1a, GD1b, GM1

Abbreviations: ATCC, American Type Culture Collection; HPLC, high pressure liquid chromatography; GBD, ganglioside-binding domain; PPMP, 1R,2R-(+)-1-phenyl-2-palmitoylamino-3-N-morpholine-1-propanol.

* Corresponding author at: Interactions Moléculaires et Systèmes Membranaires, Faculté des Sciences Saint-Jérôme, Avenue Escadrille Normandie-Niemen, Service 331, 13013 Marseille, France.
E-mail address: jacques.fantini@univ-amu.fr (J. Fantini).

http://dx.doi.org/10.1016/j.bbadis.2015.11.012
0925-4439/

and GM3 [28–34]. Moreover, the Parkinson's associated protein α-synuclein has been shown to bind to GM1 [35–37] and to several other glycosphingolipids including ganglioside GM3 [36]. The measured avidity of amyloid proteins for specific gangliosides may vary according to various parameters, including protein concentration, protein–lipid ratio and the experimental approach used. Nevertheless, the data available in the literature indicate that both Aβ and α-synuclein are able to interact with several gangliosides (reviewed in [38]). Hence, it is widely admitted that gangliosides play a key role in the pathogenesis of neurodegenerative diseases [29,30,36,39]. Using the Langmuir film balance technology with reconstituted ganglioside monolayers probed by wild-type and mutant peptides derived from Aβ and α-synuclein, we have demonstrated that the binding of amyloid proteins to gangliosides obeys a biochemical code [40]. This code is based on a common structurally-conserved domain conferring ganglioside recognition, with minor sequence variations accounting for ganglioside specificity [40]. The deciphering of this code allowed us to create a universal peptide that is constructed as a chimer between the ganglioside-binding domains of α-synuclein and Aβ. This chimeric peptide, which combines the ganglioside binding properties of both proteins, behaves as a universal ganglioside-binding compound that interacts with all the gangliosides tested so far, including the main gangliosides expressed by neurons (GM1, GD1a, GD1b and GT1b) as well as the major glial ganglioside GM3 [40].

In the present study we have evaluated the activity of this universal anti-ganglioside peptide (referred to as 'chimeric peptide') on amyloid pore formation induced by Aβ1-42 and α-synuclein in brain cells. We show that nanomolar concentrations of both wild-type and disease-associated mutant forms of these proteins induce amyloid pore formation as assessed by Ca^{2+} flux studies. These channels were inhibited by Zn^{2+}, a specific inhibitor of amyloid pores [41,42]. Most importantly, ganglioside-depleted cells could no longer sustain the formation of these pores, which indicate that gangliosides are key actors of the process. Finally we studied the anti-pore activity of the chimeric peptide against various wild-type and mutant forms of Aβ1-42 and α-synuclein, as well as its ability to prevent Aβ-induced neurotoxicity in two functional assays, i.e. microscopy evaluation of synaptic vesicle trafficking and electrophysiological recordings of the spontaneous activity of rat hippocampal slices.

2. Materials and methods

2.1. Products

SH-SY5Y cells were purchased from ATCC. DMEM/F12, HBSS, glutamine and penicillin/streptomycin were furnished by Gibco. Fluo-4AM, FM1-43 and secondary antibody were purchased from Invitrogen. The anti-ganglioside GM1 antibody was purchased from Matreya. The full-length proteins α-synuclein1-140 (wild-type and mutants) and Aβ1-42 were from rPeptide. Synthetic peptides including α-synuclein 34–50, Aβ1-16 and the chimeric peptide were obtained from Schafer (Denmark). All peptides and proteins have a purity >95% as assessed by HPLC. The chimeric peptide used in this study has been patented under the number PCT/EP2015/054968: "A chimeric peptide that interacts with cell membrane gangliosides" (inventors: Nouara Yahi and Jacques Fantini, applicant: Aix-Marseille University). Three distinct batches of this peptide have been used over a 4-year period of time with fully reproducible data.

2.2. In silico studies

Molecular dynamics simulations of ganglioside–peptide interactions have been performed with the Hyperchem program as described [40].

2.3. Cell culture

Cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F12 (DMEM/F12) supplemented with 10% fetal calf serum, glutamine (2 mM) and penicillin (50 U/mL)/streptomycin (50 μg/mL) and maintained at 37 °C with 5% CO_2. Cells were passaged twice a week and not used beyond passage 25.

2.4. Modulation of GM1 levels

Membrane GM1 levels were decreased by inhibiting cell glucosylceramide synthase with 10 μM 1R,2R-(+)-1-phenyl-2-palmitoylamino-3-N-morpholine-1-propanol (PPMP) for 48 h [43].

2.5. Ganglioside extraction and quantitation

Gangliosides were extracted and recovered from the upper phase of a Folch partition and analyzed by high performance thin layer chromatography (HPTLC) as previously described [44]. Neutral sphingolipids (ceramides, GalCer) and sphingomyelin were recovered from the lower phase of a Folch extract and analyzed by HPTLC as previously reported [44]. Glycosphingolipids were colored with orcinol and sphingomyelin and ceramide with Coomassie blue [45]. All sphingolipids were quantitated with a Gel Doc™ XR + Molecular Imager using the Image Lab™ software.

2.6. Immunocytochemistry

The cells were either submitted or not to PPMP treatment and then incubated with anti-ganglioside GM1 primary antibody (1:500) for 2 h. The cells were rinsed and subsequently treated with goat anti-rabbit Alexa Fluor 488 (1:400) for 1 h. Images were analyzed using Image J 1.45. No correction was applied to the images, and, for a better visualization, only contrast and brightness were adjusted in photography used for figures. The same adjustments were applied on all images to allow the comparison.

2.7. Lipid monolayer assay

Cell ganglioside–peptide interactions were studied with the Langmuir-film balance technique using a Kibron microtensiometer as previously described [36].

2.8. Calcium measurements

Cells were plated (45.000 cells/dish) in 35 mm culture dishes and grown during 72 h at 37 °C. They were loaded with 5 μM Fluo-4AM for 30 min in the dark, washed three times with HBSS and incubated 30 min at 37 °C [46]. The calcium fluxes were estimated by measuring the variation of cell fluorescence intensity after amyloid protein injection (220 nM) into the recording chamber directly above an upright microscope objective (BX51W Olympus) equipped with an illuminator system MT20 module. Fluorescence emission at 525 nm was imaged by a digital camera CDD (Hamanatsu ORCA-ER) after fluorescence excitation at 490 nm. Time-lapse images (1 frame/10 s) were collected using the CellR Software (Olympus). Fluorescence intensity was measured from region of interest (ROI) centered on individual cells. Signals were expressed as fluorescence after treatment (F_t) divided by the fluorescence before treatment (F_0) and multiplied by 100. The results were averaged and the fluorescence of control untreated cells is subtracted of each value. All experiments were performed at 30 °C during 1 h.