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Nimodipine selectively stimulates β-amyloid 1–42 secretion by a mechanism independent of calcium influx blockage

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Abstract

Several lines of evidence indicate that perturbed cellular Ca^{2+} homeostasis may play a prominent role in synaptic dysfunction and neuronal death in Alzheimer's disease (AD), suggesting a potential benefit of drugs capable to stabilize Ca^{2+} homeostasis. We here investigated the effects of a panel of L-type Ca^{2+} channel antagonists on the secretion of the amyloid β-peptide (Aβ), which abnormally accumulates in the senile plaques of the brain of AD patients. We found that, in primary and immortalized neuronal cells in culture, nimodipine robustly stimulated secretion (up to about four-fold at 30 μM) of the highly amyloidogenic 42-residue isoform of Aβ (Aβ42), while leaving largely unaffected total Aβ secretion. An analogous effect was also observed in vivo, as the administration of a single dose of nimodipine (10 mg/kg i.p.) induced a significant rise of Aβ42 levels in plasma of Tg2576 mice. The effect of nimodipine was independent of blockage of L-type Ca^{2+} channels and capacitative calcium entry. Accordingly, nimodipine effect was largely Ca^{2+}-independent, as neither depletion nor rise of extracellular Ca^{2+} abolished it. Hence, by showing that the effect of nimodipine on Aβ42 production is distinct from its ability to block Ca^{2+}-influx pathways, we provide evidence for a previously uncharacterized effect of this long known molecule also used in clinical practice.

Keywords: Amyloid; Alzheimer's disease; Calcium; Channels; Capacitative calcium entry; Dihydropyridine

1. Introduction

One of the most striking neuropathological features of Alzheimer's disease (AD) is the accumulation of the 4-kDa peptide amyloid β (Aβ) as fibrillar deposits in the brain parenchyma (senile plaques). The discovery that soluble Aβ is a constituent of cerebrospinal fluid [35,36] and is found in cultured cell media [11] indicates that Aβ is a normal product of cellular metabolism of amyloid precursor protein (APP). Aβ is generated from APP by a set of membrane-bound proteases, one at the amino-terminus referred to as β-secretase and one at the carboxy-terminus known as γ-secretase. The C-terminal length of Aβ generated by γ-secretase is heterogeneous, ranging from 37 to 43 aminoacids. Aβ40 is the predominant species secreted from the cells, while Aβ42 is a relatively minor isoform [40]. Pathogenic missense mutations associated with rare inherited cases of AD are found in APP itself or in presenilin (PS), type 1 and 2, which are essential components of the γ-secretase complex. The majority of these mutations result in an increased Aβ42 production in terms of either absolute levels or with respect to total Aβ (Aβtot) [3,5,37], a phenomenon correlated with the rate of amyloid deposition [18]. Aβ42 has a greater tendency to self-aggregate as compared to shorter Aβ peptides [19], rendering Aβ42, the predominant species found in senile plaque cores and in vascular amyloid deposits in AD brains [32,17].

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Dysregulation of cellular Ca^{2+} homeostasis has been proposed to play a prominent role in synaptic dysfunction and neuronal death accompanying AD [22,26] and changes in intracellular Ca^{2+} levels have been reported to influence APP processing and Aβ release [4,30]. The "Ca^{2+} overload" hypothesis suggests potential benefit of drugs capable of restoring intracellular Ca^{2+} homeostasis. Because 1,4-dihydropyridine (DHP)-based antagonists of voltage-operated Ca^{2+} channels (VOCCs) are also reportedly neuroprotective, this class of molecules has been suggested to be of potential benefit in AD. Among these, nimodipine, a VOCC antagonist belonging to the DHP class which crosses the blood–brain barrier [38], has been shown to afford neuroprotection against Aβ neurotoxicity in vitro [41] as well as against a variety of brain insults [20,15]. Nimodipine also reduces age-related perivascular anomalies and increases cerebral blood flow in experimental animals as well as cognition in aged rodents and primates [34]. For the above reasons, nimodipine has been proposed to be of potential therapeutic utility in AD [1,9,39] and included in clinical trials [24,25,27].

We here provide evidence that nimodipine robustly stimulates Aβ42 secretion in cell lines stably expressing mutant hAPP as well as in primary neuronal cultures derived from mice transgenic for mutant hAPP (Tg2576). An analogous effect was also observed for Aβ42 plasma levels in Tg2576 mice following acute administration of nimodipine. Intriguingly, nimodipine affects Aβ42 release through an elusive mechanism distinct from its ability to block Ca^{2+}-influx pathways.

2. Methods

2.1. Chemicals and reagents

6E10 and 4G8 antibodies against Aβ were purchased from Signet (Dedham, MA, USA). Fura-2/AM, Pluronic, Lysosensor ™Green-DND-189 and FM1-43 were from Molecular Probes (Leiden, The Netherlands). SKF96365 and 2-APB were from Tocris (Buckhurst Hill, UK). Dimethyl sulfoxide (DMSO) was purchased from BDH (Poole, UK). Hygromicin, antibiotics and culture media were purchased from Gibco, Invitrogen (Carlsbad, CA, USA). All the other reagents were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise stated.

2.2. Cell cultures

Human neuroglioma H4 cells stably transfected with human APP carrying the K670N, M671L Swedish double mutation (H4/APPswe) were routinely grown in Opti-MEM supplemented with 10% fetal bovine serum hygromicin (0.1 mg/ml), penicillin 100 U and streptomycin (0.1 mg/ml) in 10 cm dishes [13].

SHSY-5Y neuroblastoma cells stably transfected with the membrane-bound C-terminal fragment A4CT (C99) of human APP (SPA4CT) [23] were purchased from ABETA (Heidelberg, Germany). Cells were routinely grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 1% MEM non-essential aminoacid solution, hygromicin (0.1 mg/ml), penicillin 100 U and streptomycin (0.1 mg/ml) in 10 cm dishes.

For Aβ-release experiments, H4 or SHSY-5Y cells were plated at the density of 10^5 cells/cm^2 in 24-well plates and then allowed to grow to confluence. The medium was then replaced with 0.5 ml fresh medium and incubated with or without the compounds of interest or the vehicle for the desired amount of time. Afterwards, the medium was collected, cleared by centrifugation (12,000 × *g* for 5 min at 4 °C), and immediately analyzed for Aβ by sandwich ELISA.

Primary granule cell cultures were isolated from cerebella of 7–8 day old mice pups obtained by breeding female B6SJL F1 mice with hemizygous male Tg2576 mice [16]. Animals were purchased by Taconic (Germantown, NY, USA). Genomic DNA of each pup was isolated from a tail biopsy by proteinase K digestion followed by phenol/chloroform extraction as described previously [8]. Genomic DNA from each embryo was analyzed by PCR amplification primed with oligomers specific for the human APP695 gene to identify its transgenic status.

Cerebella were incubated in a 0.025% trypsin solution and dissociated by trituration as described previously [7]. All the experiments were performed on cultures kept 10–12 days in vitro. The medium was then replaced with 0.5 ml of medium obtained from non-transgenic sister cultures and incubated for the desired amount of time with the compounds of interest. Afterwards, the medium was collected, cleared by centrifugation (12,000 × *g* for 5 min at 4 °C), and immediately analyzed for Aβ by sandwich ELISA.

2.3. Animals and in vivo drug administration

Nine-month old, female, transgenic B6SJLF17J mice (Tg2576 line) [16] were purchased from Taconic (Germantown, NY, USA). Animals were administered with nimodipine (10 mg/ml, i.p.) dissolved in DMSO or with the vehicle. Three hours after nimodipine or vehicle administration, animals were sacrificed and blood samples were collected in EDTA-coated tubes and centrifuged (800 × *g* for 20 min) to separate the plasma. Plasma samples were immediately assayed for Aβ detection by sandwich ELISA. All experiments were performed in compliance with the guidelines of the European Union and of the Italian Decreto Legislativo 27/1/92, Number 116, Article 7 for the use of laboratory animals.

2.4. Sandwich ELISA for Aβ detection

Sandwich ELISA for total Aβ used monoclonal 6E10 (5 μg/ml) against Aβ aminoacids 1–17 as the capturing an-