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Presenilin mutations linked to familial Alzheimer's disease reduce endoplasmic reticulum and Golgi apparatus calcium levels☆

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Abstract

Presenilin-1 and -2 (PS1 and PS2) mutations, the major cause of familial Alzheimer's disease (FAD), have been causally implicated in the pathogenesis of neuronal cell death through a perturbation of cellular Ca^{2+} homeostasis. We have recently shown that, at variance with previous suggestions obtained in cells expressing other FAD-linked PS mutations, PS2-M239I and PS2-T122R cause a reduction and not an increase in cytosolic Ca^{2+} rises induced by Ca^{2+} release from stores. In this contribution we have used different cell models: human fibroblasts from controls and FAD patients, cell lines (SH-SY5Y, HeLa, HEK293, MEFs) and rat primary neurons expressing a number of PS mutations, e.g. P117L, M146L, L286V, and A246E in PS1 and M239I, T122R, and N141I in PS2. The effects of FAD-linked PS mutations on cytosolic Ca^{2+} changes have been monitored either by using fura-2 or recombinant cytosolic aequorin as the probe. Independently of the cell model or the employed probe, the cytosolic Ca^{2+} increases, caused by agonist stimulation or full store depletion by drug treatment, were reduced or unchanged in cells expressing the PS mutations. Using aequorins, targeted to the endoplasmic reticulum or the Golgi apparatus, we here show that FAD-linked PS mutants lower the Ca^{2+} content of intracellular stores. The phenomenon was most prominent in cells expressing PS2 mutants, and was observed also in cells expressing the non-pathogenic, "loss-of-function" PS2-D366A mutation. Taken as a whole, our findings, while confirming the capability of presenilins to modify Ca^{2+} homeostasis, suggest a re-evaluation of the "Ca^{2+} overload" hypothesis in AD and a new working hypothesis is presented.

Keywords: Presenilin; Calcium stores; Alzheimer's disease; Aequorin; Fura-2; Capacitative calcium entry

Abbreviations: AD, Alzheimer's disease; FAD, familial form of Alzheimer's disease; Aβ, amyloid β-peptide; APP, amyloid precursor protein; PS, presenilin; ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; Bk, bradykinin; Hist, histamine; Cch, carbachol; Tg, thapsigargin; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase; CCE, capacitative Ca^{2+} entry; CPA, cyclopiazonic acid; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; $[Ca^{2+}]_{ER}$, endoplasmic reticulum Ca^{2+} concentration; $[Ca^{2+}]_{Go}$, Golgi apparatus Ca^{2+} concentration; mKRB, modified Krebs–Ringer buffer; Aeq, aequorin; cyt-Aeq, cytosolic aequorin; ER-Aeq, endoplasmic reticulum-targeted Aeq; Go-Aeq, Golgi apparatus-targeted Aeq; GFP, green fluorescent protein

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to dementia. Deposition of the β-amyloid (Aβ) peptide in senile plaques and neurofibrillary degeneration are common features to all forms of the disease [1]. Mutations in three different genes are responsible for the familial forms of the disorder (FAD) [2]. The first gene encodes the β-amyloid precursor protein (APP), a type I membrane protein from which Aβ is derived by proteolytic cleavage. The other genes encode two homologous proteins, presenilin-1 (PS1) and presenilin-2 (PS2), which are essential components of the APP cleavage complex [3]. All the FAD-linked mutations were shown to favour the generation of longer, more amyloidogenic Aβ species. As integral membrane proteins, presenilins (PSs) are abundantly expressed with markers of the endoplasmic reticulum (ER), the Golgi complex and endo-exocytic vesicles; only a minor fraction is present at the plasma membrane level [4,5]. Several lines of evidence suggest that PSs are also involved in the regulation of cellular Ca^{2+} homeostasis. Exaggerated Ca^{2+} responses to agonists that stimulate release of Ca^{2+} from the ER were reported in PC12 cells expressing the FAD-linked PS1-L286V [6]. Similarly, potentiation of inositol-1,4,5-trisphosphate (IP_3) evoked Ca^{2+} release was reported in *Xenopus* oocytes expressing FAD-linked PS1 or PS2 mutants, in particular, PS1-M146V, PS2-N141I and PS2-M239V [7,8]. In oocytes expressing PS1-M146V, a lower IP_3 threshold level was also found by confocal analysis of elementary Ca^{2+} release events, an effect that was attributed to an abnormal elevation of the ER Ca^{2+} content ("Ca^{2+} overload") [9]. Enhanced Ca^{2+} responses were also triggered in neurons derived from transgenic mice [10]. The increased Ca^{2+} release was often coupled to a reduced capacitative Ca^{2+} entry (CCE), the Ca^{2+} influx pathway that is activated by emptied stores [11]. The same authors also suggested that CCE reduction was causally linked to Aβ production since in PS1 expressing cell lines, prolonged treatment with the CCE inhibitor SKF96365 induced an increase in the synthesis of the amyloidogenic $Aβ_{42}$ peptide [11, but see also 12]. Altogether these findings allowed the breakthrough of the "Ca^{2+} overload" hypothesis as a central, pathogenetic event in AD [10,13].

We have recently demonstrated that the FAD-linked PS2 mutations M239I and T122R reduce rather than increase Ca^{2+} release in human fibroblasts and cell lines stably or transiently expressing the PS2 mutants [14,15]. Given the anti-apoptotic effect of a low store Ca^{2+} level [16], we here investigate first whether a reduced Ca^{2+} release could be associated to other PS mutants, and secondly, whether it corresponds to a reduced store Ca^{2+} level. Cytosolic, ER and Golgi apparatus Ca^{2+} concentrations ($[Ca^{2+}]$) were thus measured by fura-2 and different recombinant, organelle-targeted aequorins in cell models expressing either FAD-linked PS1 (A246E, M146L, P117L, L286V) or PS2 (M239I, T122R, N141I) mutations.

2. Materials and methods

2.1. Human primary skin fibroblasts

Patients carrying either the PS1-P117L or the PS1-M146L mutation and control subjects were recruited at the Memory Clinic of IRCCS "Centro San Giovanni di Dio-FBF", Brescia and at the Department of Neurological and Psychiatric Sciences, University of Florence, Italy and evaluated as previously described [17]. Fibroblasts from patients and controls were obtained and cultured as previously described [14].

2.2. Ca^{2+} measurements

Both the fura-2 and the aequorin approaches were routinely employed. Whenever possible, i.e. with easy transfectable cells, the latter tool was the method of choice because it allows monitoring Ca^{2+} signals arising from a larger number of cells (10^3–10^4 times). When the two techniques were employed in HeLa cells, the obtained results were in agreement. Ca^{2+} changes when measured as peaks or areas above basal level were also in agreement. For presentation peak values are shown and expressed as mean ± S.E.M. (n = number of independent experiments; $^{*}p < 0.05$, $^{**}p < 0.001$, unpaired Student's t test). *Fura-2 approach:* Fibroblasts on coverslips were incubated with fura-2/AM (2 μM) at room temperature for 60 min in MEM containing 10% FCS, 0.04% pluronic and sulfinpyrazone (250 μM). The coverslips were washed with a modified Krebs–Ringer buffer (mKRB, in mM:140 NaCl, 2.8 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 11 glucose, pH 7.4) and processed for imaging analysis with a 20× UV permeable objective (Cell^R, Olympus Biosystems GmbH, Planegg, Germany). Neurons on coverslips were incubated with fura-2/AM (5 μM) at 37 °C and processed for imaging analysis as described for fibroblasts. For presentation, the ratios (F340/F380) were off-line averaged (30–40 cells) and normalised to the resting value. Traces are representative of 5–10 independent experiments. Where indicated, in the mKRB $CaCl_2$ was omitted and EGTA (150 μM) was added (Ca^{2+}-free medium), or NaCl was substituted by KCl (140 mM) (K^+-based medium). *Aequorin approach:* The cells on coverslips were incubated with coelenterazine (5 μM) for 1–2 h in mKRB and then transferred to the perfusion chamber. For reconstitution of ER- and Golgi (Go)-aequorin (Aeq), luminal $[Ca^{2+}]$ was reduced before coelenterazine (5 μM) addition by exposing the cells to ionomycin (5 μM) in mKRB containing EGTA (600 μM). Upon 1 h incubation at 4 °C in the same medium, the cells were extensively washed with mKRB supplemented with bovine serum albumin (BSA, 2%). All the luminescence measurements were carried out in mKRB at 37 °C. The experiments were terminated by cell permeabilization with digitonin (100 μM) in a hypotonic Ca^{2+}-rich solution (10 mM $CaCl_2$ in H_2O) to discharge the remaining unused Aeq pool. The light signal was collected as previously described [18].