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Analysis of calcium homeostasis in fresh lymphocytes from patients with sporadic Alzheimer's disease or mild cognitive impairment☆

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ABSTRACT

Alzheimer's disease (AD) is the most widespread, age-related neurodegenerative disorder. Its two subtypes are sporadic AD (SAD) of unknown etiology and genetically encoded familial AD (FAD). The onset of AD is often preceded by mild cognitive impairment (MCI). Calcium dynamics were found to be dysregulated in FAD models, but little is known about the features of calcium dynamics in SAD. To explore calcium homeostasis during the early stages of SAD, we investigated store-operated calcium entry (SOCE) and inositol triphosphate receptor (IP_3R)-mediated calcium release into the cytoplasm in unmodified B lymphocytes from MCI and SAD patients and compared them with non-demented subjects (NDS). Calcium levels in the endoplasmic reticulum and both the rising and falling SOCE slopes were very similar in all three groups. However, we found that SAD and MCI cells were more prone to IP_3R activation than NDS cells, and increases in calcium levels in the cytoplasm were almost twice as frequent in SAD cells than in NDS cells. MCI cells and SAD cells exhibited an enhanced magnitude of calcium influx during SOCE. MCI cells but not SAD cells were characterized by higher basal cellular calcium levels than NDS cells. In summary, perturbed calcium homeostasis was observed in peripheral cells from MCI and SAD patients. Thus, lymphocytes obtained from MCI subjects may be promising in the early diagnosis of individuals who will eventually develop SAD. However, no conclusions are made regarding SAD due to the limited number patients. This article is part of a Special Issue entitled: 12th European Symposium on Calcium.

1. Introduction

Alzheimer's disease (AD) is the most common age-related, neurodegenerative disorder, characterized by progressive neuronal loss that leads to cognitive, memory, and behavioral impairments. AD has been diagnosed through patient history of cognitive decline and postmortem findings of extracellular plaques of amyloid β (Aβ) protein aggregates and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein. At least two types of AD can be distinguished: sporadic Alzheimer's disease (SAD), which accounts for most cases, and familial Alzheimer's disease (FAD), which affects up to 5% of all AD patients and is caused by mutations in amyloid precursor protein (APP) or presenilin proteins 1 or 2 (PS1, PS2) [1]. These proteins participate in Aβ production, in which presenilins are part of a larger protein complex (i.e., γ-secretase) that is responsible for the final cleavage of APP to generate Aβ. Most mutations in these proteins favor the production of a toxic, amyloidogenic form of Aβ, Aβ42 [2].

The molecular mechanism of FAD pathology appears to be well understood and enabled the construction of many animal models. However, the etiology of SAD is still unknown. Until recently, the deposition of protein aggregates was widely accepted to be a proximal cause of pathogenesis in both FAD and SAD [3]. However, some differences between these two types of AD have lately been identified (reviewed in [4,5]). The appearance of plaques has been suggested to be a result of earlier pathological events and is not the only factor responsible for the pathology of the disease (reviewed in [6]). Currently, very few experimental studies have directly compared FAD and SAD or the etiology of SAD itself. SAD is particularly difficult to study because of a lack of appropriate animal models of the disease. The only material available for study is obtained from patients. Since SAD is believed to be a systemic disorder, changes might occur not only in neurons but also in peripheral cells, such as fibroblasts or blood cells [7–9].

Altered calcium homeostasis has recently emerged as one of the early events responsible for AD. Calcium can be linked both to FAD pathology, mostly by interactions between mutated presenilins and several molecules involved in ER calcium homeostasis or by loss of

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presenilin function as a leak channel (reviewed in [10–12]) as well as to SAD pathology, directly by altering Aβ generation and tau protein hyperphosphorylation or indirectly, mostly by calcium changes connected with aging (reviewed in [13,14]). The most convincing evidence showing the involvement of calcium aberration in AD is the fact, that one of the two drugs which are effective in AD treatment is memantine—a partial antagonist of N-methyl-D-aspartate receptor (NMDAR, nonselective cation channel).

The correlation between increased cytosolic calcium level, increased calcium fluxes and higher production of Aβ is well established [15,16]. It appears that almost all ER calcium related proteins are connected with Aβ production. The inhibition of SERCA pump leads to the inhibition of Aβ generation, whereas overexpression of SERCA increases Aβ accumulation [17]. Additionally inhibition of SOCE causes increased Aβ42 production [18]. Furthermore, knockdown of the IP_3R reduces [19], while expression of RyR increases [20] Aβ production. In vivo imaging of neurites and spines of APP overexpressing mice revealed, that spines which are located in proximity to Aβ plaques showed increased basal calcium levels. The consequences include abnormal spines morphology and disrupted calcium compartmentalization [21].

Many recent studies have shown a strong correlation between PS1 and PS2 mutations in FAD models and calcium dyshomeostasis. Such changes in calcium handling are mostly associated with calcium ion flux from the endoplasmic reticulum (ER) via two types of calcium channels: inositol triphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs).

IP_3 receptor activity was found to be significantly increased in *Xenopus laevis* oocytes that overexpress mutant PS1 and PS2 [22,23], mouse PS1 knock-in cortical neurons [24], and lymphoblasts derived from FAD patients [25]. A recent study showed that the expression of the FAD mutant PS constitutively activates cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and influences CREB-responsive gene expression in both cultured neuronal cells and mouse models of AD [26]. This was one of the first studies that showed a causal link between calcium dyshomeostasis and alterations in signaling pathways that possibly lead to neuronal dysfunction.

Increased RyR expression and exaggerated RyR calcium signaling was found in several AD mouse models that bore mutated PS1 [27–30]. An altered RyR expression pattern was found in human brain AD specimens and correlated with tau and amyloid pathology [31]. Presenilin-linked disruptions in RyR signaling and subsequent calcium-induced calcium release via NMDAR-mediated calcium influx was shown to alter synaptic function, providing additional evidence of a strong link between calcium handling and the functioning of neurons in FAD [32].

Store-operated calcium entry (SOCE) is a mechanism that enables the replenishment of depleted ER calcium stores. SOCE was shown to be downregulated in mouse PS1 knock-in fibroblasts [33], human neuroblastoma that overexpressed PS1 [34], cortical neurons from different FAD models [35], human microglia [36], and lymphoblasts obtained from FAD patients [37]. Mutations in presenilins, therefore, may disrupt various aspects of calcium homeostasis and contribute to neuronal failure.

Interestingly, some changes in calcium homeostasis were also observed in peripheral cells obtained from SAD patients [38–41]. A more comprehensive dataset that reveals aberrant calcium signaling in peripheral cells derived from SAD patients may help better understand the molecular basis of the disease and relationship between FAD and SAD.

Mild cognitive impairment (MCI) is defined as the presence of cognitive impairments that are more severe than those expected at a certain age but not significant enough to interfere with everyday activities [42]. Mild cognitive impairment does not fulfill the criteria of AD or any other dementia. It is often observed in individuals who develop AD later in life and therefore may be considered a risk factor for AD [42–44]. Little is known about calcium dysregulation in MCI, but RyR expression patterns were shown to be different between MCI and AD groups [45].

The present study investigated the changes in calcium handling observed in unmodified lymphocytes derived from SAD patients and MCI subjects. We focused on the two major cytoplasmic calcium entry mechanisms: SOCE and IP_3R-dependent flux. To our best knowledge, there was no study concerning IP_3R mediated signals in unmodified lymphocytes B from SAD patients, despite very promising results obtained for FAD lymphocytes bearing presenilin mutations. Also, no broad spectrum analysis including kinetics of the SOCE reaction was made in cells obtained from SAD patients. The use of fresh lymphocytes B is critical, because most of the experiments to date were carried on immortalized lymphocytes B from AD patients and it is known, that the process of immortalization interferes with cell metabolism and calcium handling [46,47]. Our results showed that cells derived from the MCI group were characterized by increased SOCE calcium influx and basal calcium levels. The cells derived from SAD patients exhibited a higher response to IP_3R stimulation. These data confirm some of the previous observations in FAD cells and provide new information about early calcium aberrations that likely lead to SAD.

2. Materials and methods

2.1. Subjects

Samples of peripheral venous blood were obtained from 16 SAD patients aged 57–86 years (mean, 75 ± 8 years), 28 MCI subjects aged 58–84 years (mean, 69 ± 9 years), and 22 non-demented subjects (NDS) aged 60–84 years (mean, 73 ± 7 years). All of the subjects were considered for the IP_3R calcium signaling measurements. Due to the failure of hardware during SOCE measurements the samples from three SAD patients and one MCI subject could not be collected.

The subjects' demographic and clinical characteristics are reported in Table 1. The SAD patients and MCI subjects were recruited from the Alzheimer's Disease Department at the Ministry of Internal Affairs Hospital, Warsaw, Poland. The non-demented subjects were recruited from the Cardiovascular Disease Clinic at Banacha's Hospital, Warsaw, Poland. The SAD and MCI subjects underwent detailed medical, neuropsychological, neurological, and psychiatric examinations. Close informants were interviewed, and laboratory testing and computed tomography or magnetic resonance imaging of the brain were performed. The clinical diagnosis of probable AD was made according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association [48]. Mayo Clinic criteria were used to diagnose MCI [49]. Two MCI and two AD patients suffered from infections or immune diseases. The exclusion of the results obtained for these subjects doesn't change the statistical significance of the results. The NDS group consisted of subjects with no apparent neurological disease or psychiatric syndrome, no complaints of memory impairment, and a Mini-Mental State Examination score ≥ 27. The exclusion criteria for NDS included autoimmune diseases, neoplasms, and acute or chronic inflammation. Because NDS were recruited from the Cardiovascular Disease Clinic, most of them suffered from cardiovascular diseases (CVD). CVD is one of the many risk factors for AD [50] and aging is one of the risk factors for both AD and CVD [50]. Therefore, aged cardiovascular patients who showed no signs of dementia can be considered as AD "escapees". All of the participants were informed of the purpose of the study and gave their informed written consent. The project was approved by the Bioethical Committee for Studies on Human Subjects at Central Clinical Hospital MSWiA in Warsaw (approval no. 08/2011).

2.2. Preparation of cells

Blood samples were collected between 8:00 and 9:00 AM. Peripheral blood mononuclear cells were isolated from 6 ml of venous blood collected in lithium heparin-treated tubes (BD Vacutainer, BD Biosciences) by standard density gradient centrifugation (LSM 1077 Lymphocyte, PAA). Briefly, blood was mixed in a 1:1 ratio with phosphate-buffered