

Alterations in Calcium Homeostasis as Biological Marker for Mild Alzheimer's Disease?

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Received May 5, 2003

Accepted September 15, 2003

Summary

The calcium hypothesis of neurodegenerative disorders such as Alzheimer's disease (AD) suggests that altered cytosolic Ca^{2+} levels ($[Ca^{2+}]_i$) and/or disturbances in Ca^{2+} homeostasis concern cellular mechanisms underlying neuronal pathology. To search for a diagnostic marker of Alzheimer's disease, we measured cytosolic calcium concentrations in platelets of AD patients, age-matched control subjects (AMC), and vascular dementia (VD) patients. The ($[Ca^{2+}]_i$) was determined using long wavelength indicator Fluo-3AM in 21 mild AD patients, 17 AMC, and 23 patients with VD. The basal values of $[Ca^{2+}]_i$ were significantly lower in AD compared to AMC. After the addition of 1 mM calcium, the $[Ca^{2+}]_i$ markedly increased in platelets of AD compared to AMC and VD. Measurement of calcium homeostasis could provide a very sensitive, but less specific biological marker of AD. These results support the hypothesis that influencing calcium homeostasis may provide a therapeutic strategy in dementia.

Key words

Alzheimer's disease • Vascular dementia • Platelets • Calcium homeostasis • Biological marker

Introduction

Among other possible pathogenic factors, a progressive alteration in the ability of neurons to regulate intracellular calcium homeostasis may be a crucial signal transduction event linked strongly to the initiation and development of Alzheimer's disease (AD) pathology (O'Neill *et al.* 2001, Strunecká *et al.* 2002). Precise details of the processes that may lead to calcium homeostasis destabilization in the brain are not known. Many of the pathophysiological changes are not specific for neurons, but also occur (although with some

differences) in other cell types (Řípková *et al.* 2000, Kozubski *et al.* 2002). The search for correlations between biological changes at the cell level and clinical manifestation of AD could allow clinicians to make a more objective diagnosis *ante-mortem*. According to the Consensus Report of the Working Group on "Molecular and Biochemical Markers of Alzheimer's Disease" (1998), sponsored by the Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging, a diagnostic test for AD should be sensitive, specific, based on well available body fluids (Řípková and Strunecká 2001, Csernansky *et al.* 2002), able to detect

AD early in its course, be simple and user-friendly. To search for a diagnostic marker meeting these criteria, we examined calcium homeostatic mechanisms in platelets of patients with mild AD, age-matched controls (AMC), and vascular dementia (VD) patients.

Methods

Intracellular free calcium level ($[Ca^{2+}]_i$) in the absence of extracellular Ca^{2+} and after the addition of 1 mM Ca^{2+} into the incubation medium was estimated in 21 mild AD patients (Mini Mental State Examination (MMSE) 27-22), 17 AMC free of cognitive impairment and 23 patients with VD. The AD and VD patients were diagnosed according to Diagnostic and Statistical Manual of Mental Disorders IV (1994), National Institute of Neurological and Communicative Disorders/Alzheimer's Disease and Related Dementias Association criteria (McKhann *et al.* 1984), Hachinski Ischemic Score (Hachinski *et al.* 1975), Gottfries-Brane-Steen scale (Gottfries *et al.* 1982), and MMSE (Folstein *et al.* 1975). Genetic investigation revealed 42.9 % of ApoE4 homo- or heterozygous AD patients. A full range of blood tests was performed to exclude metabolic causes of dementia. Moreover, the VD patients were also diagnosed according to National Institute of Neurological Diseases and Stroke criteria (Roman *et al.* 1993). All patients and age-matched controls had brain CT scans (with the results supporting the clinical diagnosis) and had no evidence of thyroid or vitamin B₁₂ deficiency. No subject of this study had concurrent somatic illness (e.g., diabetes and hypertension; previously reported hypertension in some VD cases was fully compensated without medication at the time of examination), substance abuse history, ongoing infection, or received calcium channel blocking agents. All control subjects were without history of neurological or psychiatric disorder, AMC had MMSE scale range from 29 to 30. The Ethic Committee of the Prague Psychiatric Center approved the study. Before admission to the study a written informed consent was obtained from all subjects.

Venous citrated blood samples were drawn from each subject after overnight fasting, and immediately centrifuged twice at 130 x g for 15 min. Platelet-rich plasma was combined and centrifuged at 500 x g for 15 min. $[Ca^{2+}]_i$ was determined immediately using long wavelength indicator Fluo-3 as described earlier (Řípková *et al.* 1997). Briefly, resuspended platelets (1-3 x 10⁸ cells/ml) were loaded by incubation with 2.0 μM Fluo-

3AM for 30 min at 37 °C. Loaded platelets were washed twice and resuspended in the incubation medium without Fluo-3AM. Fluorescence was excited at 490 nm and emitted light read at 530 nm (spectrofluorometer RF-5000 Shimadzu, Japan, continuous stirring). After emitted light of basal $[Ca^{2+}]_i$ was recorded, the $CaCl_2$ (at a final concentration of 1mM) was added and emitted light was recorded after one minute. The calibration of the signal was performed by addition of Triton X-100 and EGTA, after which calculation of calcium concentrations was performed. To verify that any differences between the $[Ca^{2+}]_i$ in platelets of patients and those of controls can not be due to differences in Fluo-3AM loading, the cytosolic Fluo-3 concentration was measured. Data were analysed using one-way analysis of variance (ANOVA) for the global group comparison. Pairwise group comparisons were performed using separate t-tests. The BMDP software package (Dixon *et al.* 1992) was used for the analyses.

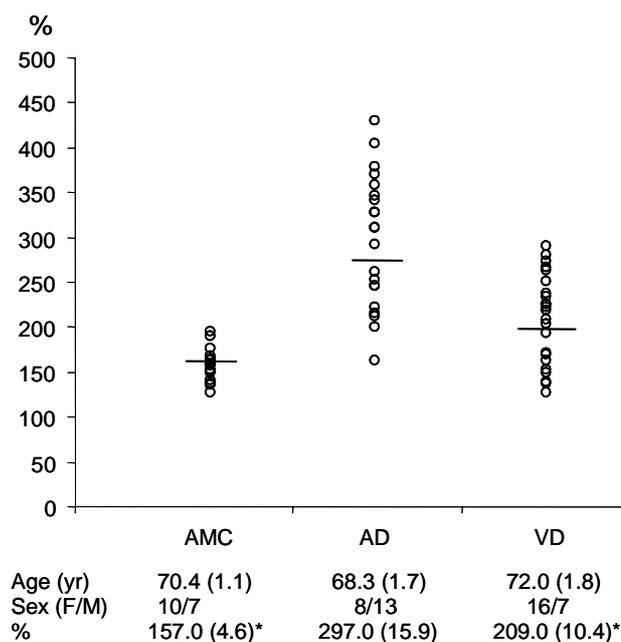


Fig. 1. Changes in calcium homeostasis in platelets of AMC, mild AD patients, and VD patients. Data are expressed in % of basal control value \pm S.E.M. (basal value was measured under the same conditions without 1 mM calcium addition). *Significant differences ($P < 0.001$): AMC and VD vs AD; AMC vs VD.

Results

In the AD patients the basal $[Ca^{2+}]_i$ values (in the absence of extracellular Ca^{2+}) were significantly lower (81 ± 5.9 nM) in comparison with AMC (122 ± 4.7 nM; $t = 5.45$, $df = 35$, $P < 0.001$), but not with VD (102 ± 6.6 nM)

subjects (ANOVA: $F=10.84$; $df=2, 58$; $P=0.0001$). After the addition of 1 mM calcium to the incubation medium, the $[Ca^{2+}]_i$ markedly increased in platelets of AD patients ($297\pm 15.9\%$) while the $[Ca^{2+}]_i$ increased only to a smaller extent in AMC ($157\pm 4.6\%$) and VD patients ($209\pm 10.4\%$) (AMC vs AD: $t=8.42$, $df=23$, $P<0.001$; AMC vs VD: $t=4.57$, $df=29$, $P<0.001$; AD vs VD: $t=4.59$, $df=35$, $P<0.001$; ANOVA: $F=33.22$; $df=2, 58$; $P=0.0001$) (Fig. 1). There were no significant differences in cytosolic Fluo-3 concentrations between patients and control subjects.

Discussion

The alterations of calcium homeostasis observed in platelets of patients with mild Alzheimer disease result in decreased basal $[Ca^{2+}]_i$ estimated in medium with low Ca^{2+} concentration, while the presence of calcium in the extracellular medium would lead to markedly increased $[Ca^{2+}]_i$ in comparison with age-matched controls. Our measurements indicate that platelets of AD patients have impaired buffering capacity, resulting in the rise of $[Ca^{2+}]_i$ towards potentially harmful levels. The percentage of increase of $[Ca^{2+}]_i$ in platelets after the addition of

1 mM calcium to the incubation medium could provide a biological marker of AD which is very sensitive (95 %) and fulfills most of the other criteria defining the desired biological marker (see above). Unlike *post-mortem* examinations (Sáez-Valero *et al.* 1997), this measurement is non-invasive and able to detect AD early in its course. However, there is an overlap between the AD and VD patients, although the difference between these two groups is statistically significant. Calcium depletion as well as a calcium overload may be detrimental to neurons, impairing the processes of neurotransmission and long-term potentiation. Although our results do not offer a highly specific diagnostic marker for the differential diagnosis of AD, they further demonstrate that disturbances in Ca^{2+} buffering may represent the common denominator in the pathophysiology of dementia (Kario and Pickering 1999) and allow the search for a common therapeutic strategy of mild AD and VD.

Acknowledgements

Supported by the Ministry of Health of the Czech Republic (Project No. MZ00000023752)

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Reprint requests

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