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Similar age-related changes of free intracellular calcium in lymphocytes and central neurons: effects of Alzheimer's disease

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Summary. Several studies suggest that alterations of cytosolic free calcium concentration ($[Ca^{2+}]_i$) are involved in the pathophysiology of aging and Alzheimer's disease (AD). However, only few data are presently available giving detailed information about specific characteristics of age-related or AD-specific changes in cellular Ca^{2+}-homeostasis. To allow a comprehensive evaluation of age-related changes in $[Ca^{2+}]_i$, we performed parallel investigations in central mouse brain cells and mouse spleen lymphocytes of young and aged animals and also in human lymphocytes and granulocytes of young and aged donors and additionally of AD patients. In aged animals, basal $[Ca^{2+}]_i$ was decreased in brain cells but increased in spleen lymphocytes. No age-related alterations in baseline $[Ca^{2+}]_i$ was found in human lymphocytes or granulocytes. However, comparison of activation-induced rise in $[Ca^{2+}]_i$ revealed parallel age-related changes in the different cell-types investigated. The increase in $[Ca^{2+}]_i$ after depolarization of mouse brain cells with KCl and after stimulation of mouse lymphocytes with phytohaemagglutinin (PHA) was significantly impaired in aged animals. Moreover, activation of human lymphocytes with PHA also revealed a reduced increase in $[Ca^{2+}]_i$ in cells of aged donors. In lymphocytes of AD-patients there was a tendency to higher basal $[Ca^{2+}]_i$ compared to their aged matched controls, but no specific alterations in $[Ca^{2+}]_i$ could be found after stimulation with PHA. Also no age-related or AD-specific changes were found in granulocytes after stimulation with N-fomyl-methionyl-leucyl-phenylalanine (fMLP). Since K^+- and PHA-induced rise in $[Ca^{2+}]_i$ is mainly mediated by Ca^{2+}-influx, whereas fMLP-stimulated rise in $[Ca^{2+}]_i$ is mainly due to intracellular Ca^{2+}-release, our findings might indicate that age-related disturbances of Ca^{2+}-homeostasis especially affect mechanisms involved in Ca^{2+}-influx. The corresponding age-related alterations in mouse brain cells, mouse spleen lymphocytes and human lymphocytes after cell activation suggest a similar impairment of Ca^{2+}-homeostasis in these cells and might justify the speculation that Ca^{2+}-homeostasis in the aged human brain is affected in a comparable fashion.

Key words: Intracellular calcium – Alzheimer's disease – Lymphocyte – Brain aging

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Introduction

According to the calcium hypothesis of brain aging disturbances of the free intracellular calcium ($[Ca^{2+}]_i$) homeostasis may play a key role in the pathology of Alzheimer's disease (AD) and multiinfarct dementia (MID). They may also contribute to age-related cognitive impairment (Gibson and Peterson, 1987, Khachaturian, 1989). Thus, pharmacological mechanisms controlling the neuronal Ca^{2+}-homeostasis represent a major strategy for the development of new treatments of AD or other age-related disturbances of cognitive functions. However, few data are presently available giving detailed information about the specific characteristics of age-related or even AD-specific changes in cellular Ca^{2+}-homeostasis.

Most investigations in animals have been performed in synaptosomal preparations from old mice and rats. In these preparations, basal and depolarization-induced $^{45}Ca^{2+}$-uptake have been reported to decrease (Peterson and Gibson 1983; Leslie et al. 1985; Martinez et al. 1987), increase (Martinez et al. 1988; Michaelis et al. 1992), or even show no changes (Giovanelli and Pepeu 1987). In contrast, studies using intact, acutely dissociated central neurons revealed reduced basal $[Ca^{2+}]_i$ and reduced K^+-induced increase in $[Ca^{2+}]_i$ in aging (Hartmann et al. 1993; Hartmann and Müller 1993). Several studies about age-related changes in $[Ca^{2+}]_i$ in murine lymphocytes showed similar reductions of $[Ca^{2+}]_i$ with higher age (Miller et al. 1987, 1989; Grossmann et al. 1991).

Age related changes in Ca^{2+}-homeostasis have been investigated in human lymphocytes (Toth et al. 1989; Grossmann et al. 1989; Adunsky et al. 1991) or cultured

fibroblasts (Peterson et al. 1986; Huang et al. 1991). Again, the results from this small number of studies do not give an unequivocal answer about possible changes of Ca^{2+}-homeostasis in aged humans.

It appears premature to draw any definite conclusion from the studies cited above regarding the direction or even mechanism of age-related changes of $[Ca^{2+}]_i$ homeostasis in animals or humans, since in most of these studies different methodological aspects and different mechanisms of $[Ca^{2+}]_i$ regulation have been investigated. Accordingly, based on our initial observations on specific changes of $[Ca^{2+}]_i$ homeostasis in dissociated neurons of the mouse (Hartmann et al. 1993; Hartmann and Müller 1993), our present strategy aims at demonstrating corresponding age-related changes of $[Ca^{2+}]_i$ in mouse lymphocytes and even more importantly in human lymphocytes. Positive results would justify further speculations on comparable age-related changes of $[Ca^{2+}]_i$ homeostasis in murine and human brain and would allow the use of human lymphocytes to study $[Ca^{2+}]_i$ homeostasis in man.

Methods

Female NMRI mice (Interfauna, Tuttlingen) were killed by decapitation and the brains were immediately dissected on ice. At the same time, spleens were removed and further processed as described below (b).

(a) $[Ca^{2+}]_i$ in dissociated brain cells. Dissociated mouse brain cells were prepared from young (3 months) and aged (22 months) mice following the method of Stoll et al. (1991). The protein content of the preparation of young and aged animals was not different and averaged between 60–100 µg/ml. After washing of the cell suspension, the preparation was resuspended in 2 ml Hank's balanced salt solution (HBSS) containting 1 mmol/l $CaCl_2$ and 1 mmol/l $MgSO_4$ and was incubated with fura-2 AM in the dark (Molecualr Probes, USA) (10 µmol/l) for 45 min in a shaking water bath (37°C). After dye-loading, cells were washed twice and resuspended in 14 ml HBSS (see above). Aliquots of 2 ml were taken and kept at 37°C, each sample was washed immediately before measurement. Fluorescence was measured with a SLM Aminco 4800 spectrofluorometer, where samples were kept at 37°C under magnetic stirring. After equilibration to get the basal $[Ca^{2+}]_i$ the appropriate concentration of KCl (50 µl) was added. $[Ca^{2+}]_i$ was calculated according to Grynkiewiecz et al. (1985) (R_{max}, 0.2% SDS, R_{min} EGTA 6 mmol/l, TRIS 30 mmol/l).

(b) Isolation of mouse spleen T-lymphocytes. Spleen cells were depleted of B-lymphocytes by passage over nylon wool columns (Julius et al. 1973) and subsequently further depleted of erythrocytes by NH_4Cl lysis. Lymphocytes prepared in this way were analysed by flow cytometric analysis using fluorescein isothiocyanate- conjugated (FITC) hamster anti-mouse CD3-e monoclonal antibody (Dianova, Hamburg). The percentage of T-cells in this preparation was found to be approximately 88%.

(c) Healthy volunteers and Alzheimer patients. Eleven patients (5 men, 7 women) with 'probable' or 'possible' Alzheimer's disease according to NINCDS-ADRDA criteria (McKhann et al. 1984) were recruited from an ongoing longitudinal study (Förstl et al., in press). Their ages were between 53 to 86 years (median 72.5 years), the duration of illness ranged from 1 to 20 years (median 2 years) and the severity of illness was moderate in the majority of cases with clinical dementia ratings from mild to severe (CDR = 1–3; Berg 1984). Their findings were compared with those of age-appropriate group of non-demented controls (age-median 68 years; range 42–88 years; 6 men, 6 women). Patients and controls did not receive treatments with calcium antagonists. The 14 aged controls were compared with 14 sex-matched young subjects. Young donors were volonteers of age 20–30 years (mean age = 27) without chronic illness and did not take any medications except one taking oral contraceptives.

(d) Isolation of peripheral blood lymphocytes (PBL) and granulocytes. PBL were isolated from heparinized blood by centrifugation on Ficoll-Hypaque (Lymphoprep^R, Immuno, Heidelberg). Granulocytes in the bottom fraction were separated from erythrocytes by sedimentation (Boyum et al. 1984).

(e) Measurement of $[Ca^{2+}]_i$ in blood cells. For Ca^{2+} measurements murine and human lymphocytes at a density of 10^6 cells/ml were loaded with fura-2 AM (3 µmol/l) for 40 min at 37°C, human granulocytes were incubated for 30 min at room temperature. After washing the samples were equilibrated in a cuvette at 37°C for 3 min (cell density 2.5×10^6 cells/ml). Lymphocytes were stimulated with phytohaemagglutinin (PHA) (Sigma, München, FRG) at a final concentration of 15 or 100 µg/ml. Granulocytes were stimulated with N-formyl-methionyl-leucyl-phenylalanin (fMLP) (Sigma, München, FRG), final concentration 1 µmol/l. For calculation of $[Ca^{2+}]_i$ see above.

(f) Statistics. Differences were assessed by analysis of variance (ANOVA) (SAS Institute, Cary, North Carolina) or by unpaired Students's *t*-test.

Results

Measurement of $[Ca^{2+}]_i$ in intact mouse brain cells by fluorescence spectroscopy revealed baseline levels of $[Ca^{2+}]_i$ about 350 nmol/l which is in accordance with findings reported by Villalba et al. (1992) in a similar preparation of rat brain and with our previous findings in the mouse brain (Hartmann et al. 1993; Hartmann and Müller 1993). A significant reduction of basal $[Ca^{2+}]_i$ was found in neurons of aged animals where $[Ca^{2+}]_i$ averaged at 265 nmol/l (Fig. 1, inset). Depolarization of neuronal cells with KCl induced a fast increase in $[Ca^{2+}]_i$ which was dependent on the K^+-concentration used. Comparison of the K^+-activated elevation of $[Ca^{2+}]_i$ in cells of young and aged mice revealed a significant lower rise in $[Ca^{2+}]_i$ in brain cells of aged mice (Fig. 1).

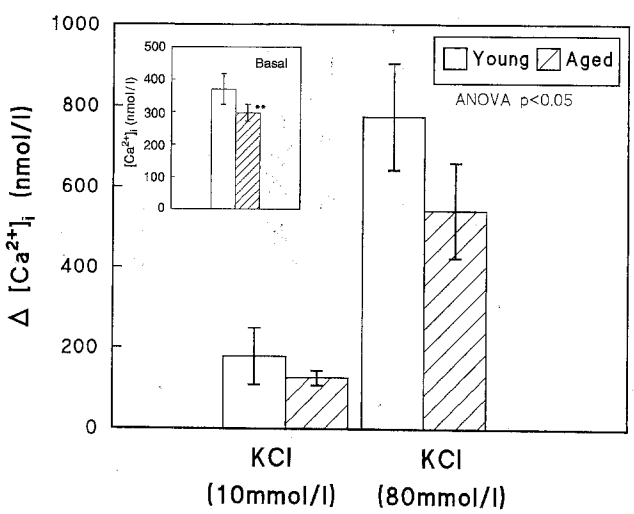

Fig. 1. Potassium-induced increase in $[Ca^{2+}]_i$ in mechanically dissociated neurons from young (3 months) and aged (22 months) female NMRI mice. Values represent the maximal initial response and are means ± SD (n = 6–7), each representing an individual animal. Response in aged brain cells of aged animals is significantly reduced (ANOVA $P = 0.022$, $F = 7.50$). *Inset:* Basal $[Ca^{2+}]_i$ in dissociated neurons from aged mice is significantly reduced (n = 6–7, $P < 0.001$)

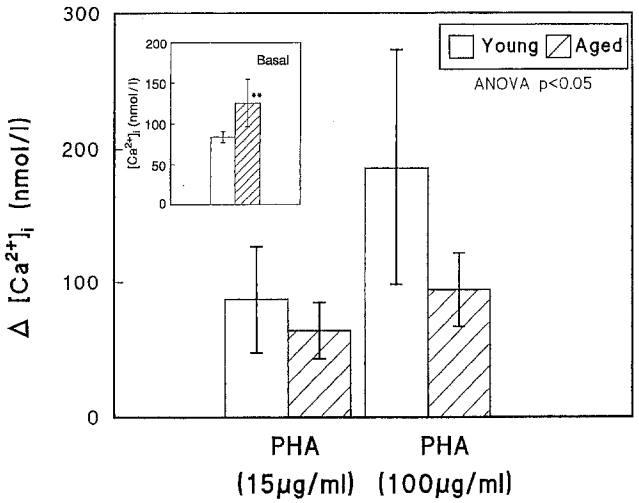

Fig. 2. PHA-stimulated increase in $[Ca^{2+}]_i$ in spleen T-lymphocytes of young (3 months) and aged (22 months) female NMRI mice. Values represent the maximal response and are means ± SD (n = 6–7), each representing an individual animal. Response in aged animals is significantly reduced (ANOVA $P = 0.057$, $F = 4.52$). *Inset:* Basal $[Ca^{2+}]_i$ in spleen T-lymphocytes of aged animals is significantly increased (n = 6–7, $P < 0.01$)

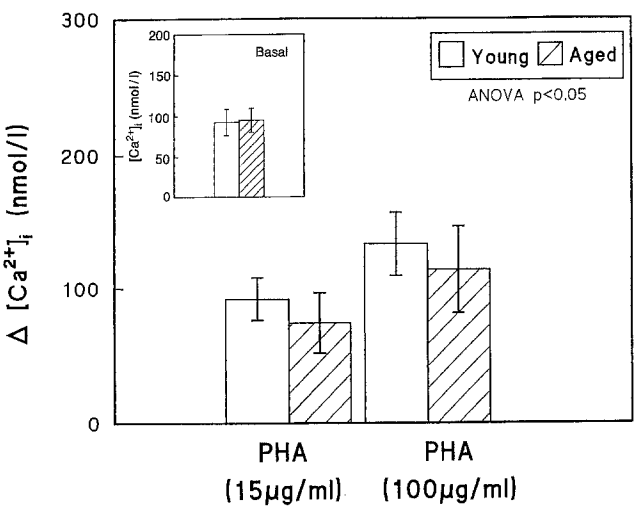

Fig. 4. PHA-stimulated increase in $[Ca^{2+}]_i$ in human lymphocytes of young and aged subjects. Values represent the maximal response and are means ± SD (n = 14). Response in lymphocytes of aged donors is significantly reduced (ANOVA P = 0.0377, F = 4.89). *Inset:* Basal $[Ca^{2+}]_i$ in lymphocytes of young and aged donors (n = 14)

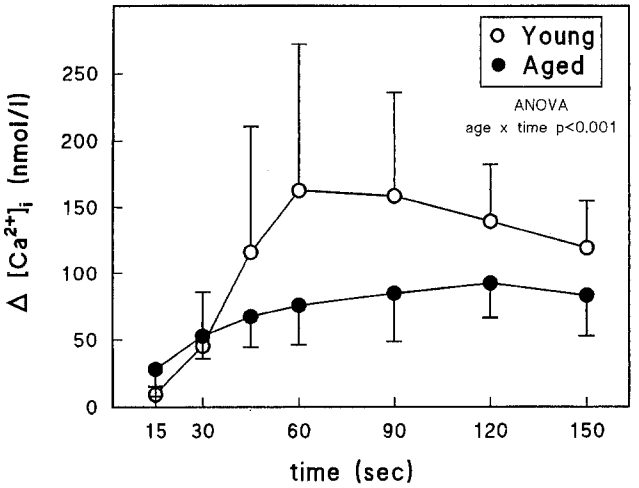

Fig. 3. Time-course of PHA-induced (100 µg/ml) increase in $[Ca^{2+}]_i$ in spleen T-lymphocytes of young (3 months) and aged (22 months) mice. Response in cells of aged animals is significantly reduced (n = 6–7, ANOVA $P = 0.0004$, $F = 4.86$, age × time interaction)

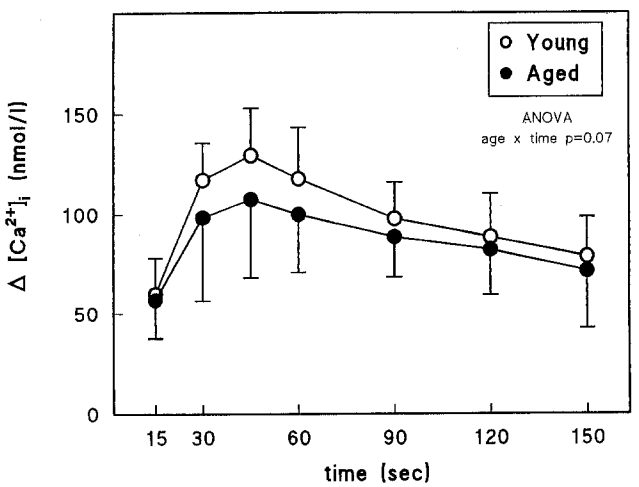

Fig. 5. Time course of PHA-induced (100 µg/ml) increase in $[Ca^{2+}]_i$ in human lymphocytes of young and aged subjects. Response in cells of aged donors is reduced (n = 14, ANOVA P = 0.07, F = 2.0, age × time interaction)

To compare age-related alterations of Ca^{2+}-regulating mechanisms in central neurons and peripheral cells, T-lymphocytes were isolated from spleens of the same animals. In contrast to results obtained in neurons, basal $[Ca^{2+}]_i$ in T-lymphocytes of aged mice was significantly increased (Fig. 2, inset). Activation of T-cells with the lectin PHA resulted in a concentration-dependent rise in $[Ca^{2+}]_i$, which was altered by aging in the same direction as in neuronal cells, namely a significant lower increase in $[Ca^{2+}]_i$ after cell activation (Fig. 2).

In contrast to the fast rise in $[Ca^{2+}]_i$ after depolarization of brain cells, where maximal response is usually seen after a few seconds (Hartmann et al. 1993) maximal increase after PHA-stimulation is reached only after about 60 s (Fig. 3). This can be explained by the contribution of two different mechanisms, an IP_3-activated intracellular Ca^{2+}-release, which dominates the first phase, and a Ca^{2+}-influx from the extracellular space, which represents the main component of the plateau phase (Michel et al. 1992). Similar to the reduced depolarization-induced Ca^{2+}-influx in aged brain cells, age-related impairment of PHA-induced elevation of $[Ca^{2+}]_i$ mainly affects the plateau phase (Fig. 3).

In the second part of our study, alterations of $[Ca^{2+}]_i$ were investigated in human blood cells of young and aged subjects. Measurement of basal $[Ca^{2+}]_i$ in lymphocytes revealed no difference between the two groups (Fig. 4, inset). However, parallel to our findings in mouse lympho-

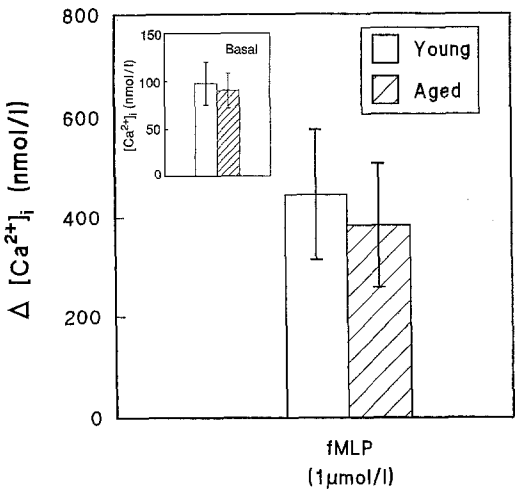

Fig. 6. fMLP-induced increase in $[Ca^{2+}]_i$ in human granulocytes of young and aged subjects. Values represent the maximal response and are means ± SD (n = 14). *Inset:* Basal $[Ca^{2+}]_i$ in granulocytes of young and aged donors

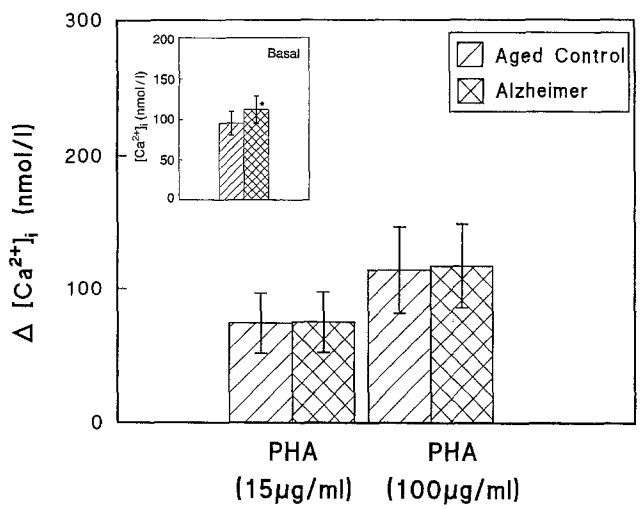

Fig. 7. PHA-stimulated increase in $[Ca^{2+}]_i$ in human lymphocytes of aged controls (n = 14) and of AD patients (n = 14). Values represent the maximal response and are means ± SD. *Inset:* Basal $[Ca^{2+}]_i$ in lymphocytes of AD patients is significantly increased ($P < 0.05$)

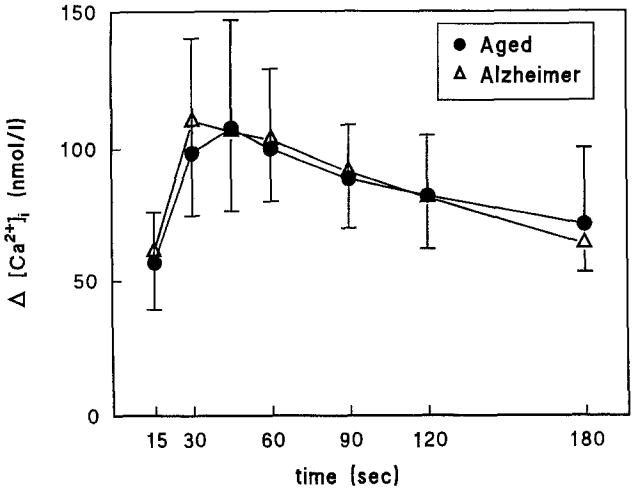

Fig. 8. Time-course of PHA-induced (100 μg/ml) increase in $[Ca^{2+}]_i$ in human lymphocytes of aged controls (n = 14) and of AD patients (n = 12)

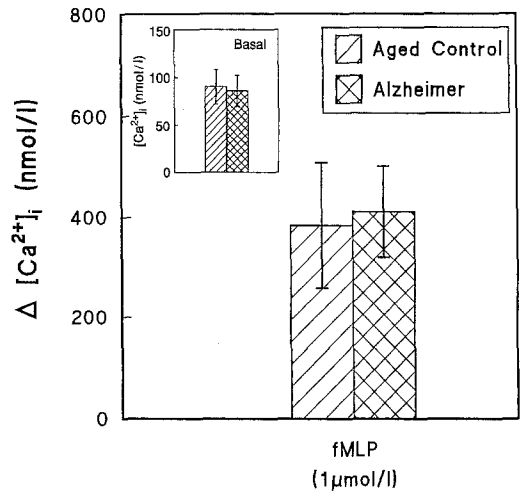

Fig. 9. fMLP-induced increase in $[Ca^{2+}]_i$ in human granulocytes of aged controls (n = 14) and of AD patients (n = 12). Values represent the maximal response and are means ± SD. *Inset:* Basal $[Ca^{2+}]_i$ in granulocytes of aged controls and of AD patients

cytes, the PHA-activated rise in $[Ca^{2+}]_i$ was significantly lower in cells of aged subjects (Fig. 4). The kinetics of the PHA-induced increase in $[Ca^{2+}]_i$ are different in human lymphocytes, since the maximal increase is already reached after 30–45 s. However, comparison of the time courses in cells of young and aged subjects show similar alterations as observed in mice, i.e. impaired PHA-activated increase in $[Ca^{2+}]_i$ in cells of aged subjects is more pronounced in the plateau phase (Fig. 5). We performed some experiments about age-related alterations of Ca^{2+}-homeostasis in human granulocytes. In this cell type, no difference in basal $[Ca^{2+}]_i$ could be found and $[Ca^{2+}]_i$ after stimulation with fMLP was also not different between the two groups (Fig. 6).

As mentioned above, alterations of cellular Ca^{2+}-homeostasis may be involved in the pathology of AD. Therefore, we compared $[Ca^{2+}]_i$ in lymphocytes of AD patients and of aged-matched controls. A mild but significant increase of basal $[Ca^{2+}]_i$ was found in AD (Fig. 7, inset). However, PHA-induced rise in $[Ca^{2+}]_i$ was not different. Even after stimulation with a very high concentration of PHA (100 μg/ml) no AD-specific alteration could be found (Fig. 7). This is in contrast to findings of Adunsky et al. (1991) who described a dramatically increased response in lymphocytes of AD patients under this condition. The time course of the PHA-induced increase in $[Ca^{2+}]_i$ was also not altered in AD (Fig. 8) as the fMLP-induced rise in $[Ca^{2+}]_i$ in granulocytes (Fig. 9).

Discussion

We carried out experiments on age-related alterations of Ca^{2+}-homeostasis in dissociated mouse brain cells, mouse

lymphocytes, human lymphocytes and human granulocytes. These cells were stimulated under appropriate conditions similar to physiological stimulation to allow the evaluation of common mechanisms presumedly involved in the disturbed Ca^{2+}-homeostasis in aging. Comparable age-related alterations of stimulus-induced rise in $[Ca^{2+}]_i$ were observed in the first three cell types mentioned.

The relevance of reduced basal $[Ca^{2+}]_i$ and reduced depolarization-induced elevations of $[Ca^{2+}]_i$ in dissociated neurons of aged mice has been discussed recently (Hartmann et al. 1993; Hartmann and Müller, 1993). A reduced elevation of $[Ca^{2+}]_i$ following murine T-cell activation in aging was also found by Miller et al. (1987, 1989) after stimulation with ConA or ionomycin and by Grossmann et al. (1991) after T-cell receptor stimulation with anti-CD3. Franklin et al. (1990) did not report age-related alterations when spleen cells were stimulated with ionomycin, but it must be taken into account that ionomycin- and lectin-induced elevations of $[Ca^{2+}]_i$ are mediated by different mechanisms. Few, but heterogenous, data are available about age-related alterations of $[Ca^{2+}]_i$ homeostasis in human lymphocytes. Lustyik and O'Leary (1989) found no age-related alterations following cell activation, whereas Grossmann et al. (1989) found reduced $[Ca^{2+}]_i$ after stimulation with PHA.

On the basis of our corresponding findings in different cell types, several speculations can be made about common characteristics of impaired Ca^{2+}-homeostasis in aged lymphocytes of mice and humans on the one hand, and in central neurons and peripheral lymphocytes on the other. The PHA-activated rise in $[Ca^{2+}]_i$ in T-cells is initially due to IP_3-induced release of intracellular Ca^{2+} and secondly, during signal maintenance, caused by Ca^{2+}-influx from the extracellular space (Michel et al. 1992). In lymphocytes of aged mice or humans, mainly the Ca^{2+}-influx during the plateau phase is reduced, whereas intracellular Ca^{2+}-release during the initial phase seems to be less affected. Similarly, no age-related alteration in fMLP-induced increase in $[Ca^{2+}]_i$ were found in human granulocytes. Since fMLP elevates $[Ca^{2+}]_i$ mainly by IP_3-induced intracellular Ca^{2+}-release (Thompson et al. 1991) this observation fits into the interpretation of the findings on lymphocytes suggesting that age-related disturbances of $[Ca^{2+}]_i$ after PHA stimulation must be related to changes of Ca^{2+}-influx. Depolarization-induced rise in $[Ca^{2+}]_i$ in brain cells, which is also impaired in neurons of aged mice, is highly dependent on extracellular calcium and therefore the signal is dominated by a Ca^{2+}-influx.

Even if some reservations must be made regarding a direct comparison of results obtained in central neurons and in peripheral cells, both cell types share common features concerning the regulation of Ca^{2+} homeostasis. Therefore, the conclusion seems to be legitimate that aging affects mechanisms regulating Ca^{2+} influx in neurons and lymphocytes in a comparable fashion. Moreover, it seems to be justified to speculate that aging might also change $[Ca^{2+}]_i$ homeostasis in human brain in a comparable fashion to that observed in human lymphocytes.

The divergent age-related alterations in baseline $[Ca^{2+}]_i$ in mouse brain cells (reduced), in mouse lymphocytes (increased) and in human lymphocytes (no change) strongly suggest that even if one mechanism controlling $[Ca^{2+}]_i$ is similarly altered, the consequences for $[Ca^{2+}]_i$ homeostasis must not necessarily be identical in each cell type. Moreover, participation of other Ca^{2+}-regulating mechanisms (e.g. Ca^{2+}-pump) cannot be excluded. Similar to our findings, no age-related change in basal $[Ca^{2+}]_i$ in human lymphoctes were found by Lustyik and O'Leary (1989) or by Grossmann et al. (1989). Toth et al. (1989) reported a reduced basal $[Ca^{2+}]_i$ in lymphocytes of aged subjects, which parallels our findings in murine lymphocytes.

In respect to AD, in the present study, baseline $[Ca^{2+}]_i$ in lymphocytes of AD-patients is significantly increased. This is in accordance with findings of Adunsky et al. (1991) who also found elevated baseline $[Ca^{2+}]_i$ in AD patients. A similar tendency was also observed by Bondy et al. (this issue). In contrast to findings of Adunsky et al. (1991) no AD-specific enhanced response following T-cell activation with high doses of PHA (100 µg/ml) could be demonstrated. This unaltered response is in accordance with findings of Bondy et al. (this issue). Ca^{2+} homeostasis in granulocytes is not altered in AD patients. All findings taken together might suggest small but significant alterations of $[Ca^{2+}]_i$ in AD which need to be further investigated.

In summary, our findings indicate corresponding age-related alterations of $[Ca^{2+}]_i$ homeostasis in mouse brain cells, mouse spleen lymphocytes and human peripheral lymphocytes but only minor additional changes in AD. Further studies will be necessary to identify the underlying mechanisms and to characterize further similarities and differences in age-related alterations of Ca^{2+}-homeostasis in central neurons and peripheral cells.

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References

Adunsky A, Baram D, Hershkowitz M, Mekori YA (1991) Increased cytosolic calcium in lymphocytes of Alzheimer patients. J Neuroimmunol 33:167–172

Berg L (1984) Clinical dementia rating (Letter) Br J Psychiatry 145:339

Bondy B, Klages U, Müller-Spahn F, Hock C (1994) Cytosolic free $[Ca^{2+}]$ in mononuclear blood cells from demented patients and healthy controls. Eur Arch Psychiatry Clin Neurosci 243:224–228

Boyum A (1984) Separation of lymphocytes, granulocytes and monocytes from human blood using iodinated density gradient media. Methods Enzymol 108:88–102

Förstl H, Besthorn C, Geiger-Kabisch C, Sattel H, Schreiter-Gasser U (1993) Psychotic features and the course of Alzheimer's disease: relationship to cognitive, EEG and CT-Scan findings. Acta Psychiatr Scand 87:395–399

Franklin RA, Arkins S, Kelley KW (1990) The proliferative response of rat T cells to calcium ionophores increase with age. Cell Immunol 130:416–428

Gibson GE, Peterson C (1987) Calcium and the aging nervous system. Neurobiol Aging 8:319–343

Giovanelli L, Pepeu G (1989) Effect of age on K^+-induced cytosolic Ca^{2+} changes in rat cortical synaptosomes. J Neurochem 53:392–398

Grossmann A, Ledbetter JA, Rabinovitch PS (1989) Reduced proliferation of T lymphocytes in aged humans is predominantly in the $CD8^+$ subset and is unrelated to defects in transmembrane signaling which are predominantly in the $CD4^+$ subset. Exp Cell Res 180:367–382

Grossmann A, Maggio-Price L, Jinneman JC, Rabinovitch PS (1991) Influence of aging on intracellular free calcium and proliferation of mouse T-cell subsets from various lymphoid organs. Cell Immunol 135:188–131

Grynkiewiecz G, Poenie M, Tsien RY (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440–3450

Hartmann H, Eckert A, Müller WE (1993) Aging enhances the calcium sensitivity of central neurons of the mouse as an adaptive response to reduced free intracellular calcium. Neurosci Lett 152:181–184

Hartmann H, Müller WE (1993) Age-related changes in receptor-mediated and depolarizaiton-induced phosphatidylinositol turnover in mouse brain. Brain Res 622:86–92

Huang HM, Toral-Barza L, Thaler H, Tofel-Grehl B, Gibson GE (1991) Inositol phosphates and intracellular calcium after bradykinin stimulation in fibroblasts from young, normal aged and Alzheimer donors. Neurobiol Aging 12:469–473

Julius MH, Simpson E, Herzenberg LA (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur J Immunol 3:645–653

Khachaturian ZS (1989) The role of calcium regulation in brain aging: reexamination of a hypothesis. Aging 1:17–34

Leslie SW, Chandler LJ, Barr EM, Farrar RP (1985) Reduced calcium uptake by rat brain mitochondria and synaptosomes in response to aging. Brain Res 329:177–183

Lustyik G, O'Leary JJ (1989) Aging and the mobilitation of intracellular calcium by phytohemmagglutinin in human T cells. J Gerontol 44:B30–36

Martinez A, Vitorica J, Bogonez E, Satrustegui J (1987) Differential effects of age on the pathways of calcium influx into nerve terminals. Brain Res 435:249–257

Martinez A, Vitorica J, Satrustegui J (1988) Cytosolic free calcium levels increase with age in rat brain synaptosomes. Neurosci Lett 88:336–342

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: a report of the NINCDS-ADRDA work group under the Anspices of the Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 34:939–944

Michaelis ML, Foster CT, Jayawickreme C (1992) Regulation of calcium levels in brain tissue from adult and aged rat. Mech Ageing Dev 62:291–306

Michel MC, VanTits LJH, Trenn G, Sykora J, Brodde OE (1992) Dissociation between phytohemagglutinin-stimulated generation of inositol phosphates in Ca^{2+}-increase in human mononuclear leukocytes. Biochem J 285:137–141

Miller RA, Jacobson B, Weil G, Simons ER (1987) Diminished calcium influx in lectin-stimulated T cells from old mice. J Cell Physiol 132:337–342

Miller RA, Philosophe B, Ginis I, Weil G, Jacobson B (1989) Defective control of cytoplasmic calcium concentration in T lymphocytes from old mice. J Cell Physiol 138:175–182

Peterson C, Gibson GE (1983) Aging and 3,4 diaminopyridine alter synaptosomal calcium uptake. J Biol Chem 258:11482–11486

Peterson C, Ratan RR, Shelanski M, Goldman JE (1986) Cytosolic free calcium and cell spreading decrease in fibroblasts from aged and Alzheimer donors. Proc Natl Acad Sci USA 83:7999–8001

Stoll L, Schubert T, Müller WE (1991) Age-related deficits of central muscarinic cholinergic receptor function in the mouse: partial restoration by chronic piracetam treatment. Neurobiol Aging 13:39–44

Thompson NT, Bonser RW, Tateson JE, Spacey GD, Randall RW, Hodson HF, Garland LG (1991) A quantitative investigation into the dependence of Ca^{2+} mobilization on changes in inositol 1,4, 5-trisphosphate levels in the stimulated neutrophil. Br J Pharmacol 103:1592–1596

Toth S, Csermely P, Beregi E, Szkladanyi A, Szabo LD (1989) Decreased cytosolic free calcium concentration of aged human lymphocytes in resting state. Compr Gerontol Suppl Issue A+B 3:16–22

Villalba MA, Martinez-Serrano C, Brner P, Blanco P, Satrustegui J (1992) NMDA-induced increase in $[Ca^{2+}]_i$ and $^{45}Ca^{2+}$uptake in acutely dissociated brain cells derived from adult rats. Brain Res 570:347–353