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Cytosolic free [Ca^{2+}] in single T-lymphocytes from depressed patients and healthy controls

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Summary. Human lymphocytes are widely used as peripheral models for central neurones. Alterations in immune function have been reported in depressed patients, e.g. mitogen-induced proliferation is impaired during depression. One possible causative mechanism could be altered $[Ca^{2+}]_i$ regulation. Phytohaemagglutinin (PHA)-induced rise of $[Ca^{2+}]_i$ has been found to be diminished in lymphocyte suspensions from depressed patients (Ecker et al., this issue). We measured PHA-induced rise of $[Ca^{2+}]_i$ in single Fura-2 AM-loaded $T11^+$ lymphocytes of patients with major depression and controls to further analyse $[Ca^{2+}]_i$ regulation in depression.

The $[Ca^{2+}]_i$ of resting lymphocytes was 57 ± 2 nmol/l (mean ± SEM). There was no difference in resting $[Ca^{2+}]_i$ of resting lymphocytes of patients and controls. PHA evoked an increase of $[Ca^{2+}]_i$ an 7 out of 14 cells from control subjects up to 400–500 nmol/l. In contrast, only 4 out of 13 cells from depressed patients showed an increase of $[Ca^{2+}]_i$ up to 200 nmol/l. In a small fraction of cells from both groups the $[Ca^{2+}]_i$ signal is oscillating.

Our preliminary data confirm alteration of $[Ca^{2+}]_i$ regulation in lymphocytes of depressed patients.

Key words: Intracellular free calcium – Major depression – Lymphocytes

Introduction

An important breakthrough in the understanding of neuronal function was achieved with the technique of single cell recording. Thereby cellular mechanisms such as calcium dependence were detected with identical importance for the functional homeostasis of central neurons as of several peripheral cells. A specific example is the surprising similarity between lymphocytes and neurons with respect to surface receptors (Wiedermann 1987, Plaut 1987), membrane channels (Lewis and Cahalan 1988) or to the oscillating mode of $[Ca^{2+}]_i$ changes (Friel and Tsien 1992), suggesting T lymphocytes as a peripheral model of neuronal function.

This analogous situation in T-lymphocytes led us to the measurement of free intracellular calcium concentration ($[Ca^{2+}]_i$) in single T-lymphocytes of normal volunteers and depressed patients. This approach is further supported by the following observations:

– In depression, impaired immunological function has been reported, such as reduced cell proliferation (Kronfol et al. 1983, 1989; Schleifer et al. 1984, 1989) or reduced natural killer cell activity (Miller et al. 1987; Evans et al. 1992; Maes et al. 1992).
– These immunological functions are regulated in a sensitive manner by calcium dependent mechanisms (Meuer and Resch 1989).
– Calcium homeostasis seems to be distrubed in affective disorders (Dubovsky et al. 1991; Kusumi et al. 1991, 1992).

The interpretation of the data presented have will compare results on single cell level with the data of cells in suspension as reported in other contributions to this issue.

Subjects and methods

Depressed female patients (age 29, 34, 71 and 76 years) were compared with four age-matched female controls. The patients fulfilled DSM III R criteria for unipolar depression and had a HAMD score between 18 and 24. No subject had received psychotropic medication for at least one week.

From 10 ml heparinized blood mononuclear cells were separated by Ficoll-Hypaque (Biochrom, Berlin, Germany) centrifugation. The cells were washed in RPMI 1640 (Biochrom) + 5% fetal calf serum (FCS, Boehringer, Mannheim, Germany). Monocytes were separated by adherence to plastic culture dishes. Non-adherent cells were incubated with anti-T11 antibody (gift from S. Meuer, DKFZ Heidelberg, Germany) for 60 min at 4°C. $T11^+$ cells were panned to precoated class coverslips for at least 60 min at 4°C. Coverslips were coated with goat anti-mouse antibody (DAKO, Hamburg, Germany) at 4°C overnight. For $[Ca^{2+}]_i$ measurements cells were loaded with 5 µmol/l Fura-2 AM (Molecular Probes, Eugene, OR, USA) for 30 min at 37°C. Measurements were per-

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formed in NaCl 160, KCl 4.5, $CaCl_2$ 2, $MgCl_2$ 1, Hepes-NaOH 5 (pH7.4) at 20–22°C within 30 min.

The experiments were controlled, recorded and analysed using TIDA5 software and hardware (Batelle Institute, Frankfurt, Germany) with a 4/86 computer. Cells were alternately illuminated with 340 and 380 nm light flashes. These were generated by two Hamamatsu flash bulbs, each of which illuminated a 1.25 mm diameter quartz fibre. The two fibres were placed in the entrance slit region of a concave holographic grating (Jobin Yvon). A third fibre in the exit slit area collected the light (340 nm when flash 1 was triggered, 380 nm when flash 2 was triggered) and transferred it to the microscope. A Nikon Fluor ×40 objective was used. Emitted light was collected at > 420 nm in a 10 µm area by a photodiode (Hamamatsu). This optical setup was constructed by R. Uhl, Technology Transfer, München, Germany (Uhl et al., personal communication). For calibration 10 mmol/l $CaCl_2$-solution (10 mmol/l EGTA-solution respectively) with 25 µmol/l Fura-2 was used to obtain R_{max} (R_{min}) and a solution containing 6 mmol/l Ca-EGTA and 3 mmol/l free EGTA was used to obtain $K_D \times S_{b380}/S_{f380}$. From these parameters $[Ca^{2+}]_i$ was calculated according to Almers and Neher (1985), Grynkiewicz at el. (1985) and Negulescu and Machen (1990).

Basal $[Ca^{2+}]_i$ of 8–11 medium-sized (7–8 µm diameter) cells with smooth surface were recorded for each subject, cells with a $[Ca^{2+}]_i$ baseline > 110 were assumed to be prestimulated and were excluded. 3–4 cells of each subject were stimulated with 15 µg/ml PHA-P (Sigma, München, Germany) and $[Ca^{2+}]_i$ was recorded for 10 min.

For statisical analysis of resting $[Ca^{2+}]_i$ levels a two-tailed *t*-test ($P = 0.05$) was used.

Results

$[Ca^{2+}]_i$ of resting T-lymphocytes from control subjects was 56.7 ± 2.1 nmol/l (mean ± SEM). The cells had $[Ca^{2+}]_i$ levels between 34 and 85 nmol/l. $[Ca^{2+}]_i$ of resting T-lymphocytes from depressed patients was 51.7 ± 2.0 nmol/l (mean ± SEM). $[Ca^{2+}]_i$ levels of resting T-lymphocytes from depressed patients were not significantly different from those of T-lymphocytes from healthy controls. Figure 1 shows all resting $[Ca^{2+}]_i$ levels from control subjects and depressed patients.

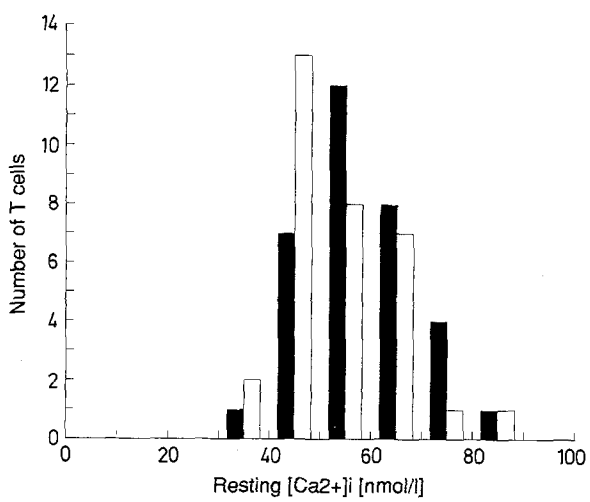

Fig. 1. Distribution of $[Ca^{2+}]_i$ levels (nmol/l) in resting T-lymphocytes from four control subjects (*filled bars*) and from four depressed patients (*white bars*)

There was no difference in absolute intensities of emitted light between cells from depressed patients or controls, indicating that Fura-2 loading was not different in cells from depressed patients or controls.

After stimulation with PHA, 7 out of 14 T-lymphocytes from control subjects (1 or 2 out of 3 or 4 stimulated cells of each control subject) showed an increase of $[Ca^{2+}]_i$. The latency between application of PHA and the $[Ca^{2+}]_i$ rise was between 50 and 200 s. The pattern of the $[Ca^{2+}]_i$ signal was variable. Four T-cells displayed a slow and smooth increase of $[Ca^{2+}]_i$ to about 100 nmol/l, in 2 T-cells $[Ca^{2+}]_i$ rose continuously to 400–500 nmol/l, in 1 T-cell $[Ca^{2+}]_i$ rose to 300–400 nmol/l with a few oscillations (amplitude 50–100 nmol/l, frequency 1/50–100 s) (Fig. 2).

Only 4 out of 13 T-lymphocytes from depressed patients showed an increase of $[Ca^{2+}]_i$ after stimulation with PHA (2/4 from patient 1, 1/3 from patient 2 and 3 each,

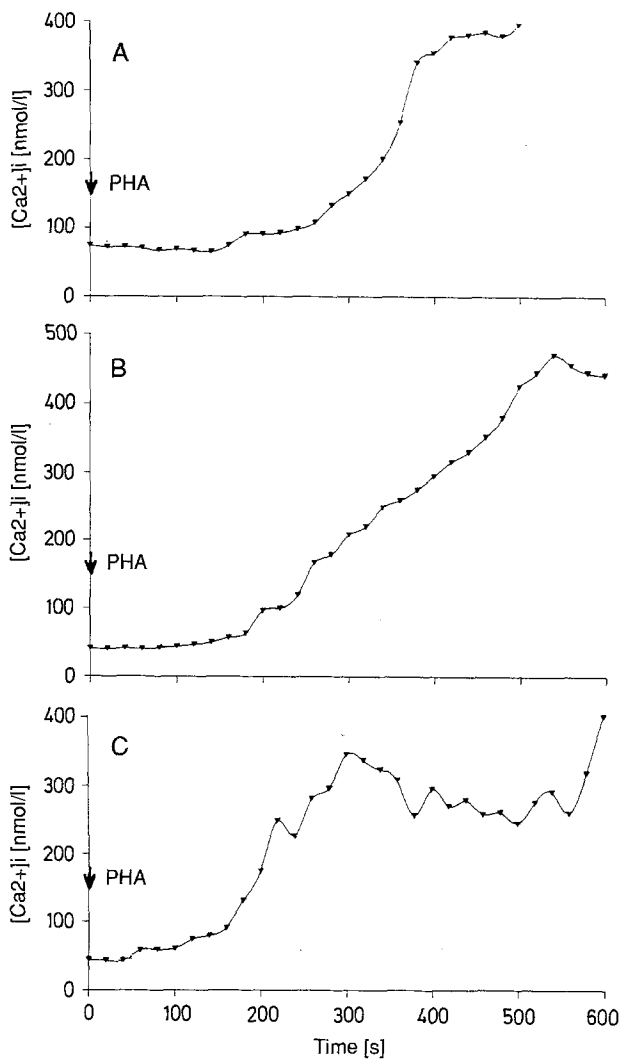

Fig. 2. After stimulation with 15 µg/ml PHA-P (at time = 0), 7 out of 14 stimulated T-lymphocytes from four control subjects displayed an increase of $[Ca^{2+}]_i$ (nmol/l), in three cells the $[Ca^{2+}]_i$ rose to at least twice the resting value. Only these responding cells are shown. Two cells (*A,B*) showed a continuous rise without oscillations to $[Ca^{2+}]_i$ levels around 400 nmol/l, 1 cell (*C*) showed an oscillatory $[Ca^{2+}]_i$ signal

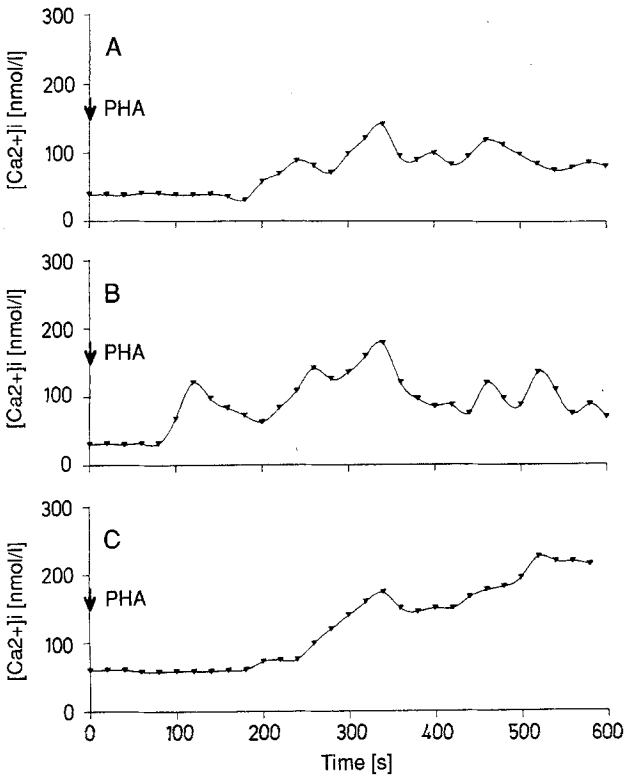

Fig. 3. After stimulation with 15 µg/ml PHA-P (at time = 0), 4 out of 13 stimulated T-lymphocytes from four depressed patients displayed an increase of $[Ca^{2+}]_i$ (nmol/l), in three cells the $[Ca^{2+}]_i$ rose to at least twice the resting value. Only these responding cells are shown. The cells (A–C) showed an oscillatory $[Ca^{2+}]_i$ signal with peak levels up to 200 nmol/l

0/3 from patient 4). The latency was the same as in cells from control subjects. One T-cell displayed a smooth rise of $[Ca^{2+}]_i$ to about 100 nmol/l, 3 T-cells showed oscillations of $[Ca^{2+}]_i$ up to 150–200 nmol/l (amplitude 30–70 nmol/l, frequency 1/50–100 s) (Fig. 3). $[Ca^{2+}]_i$ did not reach as high levels as in T-cells from control subjects, but there was a tendency to more oscillations in T-cells from depressed patients.

The resting $[Ca^{2+}]_i$ levels of responding cells were not different from resting $[Ca^{2+}]_i$ levels of non-responsive cells from controls and depressed patients.

Discussion

Panning with anti-T11 antibodies selected a population consisting of resting and activated T-lymphocytes as well as large granular lymphocytes. To examine a homogeneous population medium-sized cells with a perfectly smooth surface were assumed to be the T-lymphocyte population. Previous studies had demonstrated that the anti-T11 antibody used did not activate $[Ca^{2+}]_i$ by itself nor did it inhibit activation of T-lymphocytes by PHA in suspension (data not shown). Further, slight preactivation of the cells by crosslinking of the anti-T11 antibody during panning is unlikely since the obtained resting $[Ca^{2+}]_i$ levels were in the same range as data obtained previously (see below). PHA induced a non-specific stimulation of T-lymphocytes, further studies are planned with specific T-cell stimulators or neurotransmitters.

Single cell measurement reveals variability of the resting $[Ca^{2+}]_i$ between 34 and 85 nmol/l from cell to cell. These data are in good accordance with Hess et al. (1993) concerning absolute levels and variability. The mean resting $[Ca^{2+}]_i$ of our cells is somewhat lower than resting $[Ca^{2+}]_i$ obtained from measurements in suspension (Tsien et al. 1982, Eckert et al., this issue), which may be due to different calibration procedures.

With respect to resting $[Ca^{2+}]_i$ levels, cells from depressed patients and controls were not different. This confirms data from cells in suspension (Bondy et al., this issue; Eckert et al., this issue).

Mitogenic stimulation by PHA increased the resting $[Ca^{2+}]_i$ in 7 out of 14 cells from healthy controls. In 3 cells $[Ca^{2+}]_i$ rose to values of 400–500 nmol/l, in 4 cells there was a weak rise of $[Ca^{2+}]_i$ to 100–150 nmol/l. This fraction of responding cells fits with the estimation of prestimulated T-lymphocytes which can be activated by 15 µg/ml PHA in the absence of monocytes (S. Meuer, personal communication). Similarly Hess et al. (1993) found that 46% of T-cells responded to 10 µg/ml PHA in a slightly different preparation (T-lymphocytes were separated by nylon wool and plated on poly-D-lysine-coated glass). In the depressed patients, fewer lymphocytes (only 4 out of 13) showed an increase in $[Ca^{2+}]_i$ due to PHA, 3 cells displayed a $[Ca^{2+}]_i$ rise to about 200 nmol/l, 1 cell displayed a weak $[Ca^{2+}]_i$ rise to about 100 nmol/l. Hence, in depressed patients particularly the fraction of cells with a weak $[Ca^{2+}]_i$ rise was smaller. This possibly suggests a lower number of prestimulated peripheral T-cells in depressed patients. Further, in the responding lymphocytes from depressed patients we never observed high rises of $[Ca^{2+}]_i$ up to 500 nmol/l but only dampened oscillations up to about 200 nmol/l. Both findings fit well with the finding of a decreased $[Ca^{2+}]_i$ response of lymphocytes from depressed patients in suspension (Eckert et al., this issue). Since the increase of $[Ca^{2+}]_i$ is an essential step in the proliferation of lymphocytes (Meuer and Resch 1989) the reduced rise of $[Ca^{2+}]_i$ in cells of depressives could possibly explain the reduced proliferation reported elsewhere (Kronfol et al. 1983, 1989; Schleifer et al. 1984).

In accordance with Hess et al. we observed an oscillating $[Ca^{2+}]_i$ signal in response to PHA in a fraction of lymphocytes, which seems to be linked to cellular calcium physiology in a fundamental way. Whereas the initial rise of $[Ca^{2+}]_i$ is related to Ca^{2+} release from intracellular stores depending on receptor binding and signal transduction via second messengers, true oscillations reflect the interaction between Ca^{2+} release and Ca^{2+} influx (Berridge 1993, Lewis and Cahalan 1989). Ca^{2+} influx depends on the membrane potential (Lewis and Cahalan 1990) and on the filling of intracellular stores (Hoth and Penner 1992). Further studies may reveal which of these mechanisms are responsible for the lower high peak levels of Ca^{2+} oscillations in depressed patients which yet seem to be more frequent than in controls.

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References

Almers W, Neher E (1985) The Ca signal from fura-2 loaded mast cells depends strongly on the method of dye-loading. FEBS Lett 192(1):13–18

Berridge MJ (1993) Inositol trisphosphate and calcium signalling. Nature 361:315–325

Dubovsky SL, Lee C, Christiano J, Murphy J (1991) Elevated platelet intracellular calcium concentration in bipolar depression. Biol Psychiatry 29:441–450

Evans DL, Folds JD, Petitto JM, Golden RN, Pedersen CA, Corrigan M, Gilmore JH, Silva SG, Quade D, Ozer H (1992) Circulating natural killer cell phenotypes in men and women with major depression. Arch Gen Psychiatry 49:388–395

Friel DD, Tsien RW (1992) Phase-dependent contributions from Ca2+ entry and Ca2+ release to caffeine-induced [Ca2+]i oscillations in bullfrog sympathetic neurons. Neuron 8:1109–1125

Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem 260(6):3440:3450

Hess SD, Oortgiesen M, Cahalan MD (1993) Calcium oscillations in human T-and NK-cells depend upon membrane potential and calcium influx. J Immunol, in press

Hoth M, Penner R (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 355:353–356

Kronfol Z, Silva J Jr., Greden J, Dembinski S, Gardner R, Carroll B (1983) Impaired lymphocyte function in depressive illness. Life Sci 33(3):241–247

Kronfol Z, House JD (1989) Lymphocyte mitogenesis, immunoglobulin and complement levels in depressed patients and normal controls. Acta Psychiatr Scand 80:142–147

Kusumi I, Koyama T, Yamashita I (1991) Serotonin-stimulated Ca2+ response is increased in the blood platelets of depressed patients. Biol Psychiatry 30:310–312

Kusumi I, Koyama T, Yamashita I (1992) Serotonin-stimulated Ca2+ response in the platelets of depressed patients. Clin Neuropharmacol 15 [Suppl 1]:P-124, 588–689

Lewis RS, Cahalan MD (1988) The plasticity of ion channels: parallels between the nervous and immune systems. TINS 11(5):214–218

Lewis RS, Cahalan MD (1989) Mitogen-induced oscillations of cytosolic Ca2+ and transmembrane Ca2+ current in human leukemic T-cells. Cell Regulation 1:99–112

Lewis RS, Cahalan MD (1990) Ion channels and signal transduction in lymphocytes. Ann Rev Physiol 52:415

Maes M, Stevens W, Peeters D, DeClerk L, Scharpe S, Bridts C, Schotte C, Cosyns P (1992) A study on the blunted natural killer cell activity in severly depressed patients. Life Sci 50:503–511

Meuer S, Resch K (1989) Cellular signalling in T-lymphocytes. Immunology Today 10(8):S23–S25

Miller AH, Asnis GM, Lackner C, Norin AJ (1987) The in vitro effect of cortisol on natural killer cell activity in patients with major depressive disorder. Psychopharmacol Bull 23:502–504

Negulescu PA, Machen TE (1990) Intracellular ion activities and membrane transport in parietal cells measured with fluorescent dyes. Methods Enzymol 102:38–81

Plaut M (1987) Lymphocyte hormone receptors. Ann Rev Immunol 58:621–669

Schleifer SJ, Keller SE, Meyerson AT, Raskin MJ, Davis KL, Stein M (1984) Lymphocyte function in major depressive disorder. Arch Gen Psychiatry 41:484–486

Schleifer JS, Keller SE, Bond RN, Cohen J, Stein M (1989) Major depressive disorder and immunity; role of age, sex, severity, and hospitalization. Arch Gen Psychiatry 46:81–87

Tsien RY, Pozzan T, Rink TJ (1982) T-cell mitogens cause early changes in cytoplasmic free Ca2+ and membrane potential in lymphocytes. Nature 295:68–71

Wiedermann CJ (1987) Shared recognition molecules in the brain and lymphoid tissues: the polypeptide mediator network of psychoneuroimmunology. Immunol Lett 16:371–378