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Platelet and lymphocyte free intracellular calcium in affective disorders

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Summary. Many studies have demonstrated pharmacologic similarities between platelet and brain 5-HT_2 binding sites. Therefore it may be possible to use platelets as a model for the central serotonergic neuron. Accordingly, a previous report (Kusumi et al. 1991b) about elevated $[Ca^{2+}]_i$ after serotonin stimulation in platelets of depressed patients was interpreted as further evidence for enhanced serotonergic sensitivity in depression. However, a very recent study showed an enhanced thrombin-induced platelet Ca^{2+} response, rather suggesting abnormalities of intracellular Ca^{2+} regulation in affective disorders. In the present study we have determined 5-HT_2- and thrombin-induced Ca^{2+} responses in platelets and additionally phytohemagglutin (PHA)-induced Ca^{2+} increase in lymphocytes of medicated depressed patients (8 mono- and 2 bipolar, HRSD > 17) and of ten sex- and age-matched controls. The results showed no significant difference in basal calcium levels between the two groups and no significant difference in the Ca^{2+} response to thrombin although the response was higher in the patients. The Ca^{2+} increase after serotonin stimulation in depressed patients was significantly ($P < 0.05$) higher than in healthy controls. By contrast, the Ca^{2+} response to PHA in lymphocytes was significantly decreased in the patients. Our data confirm elevated Ca^{2+} responses after 5-HT_2 receptor activation even in medicated depressed patients. However, Ca^{2+} responses in lymphocytes were decreased. Together with the observations of an enhanced Ca^{2+} response in platelets after thrombin stimulation, we speculate that the findings rather suggest alterations of $[Ca^{2+}]_i$ regulation in depression than specific changes of serotonergic sensitivity.

Key words: Intracellular calcium –Affective disease – Platelets – Lymphocytes

Introduction

Many studies have demonstrated pharmacological similarities between platelet and brain serotonin$_2$ (5-HT_2) binding sites (Geaney et al. 1984; de Clerck et al. 1982). 5-HT_2 receptors on human platelets and on central neurons are coupled to a phospholipase C system which, via producing inositoltrisphosphate, mobilizes Ca^{2+} from internal stores (Affolter et al. 1984; Erne et al. 1985). The elevated intracellular free calcium concentration, $[Ca^{2+}]_i$, of platelets after serotonin stimulation has previously been characterized as a pure 5-HT_2 response (Kagaya et al. 1990). Because the mobilization of intracellular free calcium and the calcium influx regulate platelet function in a manner similar to that of central neurons, it may be possible to use platelets as a model for central serotonergic mechanisms.

Recent evidence suggests that abnormalities of central 5-HT_2 receptors might be involved in the biochemistry of depression and might be corrected by antidepressant drug treatment (Boyer et al. 1991; Fraser et al. 1988). These motions were supported by some recent reports indicating higher $[Ca^{2+}]_i$ in 5-HT_2 stimulated platelets of untreated depressed patients than in matched healthy controls (Kusumi et al. 1991b; Mukuni et al. 1992). However, a very recent study (Kusumi et al. 1992) also demonstrated higher thrombin-induced $[Ca^{2+}]_i$ levels in platelets of depressed bipolar patients. These findings seem to confirm observations by Dubovsky et al. (1991) and support the hypothesis of abnormalities in $[Ca^{2+}]_i$ regulation in affective disorders also based on $[Ca^{2+}]_i$ measurement in lymphocytes of depressed bipolar patients (Dubovsky et al. 1992).

In view of these findings, we investigated 5-HT_2 receptor function on platelets of depressed inpatients under current antidepressant treatment and of healthy controls (Eckert et al. 1993) to clarify whether the above mentioned observations can be confirmed and can even be extended to depressed patients already under antidepressant medication. Additionally, we studied basal and mitogen-stimulated Ca^{2+} responses in lymphocytes of the same patients as an important peripheral model to study alterations of $[Ca^{2+}]_i$ regulation in man (see Hartmann et al., this issue).

Methods

Subjects

Three male and seven female medicated depressed inpatients aged between 31 and 77 years (mean 47.5, SD 14.4 years) with a mean

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(± SD) body weight of 77 ± 17 kg entered the study. Eight patients fulfilled the requirements for unipolar major depressive disorder (MDD) according to DSM-III-R and two patients for a major depressive episode (MDE) with a history of mania (bipolar depression). Severity of depression was assessed by the 21-item Hamilton Rating Scale of Depression (HRSD). The mean (± SD) HRSD score was 24.8 ± 6.3, ranging from 17 to 36. The time of antidepressant treatment (drugs, dose/day: 3 on moclobemide, 300 mg; 1 on fluoxetine, 20 mg; 1 on doxepin, 125 mg; 1 on amitryptiline, 75 mg; 1 on maprotiline, 350 mg; 1 on trimipramine, 75 mg) differed from one to ten weeks. All patients but two received the last medication the evening before the blood sample was taken. Two patients had not received medications for 1 week. The ten sex- and age-matched controls (mean ± SD, age: 43.8 ± 13.8 years; weight: 66 ± 14 kg) had no history of psychiatric disorders and were not taking drugs. One patient and one control had taken oral contraceptives.

Cells

Platelets. Blood samples were obtained between 8 and 9 AM and drawn into acid citrate dextrose anticoagulant. Platelet-rich plasma (PRP) was prepared by centrifuging whole blood at 200 × *g* for 10 min at room temperature. The PRP was incubated with Fura2-AM (4 µmol/l) for 15 min at 37°C. After dye loading PRP was spun again at 650 × *g* for 20 min and the resulting pellet was resuspended in Hank's Balanced Salt Solution (glucose, 6 m*M*; $MgSO_4 \times 7\ H_2O$, 1 m*M*; KCl, 5 m*M*; NaCl, 137 m*M*; Na_2HPO_4, 0.3 m*M*; HEPES, 10 m*M*; pH 7.4). The final platelet count was adjusted to 1×10^8 cells/ml and 1 m*M* $CaCl_2 \times 2\ H_2O$ was added.

Lymphocytes. After removing the PRP the blood was diluted with an equal volume of RPMI-1640 and the mononuclear cells (MNC, > 95% lymphocytes) were obtained by Ficoll-Hypaque gradient centrifugation by the method of Boyum (1968). MNC were collected from the interphase, washed twice and resuspended at a density of 10^7 cells/ml in RPMI with 5 per cent fetal calf serum, without phenolred and incubated with Fura 2-AM (3 µmol/ l) for 40 min at 37°C. After washing out external dye with HBSS the lymphocyte suspension was adjusted to 2.5×10^6 cells/ml HBSS.

$[Ca^{2+}]_i$ measurement

Before fluorescence measuring in a SLM Aminco 4800 spectrofluorometer samples were equilibrated in a cuvette at 37°C for 5 min under magnetic stirring, the platelets were stimulated with serotonin (10 µmol/l) or thrombin (1 U/ml) and lymphocytes with phytohemagglutinin (PHA, 15 µg/ml). The intracellular calcium concentration $[Ca^{2+}]_i$ was calculated from the ratio of fluorescence intensities at excitation wavelengths of 340 and 380 nm with emission at 510 nm in intact platelet samples and at saturating (R_{max}, 0.05% Triton X-100) and very low (R_{min}, EGTA 6 m*M*, Tris 30 m*M*) concentrations of Ca^{2+} (Grynkiewicz et al. 1985) using a Ca^{2+}-dye dissociation constant (K_D) of 224 n*M*. Various antidepressive drugs do not seem to interfere with the $[Ca^{2+}]_i$ determination using Fura2-AM (Cai and McCaslin 1992).

Chemicals

All chemicals were obtained from Sigma, Munich, FRG, except Fura 2-AM from Molecular Probes, Ore., USA and Ficoll-Hypaque (Lymphoprep^R) from Immuno, Heidelberg, FRG.

Statistics

The results were analysed by Mann-Whitney rank sum test. Results are shown as means ± SD or as individual data.

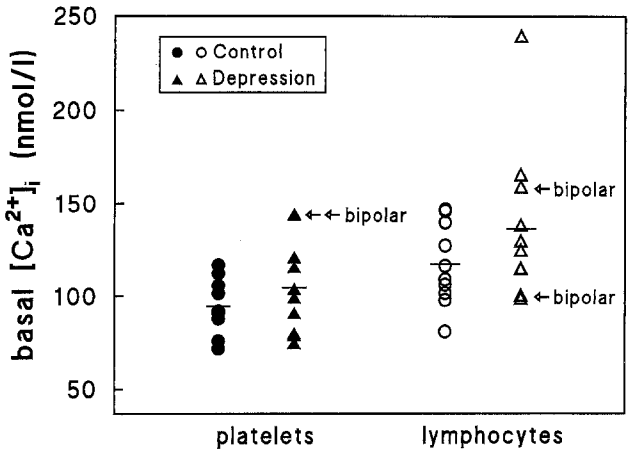

Fig. 1. Basal Ca^{2+} levels in resting platelets of controls *(solid circle)* and medicated depressed patients *(solid triangle)* and basal $[Ca^{2+}]_i$ in resting lymphocytes of controls *(open circle)* and depressed patients *(open triangle)*. *Horizontal bars* indicate the means for each group (platelets: controls 94.1 ± 18.8 nmol/l, patients 104.2 ± 25.7 nmol/l; lymphocytes: controls 117.2 ± 22.3 nmol/l, patients 137.4 ± 41.9 nmol/l). Basal $[Ca^{2+}]_i$ of the two bipolar patients are indicated by *arrows*

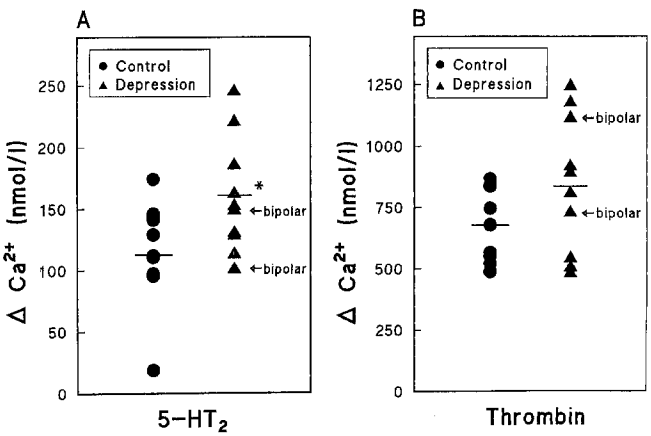

Fig. 2. A Ca^{2+} increases (ΔCa^{2+}) after 5-HT_2 stimulation (serotonin 10 µmol/l) in platelets of healthy controls *(solid circle)* and medicated depressed patients *(solid triangle)*; controls: 117.1 ± 42.4 nmol/l, patients: 158.7 ± 46.4 nmol/l. ΔCa^{2+} in depressed patients is significantly increased (* $P < 0.05$) as compared to controls. **B** Thrombin (1 U/ml)-induced Ca^{2+} increases (ΔCa^{2+}) in platelets of healthy subjects *(solid circle)* and medicated depressed patients *(solid triangle)*; controls: 678 ± 142 nmol/l, patients: 840 ± 280 nmol/l. There is no significant differences in ΔCa^{2+} between the groups

Results

In platelets, we observed no significant differences of the basal calcium levels between control and patient groups (Fig. 1). However, basal $[Ca^{2+}]_i$ of the two bipolar patients (143.4 n*M* and 143.0 n*M* respectively) was considerably higher than the mean of the eight unipolar patients (94.4 ± 17.4 n*M*). However, after serotonin stimulation the ΔCa^{2+} values (ΔCa^{2+} was the difference between resting $[Ca^{2+}]_i$ and the peak $[Ca^{2+}]_i$ after addition of agonist) in platelets of medicated depressed patients were significantly ($P < 0.05$) increased as compared with normal controls (Fig. 2

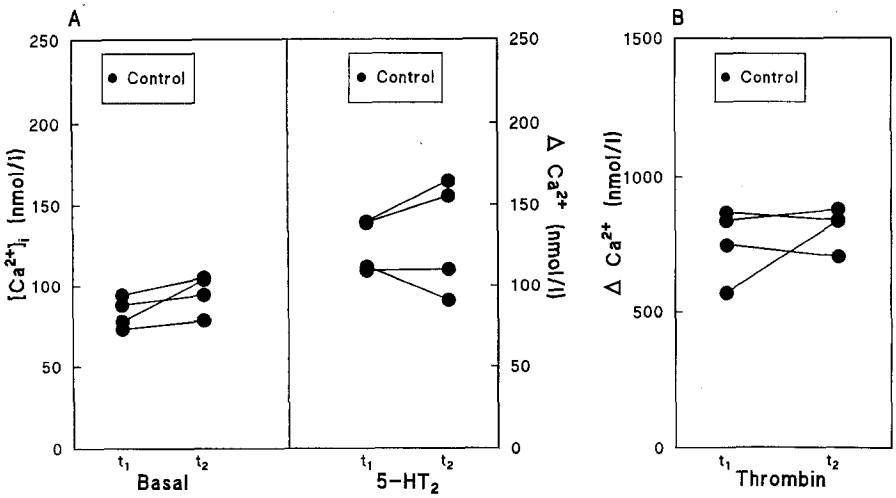

Fig. 3. Week-to-week variance of basal $[Ca^{2+}]_i$, 5-HT_2-induced Ca^{2+} increases (Fig. 3A) and thrombin-induced Ca^{2+} increases (Fig. 3B) in platelets of 4 healthy individuals

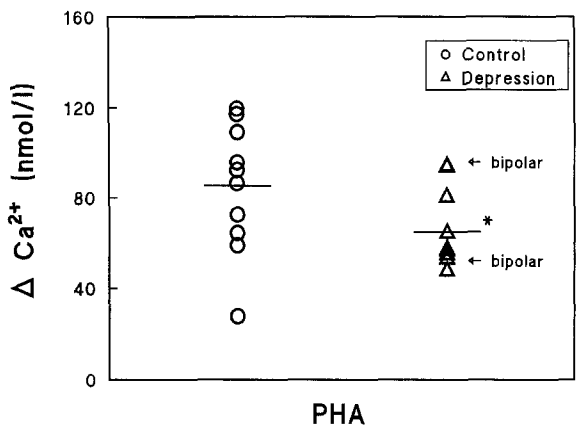

Fig. 4. PHA (15 μg/ml)-induced Ca^{2+} increases (ΔCa^{2+}) in lymphocytes of normal controls *(open circle)* and medicated depressed patients *(open triangle)*; controls: 84.4 ± 28.9 nmol/l, patients: 64.7 ± 17.9 nmol/l. ΔCa^{2+} in depressed patients is significantly decreased (* $P < 0.05$) relative to controls

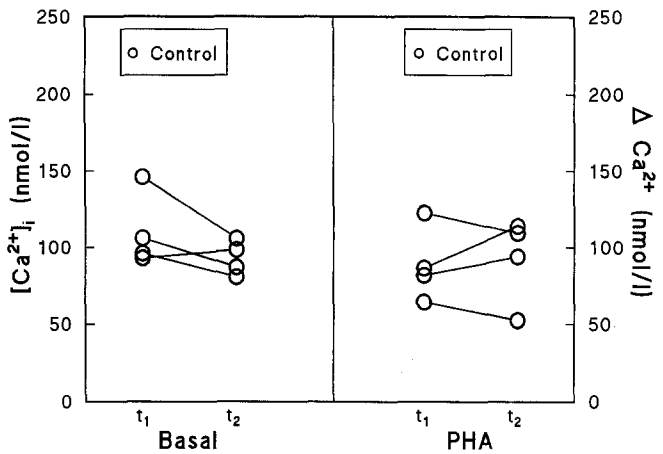

Fig. 5. Week-to-week variance of basal $[Ca^{2+}]_i$ and PHA-induced Ca^{2+} increases in lymphocytes of 4 healthy controls

A). Serotonin-induced Ca^{2+} increases were not different in platelets of the unipolar or the bipolar patients (unipolar: 166.7 ± 47.0 nmol/l versus bipolar: 152.1 and 100.8 nmol/l). Conversely, omitting the two bipolar patients did not affect the singificantly enhanced serotonin response of the depressed group (controls versus patients: 117.2 ± 42.4 versus 166.7 ± 47.0, $P < 0.05$).

By contrast, no significant differences of the calcium responses after thrombin stimulation was observed (Fig. 2 B) for either group, although the patients showed a tendency to higher Ca^{2+} responses relative to the controls. Moreover, ΔCa^{2+} after thrombin stimulation did not differ between unipolar and bipolar patients (bipolar: 728 and 1114 nmol/l; unipolar: 820 ± 296 nmol/l).

Basal $[Ca^{2+}]_i$ and agonist-induced elevations of $[Ca^{2+}]_i$ were relatively stable, individual parameters as indicated by repetition of measurements after one week (Fig. 3). Resting platelets showed a variance in basal $[Ca^{2+}]_i$ of 9.5 ± 7%, in serotonin-induced ΔCa^{2+} of 8.7 ± 6% and in thrombin-induced ΔCa^{2+} of 9.2 ± 11.8%.

There was no effect of age or sex on the 5-HT_2 induced $[Ca^{2+}]_i$ rise (data not shown).

Resting $[Ca^{2+}]_i$ in lymphocytes was also not different between medicated depressed patients and healthy controls (Fig. 1). Moreover, only one of the two bipolar patients showed an enhanced basal Ca^{2+} level as compared to the unipolar patients (bipolar: 157.8 and 99.2 nmol/l; unipolar: 139.6 ± 44.5 nmol/l). In contrast to the enhanced Ca^{2+} response in platelets, the PHA-induced $[Ca^{2+}]_i$ elevation in lymphocytes of medicated depressed patients was significantly decreased as compared with controls (Fig. 4). Repeated $[Ca^{2+}]_i$ measurements (1 week after the first determination) performed in lymphocytes of healthy volunteers again indicated a low variance (basal $[Ca^{2+}]_i$ 13.2 ± 7.6% and PHA-induced ΔCa^{2+} 12.9 ± 5.0% (Fig. 5). Intraindividual correlatations between the basal Ca^{2+} levels or the Ca^{2+} increases after serotonin- and PHA-stimulation in the two different cell types of the same patients or the same controls were not found (data not shown).

Discussion

The results of the present study confirm that the Ca^{2+} response of human platelets to a single dose of serotonin is increased in depressed patients compared with age- and

sex-matched healthy individuals (Kusumi et al. 1991b; Mikuni et al. 1992). The absolute $[Ca^{2+}]_i$ values at baseline and after serotonin stimulation found in this study are very similar to the values reported by Kusumi et al. (1991 b) and Mikuni et al. (1992) indicating that both parameters are not influenced by the different subgroups of depressed patients investigated in the three studies. Our data additionally indicate that the elevated platelet Ca^{2+} responses after 5-HT_2 stimulation are still present in depressed patients under antidepressant drug treatment and suggest that the enhanced 5-HT_2 receptor sensitivity is not generally reduced after initiating antidepressive treatment. This seems to be an important methodological advantage, since not all depressed patients can be studied under drug-free conditions. Moreover, our findings extent the observation of an enhanced Ca^{2+} response of platelets after serotonin stimulation to a very heterogenous but clinically more relevant group of depressive patients. The findings of Kusumi et al. (1991b) were obtained for monopolar and bipolar patients drug-free for at least 28 days, and the findings of Mikuni et al. (1992) on depressed first episode cases. Since all of our patients were still depressed when investigated, it needs to be clarified whether the enhanced 5-HT_2 receptor mediated platelet Ca^{2+} responses of depressed patients normalize with clinical remission.

Moreover, the small week-to-week variance of basal Ca^{2+} levels and of 5-HT_2-mediated Ca^{2+} responses within healthy individuals supports the findings of Kusumi et al. (1991a) that serotonin-stimulated Ca^{2+} responses in platelets are stable parameters and the 5-HT_2 receptor might serve as an interesting and valuable indicator for enhanced serotonergic function in depression. Together with findings about increased 5-HT_2 receptor density (Biegon et al. 1987; Arora et al. 1989) and an enhanced 5-HT_2-stimulated PI hydrolysis (Mikuni et al. 1991) in platelets of depressed patients, the data about platelet Ca^{2+} responses including our own findings suggest an enhanced sensitivity of platelet 5-HT_2 receptors in depression.

However, consistent with results of Dubovsky et al. (1991) the two bipolar patients included in our study showed an elevated intracellular free calcium concentration under baseline conditions. Moreover, Ca^{2+} responses of the patients after thrombin stimulation tended to be higher than those of the controls, although the difference did not reach statistical significance. This might suggest that alterations of $[Ca^{2+}]_i$ homeostasis originally described in bipolar patients (Dubovsky et al. 1991; Kusumi et al. 1992) might also be present in monopolar depressed patients. These observations might alternatively point to abnormalities of platelet $[Ca^{2+}]_i$ regulation in MDD probably independent of serotonergic mechanisms, an hypothesis which was further pursued by experiments using lymphocytes of the same patients.

In contrast to the findings in platelets, the Ca^{2+} response in lymphocytes after PHA stimulation was significantly decreased in lymphocytes of depressed patients relative to normal controls. These findings are clearly opposite to the previously described abnormalities of $[Ca^{2+}]_i$ regulation in platelets. This discrepancy might be explainable by the different initiation of the signalling pathway in the two cell types: the lymphocyte T cell antigen receptor (TRC) is a tyrosine kinase-linked receptor, whereas 5-HT_2- and thrombin receptors are G protein-linked receptors (Berridge 1993). Furthermore, the composition of the Ca^{2+} signal is different in the two cell types. Activation of TCR by mitogenic lectins such as PHA involves Ca^{2+} increase, mostly via influx of external Ca^{2+} (Gardner 1989; Michel et al. 1992), while serotonin- and thrombin-induced Ca^{2+} responses in platelets mainly are due to Ca^{2+} release from internal stores (Erne et al. 1985; Pollock et al. 1986; Nishio et al. 1993). Therefore, it seems possible to speculate that the different Ca^{2+} responses of the two cells types in depression can be explained on the basis of the different generation of the internal Ca^{2+} signals.

The "third" messenger Ca^{2+} plays an important role in lymphocytes as an early event in the signalling pathway cascade, which controls many cellular processes including lymphocyte proliferation. Our findings of a reduced Ca^{2+} signal after PHA stimulation in depressed patients are consistent with many previous studies (Cosyns et al. 1989; Kronfol et al. 1989; Schleifer et al. 1984) indicating a reduced proliferative response to mitogens in depression. Similarly impaired Ca^{2+} responses of lymphocytes in depression were also found by Vollmayr and Aldenhoff (this issue).

In concordance with Dubovsky et al. (1991) all findings together seem to support the speculation that there are general abnormalities of $[Ca^{2+}]_i$ regulation in depression, present in different cell types, rather than a specific alteration of 5-HT_2 receptor sensitivity.

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