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Decreased agonist-stimulated Ca^{2+} response in neutrophils from patients under chronic lithium therapy

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Summary. The agonist-stimulated increase in the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$) was determined in neutrophils from patients under chronic lithium therapy and a control group of age- and sex-matched healthy drug-free subjects. Cells were stimulated with the chemotactic peptide formylmethionylleucylphenylalanin (fMLP) and the Ca^{2+} concentrations measured with the fluorescent Ca^{2+} indicator Fura-2. The Ca^{2+} response to stimulation with fMLP was significantly attenuated in neutrophils from patients chronically treated with lithium. The data suggest that lithium treatment inhibits the inositol phospholipid second messenger generating system in human cells and support the results of earlier inositol phosphate measurements in fMLP-stimulated neutrophils.

Key words: Lithium therapy – Neutrophils – Ca^{2+} response

Introduction

Lithium salts might exert their therapeutic and prophylactic properties in manic-depressive disorders by way of dampening the generation of second messenger molecules from inositol phospholipids in a manner selective for pathologically overactive neural circuits (for review see Berridge and Irvine 1989; Berridge 1993). This idea has gained wide acceptance since this second messenger system is of utmost importance in mediating neuronal responsiveness to neurotransmitter systems that are believed to be dysregulated in affective illness (Baraban et al. 1989; Snyder 1992). However, the hypothesis is based largely on preclinical evidence obtained in experimental animals and in vitro studies. Little attention has been paid to the question whether or not chronic lithium therapy might indeed dampen the inositol phospholipid signal transducing system in manic-depressive patients. Since this second messenger system is ubiquitously expressed its activity can be measured in peripheral cells that are accessible in humans. Using this approach we have recently shown that the agonist-stimulated accumulation of inositol phosphates is indeed attenuated in neutrophils from patients under chronic lithium therapy (Greil et al. 1991). Since inositol(1, 4, 5)trisphosphate induces the release of Ca^{2+} from intracellular stores these data suggested also that the Ca^{2+} response should be reduced in these cells. Here we report that the agonist-stimulated increase in cytosolic free Ca^{2+} is indeed decreased in neutrophils from lithium-treated patients.

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Methods and materials

Materials

Fura-2AM was obtained from Molecular Probes, Eugene OR or from GIBCO, Eggenstein, F.R.G.; both samples gave identical results. Fura-2AM was dissolved as a stock solution in DMSO and stored in small aliquots at –80°C. Each portion was used only once after thawing. Ficoll-Hypaque was from Pharmacia, all other chemicals were from Sigma.

Subjects

Patients were 14 consenting individuals (12 women, mean age 45 years, range 25–62; 2 men, 41 and 42 years), who met DSM-III-R criteria (life time) for bipolar manic-depressive disorder or major depression and were on maintenance treatment with lithium carbonate for more than 6 months. All patients were remitted and symptom-free according to the "Depression-Scale", a subscale of the "Paranoid-Depression-Scale" of von Zerssen (1986) for at least 6 months. A subgroup ($n = 6$) of the patients received antidepressive medication in addition to lithium. Blood was withdrawn at periodic visits to the outpatient clinic for routine medical evaluation and determination of lithium levels (mean = 0.7 mEq/l, range 0.5–1.0). Control subjects were 14 consenting age and gender matched healthy individuals with no lifetime history of psychiatric disorder, taking no medication for at least 4 weeks before blood sampling.

Isolation of neutrophils

Human neutrophils were isolated from heparinized whole blood obtained by means of sedimentation through dextran (0.6% wt/vol)

followed by centrifugation through Ficoll-Hypaque and hypotonic lysis of contaminating erythrocytes (Boyum 1984; Dougherty et al. 1984).

Measurement of intracellular free Ca^{2+} concentrations

Intracellular Ca^{2+} concentrations were measured by use of the fluorescent Ca^{2+} indicator fura-2 (Grynkyewicz et al. 1985). Neutrophils were loaded with fura-2AM, the ester form of fura-2, by incubating neutrophils (10–20 million cells per ml) in phosphate-buffered salt solution containing 1 μ*M* fura-2AM for 30 min at 37° C. After loading the cells were centrifuged (5 min at 180 *g*), washed twice with cold buffer, resuspended in Krebs-Ringer-buffer containing 1 m*M* $CaCl_2$ at a concentration of 5 million cells/ml. Aliquots of 0.8 ml were stored on ice in the dark for up to 2 h. Directly before the measurement cells were centrifuged again and resuspended in 0.8 ml of Krebs-Ringer-buffer. The subsequent monitoring of fluorescence was performed in these 0.8 ml samples continously stirred in quartz-glass cuvettes thermostatically maintained at 37°C as described previously (van Calker et al. 1989). Cells were stimulated (for review see Omann et al. 1987) with the chemotactic tripeptide formylmethionylleucylphenylalanin (fMLP) or vehicle (controls). Fluorescence was measured in a Hitachi 2000 spectrofluorometer with excitation at 340 and 380 nm and emission at 510 nm. All measurements were done at least in triplicates (SD ≤ 10%) and were corrected for autofluorescence determined for each cell suspension. Stimulation of neutrophils with fMLP results in a rapid initial increase in the intracellular free Ca^{2+} concentration (first phase of the response) due to the release of Ca^{2+} from intracellular stores. It is followed by a lower but sustained elevation of intracellular Ca^{2+} (second phase), which slowly returns to basal levels. The second phase of the response is due to influx of extracellular Ca^{2+} (Andersson et al. 1986). The Ca^{2+} response (Δ CA^{2+}) was defined as the difference between the stimulated value (first phase of the fluorescence signal) at 0.4 μ*M* fMLP (a maximally effective fMLP concentration) and the basal value. Basal intracellular free Ca^{2+} concentrations varied between 50 and 90 n*M* (Table 1).

Results

The extent of the initial Ca^{2+} response was determined in a group of euthymic, monopolar or bipolar manic-depressive patients under chronic lithium therapy and compared with an age- and sex-matched group of healthy drug-free volunteers. The reliability of the Ca^{2+} measurement was evaluated by repeated measurements at different days in 2 normal controls and 3 lithium-treated patients. Since the intraindividual variance of the measured Ca^{2+} responses within the subjects was approximately 10–20% (Table 1) neutrophil samples were obtained and analysed on three different occasions for each subject. The Ca^{2+} response observed in neutrophils from patients under chronic lithium therapy was significantly lower than the increase observed in neutrophils from the control group (Fig. 1). No difference between the two groups was found in the basal Ca^{2+} concentrations (not shown). There was no difference in the mean Ca^{2+} response between the subgroup of patients (n = 6), who received antidepressive medication in addition to lithium (Δ Ca^{2+} = 232 ± 39) and those, who received lithium treatment alone (n = 8) (Δ Ca^{2+} = 232 ± 18).

Table 1. Intraindividual variance of basal and stimulated intracellular free Ca^{2+} concentrations in neutrophils (n*M*)[a]

Subject	Basal	fMLP-stimulated	Number of measurements
Male control (32 years)	73 ± 9 (58–87)	376 ± 29 (299–426)	12
Female control (25 years)	70 ± 11 (54–84)	343 ± 32 (281–390)	8
Female patient (40 years)	61 ± 11 (52–78)	321 ± 35 (285–362)	5
Female patient (45 years)	56 ± 9 (44–64)	310 ± 32 (292–346)	5
Female patient (52 years)	60 ± 8 (52–71)	277 ± 42 (231–346)	5

[a]To determine the reliability of the Ca^{2+}-measurements, neutrophils were isolated and measured at 5–12 different days over a period of 3 months. Values are means ± SD of these measurements. The min and max values of the measurements are shown in brackets

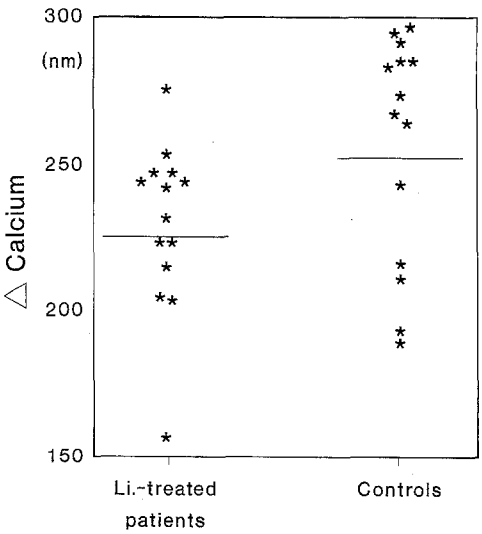

Fig. 1. fMLP-stimulated intracellular free Ca^{2+} concentrations in neutrophils from healthy, drug-free controls and lithium-treated patients. Each point represents the difference between stimulated and basal levels. Values given are the means of three determinations made with neutrophils isolated on three different days with at least 1 week intervals. Each determination was done in triplicate (SD < 10%). Neutrophils were isolated, loaded with fura-2 and measured for fluorescence with excitation at 340 nm and 380 nm and emission at 510 nm as described in Materials and Methods. The difference between mean values given is statistically significant (P < 0.05, analysis of variance)

Discussion

The results obtained in the present study show that the agonist-induced increase of the intracellular free Ca^{2+} concentration is reduced in neutrophils from patients under chronic lithium therapy. They corroborate our earlier finding that the agonist-stimulated formation of inositol phosphates in neutrophils from lithium treated patients is attenuated (Greil et al. 1991). The most likely explanation for these results is an inhibition by lithium ions of the inositol phospholipid second messenger generating system,

leading to a reduced formation of inositol(1,4,5)trisphosphates and subsequently to an attenuated release of Ca^{2+} ions from intracellular stores. Alternatively, the possibility must be considered that the reduced Ca^{2+} response in lithium-treated patients could be a characteristic feature of the illness ("trait marker") rather than the result of lithium therapy. However, we have recently determined the Ca^{2+} response in neutrophils of both acutely ill und euthymic remitted manic-depressive patients, who were free of psychiatric medication for at least two weeks. The Ca^{2+} response at 0.4 μM fMLP in the cells of these patients was not reduced as compared with controls (D. van Calker and U. Förstner, unpublished results). Furthermore, the sensitivity to stimulation with fMLP (measured by the EC_{50}-values of the dose-response curves) was increased in cells from untreated patients as compared with controls (see below), while it was decreased in cells from lithium-treated patients (van Calker et al. 1993). The reduced Ca^{2+} response in cells from lithium-treated patients is therefore not related to the illness but to the medication. Several of the lithium treated patients were treated with antidepressives in addition to lithium. However, there was no significant difference in the mean Ca^{2+} response between this group of patients ($n = 6$) and those, who received lithium treatment alone ($n = 8$). Therefore, the reduced Ca^{2+} response observed in neutrophils from lithium-treated patients is most probably an effect of lithium and not of the additional medication.

In our previous study (Greil et al. 1991) a significant decrease in the agonist induced accumulation of inositol phosphates was found in male but not in female patients. The most likely explanation for these results is the great variability that was observed in the determination of inositol phosphates, which might have obscured any possible effect of lithium in female patients (Greil et al. 1991). In the present study the Ca^{2+} response was significantly attenuated also in neutrophils from lithium-treated female patients. This is probably due to the greater reliability of the Ca^{2+} measurements. Since they are, in addition, less time consuming the inevitable variance could be further reduced by using the means of determinations performed at three different occasions.

In conclusion, the present results provide further support to the idea that the mechanism of the prophylactic action of lithium in affective psychoses might be related to its dampening effect on the intracellular Ca^{2+} signalling. An inhibition of the inositol phospholipid second messenger generating system in pathologically overactive neural pathways might also be an indirect consequence of carbamazepine's activity as an adenosine A_1 antagonist in the brain and thus represent a common final pathway in the actions of both drugs (van Calker and Berger 1993). Data from several groups (Mikuni et al. 1991, 1992; Kusumi et al. 1991, 1992; Dubovsky et al. 1991, 1992, 1993; Eckert et al. 1993) indicate that the inositol phosphate- and Ca^{2+} response to stimulation with serotonin and thrombin is increased in platelets of manic-depressive patients. Furthermore, we have recently shown that the EC_{50} values of the dose response curves of the Ca^{2+} response to fMLP are lower in neutrophils of untreated monopolar and bipolar manic-depressive patients as compared with controls (van Calker et al. 1993). In contrast to these data, which indicate an increased sensitivity to hormonal stimulation of the Ca^{2+} response in platelets and neutrophils of monopolar and bipolar patients, it has been reported that the Ca^{2+} response to stimulation with PHA is reduced in lymphocytes of depressive patients (Eckert et al. 1993; Vollmayr and Aldenhoff 1993). As discussed by Eckert et al. (1993) the difference in the results obtained with platelets and lymphocytes of depressive patients might be explained by the fact that the Ca^{2+} response in lymphocytes was initiated by the activation of the tyrosine kinase-linked antigen receptor, while the response of platelets was elicited by activation of G-protein-coupled receptors. Similar to the Ca^{2+} response in platelets to serotonin and thrombin the Ca^{2+} response to fMLP in neutrophils is also mediated by activation of a receptor-linked G-protein (Omann et al. 1987). Thus, both cell types that are stimulated via the activation of a G-protein show an increased Ca^{2+} response in manic-depressive disorder. It is therefore tempting to speculate (Avissar and Schreiber 1992) that an increased sensitivity of this signal transducing system might enhance the risk for manic-depressive illness and that lithium therapy might compensate this biochemical abnormality. Further work is clearly needed to substantiate this idea.

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References

Andersson T, Dahlgren C, Pozzan T, Stendahl O, Lew PD (1986) Characterization of fMet-Leu-Phe receptor-mediated Ca^{2+} influx across the plasma membrane of human neutrophils. Mol Pharmacol 30:437–443

Avissar S, Schreiber G (1992) The involvement of guanine nucleotide binding proteins in the pathogenesis and treatment of affective disorders. Biol Psychiatry 31:435–459

Baraban JM, Worley PF, Snyder SK (1989) Second messenger systems and psychoactive drug action: focus on the phosphoinositide system and lithium. Am J Psychiatry 146:1251–1260

Berridge MJ, Irvine RF (1989) Inositol phosphates and cell signalling. Nature 341:197–205

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

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

Dougherty RW, Godfrey PP, Hoyle PC, Putney JW, Freer RJ (1984) Secretagogue-induced phosphoinositide metabolism in human leucocytes. Biochem J 222:307–314

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

Dubovsky SL, Murphy J, Thomas M, Rademacher J (1992) Abnormal intracellular calcium ion concentration in platelets and lymphocytes of bipolar patients. Am J Psychiatry 149:118–120

Dubovsky SL, Thomas M, Hijazi A, Murphy J (1994) Intracellular calcium signalling in peripheral cells of patients with bipolar affective disorder. Eur Arch Psychiatry Clin Neurosci 243:229–234

Eckert A, Gann H, Riemann D, Müller WE (1994) Platelet and lymphocyte free intracellular calcium in affective disorders. Eur Arch Psychiatry Clin Neurosci 243:235–239

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

Greil W, Steber R, van Calker D (1991) The agonist-stimulated accumulation of inositol phosphates is attenuated in neutrophils from male patients under chronic lithium therapy. Biol Psychiatry 30:443–451

Kusumi I, Koyama T, Yamashita I (1991) Serotonin-stimulated Ca^{2+} response is increased in the blood platelets of depressed patients. Biol Psychiatry 30:310–312

Kusumi I, Koyama T, Yamashita I (1992) Thrombin-induced platelet calcium mobilization is enhanced in bipolar disorders. Biol Psychiatry 32:731–734

Mikuni M, Kusumi I, Kagaka A, Kuroda Y, Mori H, Takahashi K (1991) Increased 5-HT_2 receptor function as measured by serotonin-stimulated phosphoinositide hydrolysis in platelets of depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 15:49–61

Mikuni M, Kagaka A, Takahashi K, Meltzer HY (1992) Serotonin but not norepinephrine-induced calcium mobilization of platelets is enhanced in affective disorders. Psychopharmacology 106:311–314

Omann GM, Allen RA, Bokoch GM, Painter RG, Traynor AE, Sklar LA (1987) Signal transduction and cytoskeletal activation in the neutrophil. Physiol Rev 67:285–340

Snyder SH (1992) Second messengers and affective illness. Pharmacopsychiatry 25:25–28

van Calker D, Berger M (1993) Possible role of adenosine receptors in psychiatric diseases. Drug Dev Res 28:354–358

van Calker D, Takahata K, Heumann R (1989) Nerve growth factor potentiates the hormone-stimulated intracellular accumulation of inositol phosphates and Ca^{2+} in rat PC 12 pheochromocytoma cells: Comparison with the effect of epidermal growth factor. J Neurochem 52:38–45

van Calker D, Förstner U, Bokus M, Gebicke-Härter PJ, Wark H-J, Berger M (1993) Increased sensitivity to agonist stimulation of the Ca^{2+}-response in neutrophils of manic-depressive patients: effect of lithium therapy. Neuropsychobiology 27: 180–183

Vollmayr B, Aldenhoff JB (1994) Cytosolic free [Ca^{2+}] in single T-lymphocytes from depressed patients and healthy controls. Eur Arch Psychiatry Clin Neurosci 243:214–217

von Zerssen D (1986) Clinical Self-Rating Scales (CSRS) of the Munich psychiatric information system (PSYCHIS-München). In: Sartorius N, Ban TA (eds) Assessment of depression. Springer, Berlin Heidelberg New York, pp 270–303