RESEARCH

Effect of Mood Stabilizing Agents on Agonist-Induced Calcium Mobilization in Human Platelets

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The effect of mood stabilizing agents such as lithium, carbamazepine, valproïc acid and clonazepam on serotonin(5-HT)-or thrombin-induced intracellular calcium (Ca) mobilization was studied in the platelets of healthy subjects using the fluorescent Ca indicator fura-2. After incubating platelet-rich plasma with these drugs for one or four hours, there was no significant difference in either basal Ca^{2+} concentration or 5-HT-stimulated Ca response between each agent treatment and control. 5-HT- or thrombin-induced Ca mobilization was not altered by four weeks of lithium carbonate administration in healthy volunteers. These results indicate that these mood stabilizers fail to affect the agonist-stimulated intracellular Ca mobilizing pathway either *in vitro* or *ex vivo* in the platelets of healthy subjects.

Key Words: serotonin, thrombin, calcium, platelet, lithium, anticonvulsant

INTRODUCTION

Lithium is one of the most effective drugs available in the acute and prophylactic treatment of manic and depressive episodes. It is now increasingly apparent that the anticonvulsants such as carbamazepine, valproic acid and clonazepam also share components of this spectrum of efficacy in the affective disorders and that they are clinically effective in some lithium non-responders (Chouinard 1987; Mauri et al 1990; Post et al 1992; Prien and Gelenberg 1989; Sachs 1989). However, the biochemical basis of these mood stabilizing effects has remained unclear.

There has been increased interest in changes in GTP binding protein-coupled second messenger systems as an important factor in the pathophysiology of affective disorder (Avissar et al 1988; Berridge and Irvine 1989). Human platelet possesses several neurotransmitter receptors whose stimulations are followed by cellular metabolic processes similar to central neuron, so it seems to be an excellent model for studying these messenger systems (Pletscher 1988). Stimulation of 5-HT_2 receptor on human platelet is known to increase phosphoinositide hydrolysis (De Chaffoy de Courcelles et al 1985), producing inositol 1,4,5-triphosphate (IP_3) which mobilizes intracellular calcium (Ca) ion from internal storage sites (Affolter et al 1984). By the method of using the fluorescent Ca indicator fura-2, we have recently obtained the finding that the 5-HT-induced Ca response was significantly enhanced in bipolar depression and melancholic major depression as compared to non-melancholic major depression, dysthymia and control subjects (unpublished data). We have previously reported that thrombin-stimulated Ca mobilization was significantly higher in bipolar depression than major depression with or without melancholia and normal controls (Kusumi et al 1992). Therefore, there might be some alterations in intracellular signal transduction in

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bipolar disorder that are common to both 5-HT- and thrombin-induced Ca mobilizing pathway.

In this study we examined the effect of mood stabilizing agents on the 5-HT- or thrombin-stimulated Ca response in the platelets of normal subjects. We have previously indicated that each of the mood stabilizers such as lithium, carbamazepine, valproic acid and clonazepam, which was added to incubation medium four minutes prior to 5-HT stimulation, failed to affect the 5-HT-induced Ca response in control platelets (Kusumi et al 1991a). In the present study, we measured the 5-HT-induced Ca response after incubating platelet-rich plasma (PRP) with these drugs for a longer time. In addition, in order to investigate *ex vivo* effect of lithium, we also determined the 5-HT- or thrombin-stimulated Ca mobilization in the platelets of healthy volunteers taking lithium carbonate orally for four weeks.

METHODS

Healthy male volunteers (n = 7, mean age: 32.9 ± 5.3 years) participated in this study. They were free of psychiatric or physical illness and were not taking any drugs including aspirin that might interfere with platelet aggregation for at least four weeks prior to blood sampling. Informed consent was obtained from all subjects.

Blood samples were taken at 9:00 from the antecubital vein and were anticoagulated by addition of acid citrate dextrose. The isolation of platelets and the measurement of intracellular Ca^{2+} concentration were performed as described previously (Kusumi et al 1991a, 1991b). In order to examine *in vitro* effect of mood stabilizing agents, PRP was incubated for one or four hours at 37°C with each drug (10 mM lithium, 100 μM carbamazepine, 100 μM valproic acid or 10 μM clonazepam) which was dissolved in dimethylsulfoxide (DMSO), or an equivalent volume of DMSO as control. As *ex vivo* study, lithium carbonate, 600 mg/day (200 mg tid) was administered orally to healthy subjects for four weeks. Blood samples were collected at zero, one, two, three and four weeks after lithium administration and at one, two and four weeks after withdrawal of it. Serum lithium levels were also checked before morning administration. The platelets were stimulated with 5-HT(0.3 μM, approximately EC_{50}, or 10 μM, the concentration which induces the maximal Ca response) or thrombin (1.0 U/ml).

Results are expressed as means ± SEM. Data were analyzed for statistical significance by one-way analysis of variance for multiple comparison followed by Duncan test or one-way repeated measures analysis of variance followed by Scheffe's test.

RESULTS

After incubating PRP with the mood stabilizing agents for one hour, there was no significant difference in 5-HT-induced Ca response between each drug treatment sample and control in the platelets of normal subjects (see Fig. 1A).

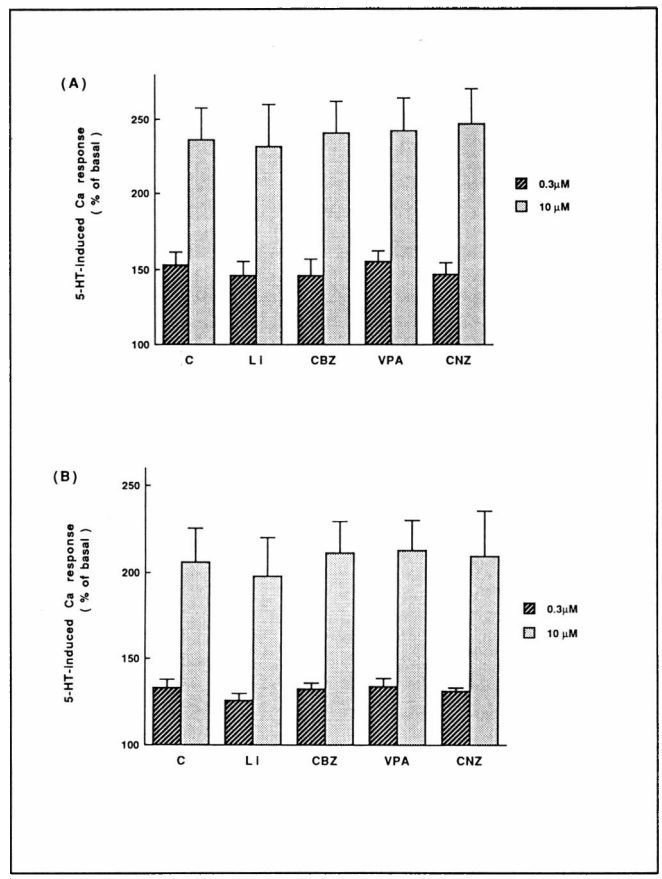

Fig. 1 Effect of incubation with mood stabilizing agents for one hour (A) or four hours (B) on 5-HT(0.3 or 10 μM)induced Ca response in the platelets of healthy subjects. Results are the means ± SEM of data obtained in four experiments and are expressed as the percentage of basal Ca^{2+} concentration. C: control, Li: 10 mM lithium, CBZ: 100 μM carbamazepine, VPA: 100 μM valproic acid, CNZ: 10 μM clonazepam.

Basal Ca^{2+} concentration was not altered by each agent treatment (75.7 ± 7.3 nM for control; 71.6 ± 8.5 nM for lithium; 69.9 ± 6.9 nM for carbamazepine; 75.2 ± 7.1 nM for valproic acid; 68.2 ± 9.0 nM for clonazepam). Similar results were obtained when incubating PRP with these compounds for a longer time of four hours (see Fig. 1B).

In *ex vivo* study, 5-HT- or thrombin-induced intracellular Ca mobilization was not significantly altered either during lithium administration or after withdrawal of it (see Fig. 2A, 2B). Resting Ca^{2+} concentration was not also significantly changed by the lithium treatment (78.7 ± 6.3 nM, 86.0 ± 8.2 nM, 69.5 ± 4.7 nM, 75.2 ± 3.8 nM and 80.1 ± 5.6 nM, respectively, at zero, one, two, three and four weeks after lithium administration; 71.2 ± 4.2 nM, 86.6 ± 9.7 nM and 82.8 ± 6.8 nM, respectively, at one, two and four weeks after withdrawal). Mean plasma lithium concentrations,

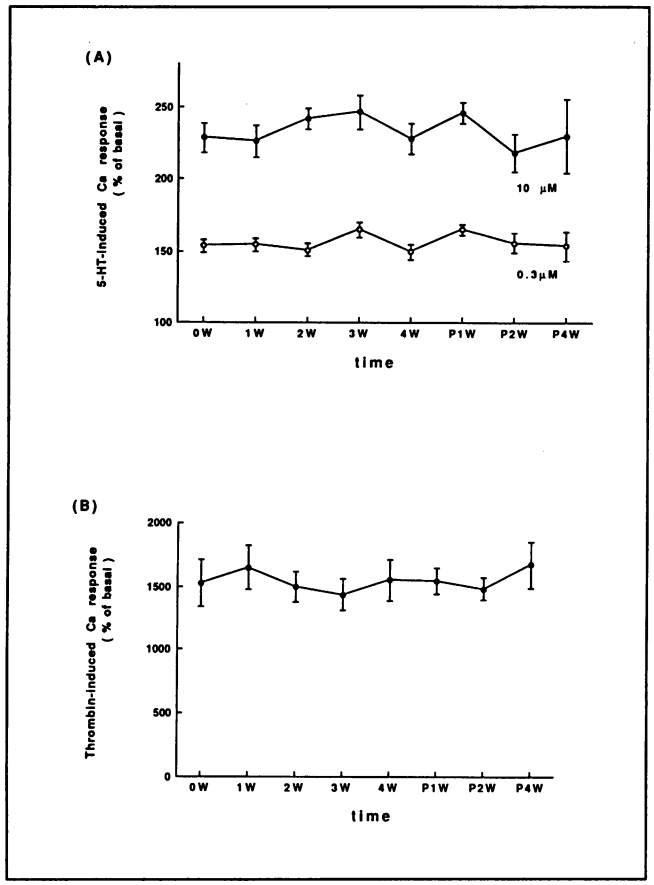

Fig. 2 Effect of four-week lithium administration on 5-HT- (A) or thrombin-induced Ca response (B) in the platelets of healthy volunteers. Lithium carbonate, 600 mg/day (200 mg tid) was administered orally to control subjects. Values are means ± SEM. P1W, P2W and P4W indicate one, two and four weeks after withdrawal of lithium treatment; N = 7.

approximately 14 hours after the evening dose, were 0.48 ± 0.04 mEq/l, 0.42 ± 0.02 mEq/l, 0.42 ± 0.03 mEq/l and 0.44 ± 0.03 mEq/l, respectively, at one, two, three and four weeks after lithium treatment.

DISCUSSION

Previous results showed that each of the mood stabilizing agents, which was added to incubation medium four minutes prior to agonist stimulation, failed to change the 5-HT-induced Ca response. This could be due to too short an incubation with these drugs, but in this study we could not find any significant difference in both basal Ca^{2+} concentration and 5-HT-induced Ca response between each agent treatment and control after incubation for one or four hours. A number of studies have demonstrated that lithium affects a broad range of components associated with signal transduction (Odagaki et al 1992). Since there may be complex interactions between different second messenger systems, leading eventually to integrated presynaptic or postsynaptic neuronal responses, we examined *ex vivo* effect of lithium on 5-HT- or thrombin-induced Ca response in this study. However, four weeks of lithium carbonate administration failed to affect the agonist stimulated Ca mobilization in the platelets of healthy volunteers.

Although marked reduction in agonist-induced IP_3 accumulation by lithium has been reported in studies of brain slices (Kennedy et al 1989; 1990; Whitworth and Kendall 1988), we could not find any effect of lithium on 5-HT- or thrombin-stimulated Ca response either *in vitro* or *ex vivo* in human platelets. The reason for this discrepancy is unknown from the present data, but previous works suggest three possible explanations. First, the effect of lithium on brain may be different from that on platelet. On the basis of inositol-depletion hypothesis, which suggests that lithium acts by lowering the level of inositol, followed by leading indirectly to receptor desensitization by limiting the synthesis of the lipid required for signalling (Berridge and Irvine 1989), tissue specific effect of lithium may critically depend upon regional variations in the supply of inositol (Nahorski et al 1991). It has been indicated that accumulation of cytidine monophosphoryl-phosphatidate, a sensitive indicator of intracellular inositol depletion, following stimulation with lithium plus agonist can be suppressed by preincubating slices with relatively high concentrations of myo-inositol (Godfrey 1989; Kennedy et al 1990). Platelets are more accessible to exogenous inositol from the plasma than brain slices, so it is possible that the inhibitory effect of lithium on inositol phosphate second messengers is obscure in the present platelet study. Second, the effect of lithium on healthy subjects may be different from that on patients with affective disorders. According to Berridge's hypothesis (Berridge and Irvine 1989), when lipid turnover is high as might occur during manic-depressive illness, there will be a much larger demand for inositol and lithium will exert a proportionally greater effect. Further studies are required to examine the effect of lithium on 5-HT-induced Ca response in the platelets of patients with affective disorders. Third, a relatively low plasma lithium level, which is on the lower side of the therapeutic range, may be one of the factors contributing to the different result between the present *ex vivo* study and animal brain study.

Multiple candidate systems have been proposed as the mechanism of action of anticonvulsant agents including carbamazepine, valproic acid and clonazepam in the treatment of bipolar affective illness (Post et al 1992). It is interesting to compare the mechanism of action of lithium and these anticonvulsants, looking for areas of common actions as well as for slight variations that may account for differences in the clinical profile of patients who respond to one agent but not to another. Since these mood stabilizing agents affect multiple signal transduction mechanisms held in common by many different neurotransmitters, they are

unique positions to affect the functional balance between neurotransmitter systems. As mentioned in the introduction, in bipolar disorder there might be some dysregulations in the postreceptor mechanism, for example, a coupling between the receptor and GTP binding protein, or some regulations of second- or third-messenger pathway involving protein kinase, phosphoprotein and phospholipid. In the present study these mood stabilizers failed to affect agonist-induced intracellular Ca mobilization either *in vitro* or *ex vivo* in the platelets of healthy subjects. It is possible that the effect these compounds have on second messenger function may be buffered by other homeostatic processes so that a robust change at the molecular level may be minimally or not at all apparent at more integrated levels. It may be important in future investigation to continue a strategy of examining multiple interacting second messenger systems and the balance between different neurotransmitters simultaneously.

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