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Intracellular calcium signalling in peripheral cells of patients with bipolar affective disorder

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Summary. Consistent with previous studies, elevated free intracellular calcium ion concentrations ($[Ca^{2+}]_i$) were found in blood platelets and lymphocytes of patients with mania and bipolar depression. Incubation with an ultrafiltrate of plasma from patients with bipolar illness had no effect on intracellular calcium ion concentration in platelets from normal subjects, suggesting that elevated $[Ca^{2+}]_i$ is not due to a circulating factor. As was true in an earlier study of the effect of lithium on platelets, incubation with therapeutic levels of carbamazepine lowered $[Ca^{2+}]_i$ in lymhocytes from affectively ill patients but not controls. Increased $[Ca^{2+}]_i$ in peripheral cells may reflect a diffuse change in cellular homeostasis and may contribute to mixtures as well as rapid alternations of activity of affective, behavioral and physiologic systems in bipolar illness. Correction of the abnormality may at least be a marker of a relevant therapeutic action if it is not the action itself.

Key words: Bipolar – Intracellular calcium – Ultrafiltrate – Lithium – Carbamazepine

Introduction

Classical neurotransmitter and receptor hypotheses of mood disorders have not explained several important features of bipolar affective disorders. For example, these disorders, which are clinically and biologically heterogeneous (Maj et al. 1989) are associated with alterations in multiple transmitter systems and the balance between them (Barban et al. 1989; Gjerris 1988; Schatzberg et al. 1989; Swann et al. 1987) and with functional changes in peripheral as well as brain cells (Irwin et al. 1990; Kronfol and House 1989). Unidirectional dysfunctions of one or even several neurotransmitter systems cannot account for the frequent occurrence of mania and depression in rapid succession or even at the same time (Post et al. 1987, 1989). It is unlikely that mood stabilizing medications such as lithium and carbamazepine have different actions on the same transmitters or receptors when they act as antimanic agents than when they have antidepressant properties.

Such observations have prompted investigations of second messengers that are capable of producing bidirectional changes in diverse affective, behavioral, and physiologic systems. One second messenger system of interest that is involved in the regulation and the action of all neurotransmitters and neuromodulators implicated in mood disorders is the phosphoinositide/calcium ion (PI/Ca^{2+}) signal transduction system. This signalling system, especially when it is hyperactive, is stabilized by antimanic agents such as lithium (Joseph et al. 1987; Berridge 1989).

Free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) normally is regulated within a very narrow range (usually around 100 n*M*) (Resnik et al. 1986; Rasmussen 1989). Neuronal stimulation by depolarization of receptor activation activates PI turnover and increases $[Ca^{2+}]_i$ by 1–2 orders of magnitude as a result of release of Ca^{2+} from intracellular stores and/or influx of Ca^{2+} through ion channels (Meyer et al. 1988, Rink 1988; Stevens 1987). Acting via intracellular proteins such as calmodulin and enzymes such as protein kinase C (PKC), calcium ions influence synthesis and release of neurotransmitters (Parnas and Segal 1989), receptor signalling (Rasmussen 1986), the action potential (Packer and Frishman 1984), neuronal periodicity (Matthews 1986), kindling (Taft et al. 1987), and the formation of functional neuronal connections (Alkon et al. 1986).

Dysregulation of intracellular Ca^{2+} could be of particular relevance to the study of mood disorders because the same elevation of $[Ca^{2+}]_i$ may facilitate or inhibit a given function, depending on the target enzyme, the phase of the cell cycle, the intracellular effector protein, and the Ca^{2+}-dependent process. In addition, higher or more sustained increases of $[Ca^{2+}]_i$ may inhibit the same function that smaller elevations facilitate (Resnik et al. 1986; Torok 1989;

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Table 1. Free intracellular calcium ion concentration ($[Ca^{2+}]_i$, nM) in platelets and lymphocytes of patients with mood disorders

Cell	Measure	Control	Control Li incubation	Untreated manic	Untreated BPD	Treated BP	BP Li incubation	UP depressed
P[a]	$[Ca^{2+}]_B$	98	–	157	136	112	–	124
	$[Ca^{2+}]_S$	427	–	855	769	513	–	388
P[b]	$[Ca^{2+}]_B$	151	–	–	231	143	–	158
	$[Ca^{2+}]_S$	330	–	–	454	349	–	362
P[c]	$[Ca^{2+}]_B$	150	115	–	221	146	157	–
	$[Ca^{2+}]_S$	304	244	–	424	362	316	–
P[d]	$[Ca^{2+}]_B$	79	–		121	–	–	–
L[d]	$[Ca^{2+}]_B$	83	–		129	–	–	–
P[e]	$[Ca^{2+}]_B$	127	134	–	–	320	330	–
	$[Ca^{2+}]_S$	370	431	–	–	688	755	–
P[f]	$[Ca^{2+}]_B$	94	–	–	143	–	–	94
	$[Ca^{2+}]_S$	211	–	–	270	–	–	261

P = platelet; L = lymphocyte; $[Ca^{2+}]_B$ resting $[Ca^{2+}]_i$; $[Ca^{2+}]_S$ agonist stimulated $[Ca^{2+}]_i$; BPD bipolar depressed; BP manic and bipolar depressed; Li lithium

[a] Dubovsky et al. 1989; [b] Dubovsky et al. 1991a; [c] Dubovsky et al. 1991b; [d] Dubovsky et al. 1992a; [e] Tan et al. 1990; [f] Eckert et al. 1992

Wolff et al. 1977) so that elevated $[Ca^{2+}]_i$ can produce excessive activation of some systems and inhibition of others.

This paper reviews evidence suggesting increased $[Ca^{2+}]_i$ in different peripheral cells of patients with mania and bipolar depression. New data are presented suggesting that the finding may indicate a diffuse dysregulation of intracellular Ca^{2+} signalling rather than a nonspecific manifestation of the stress response or a circulating factor. Results of an additional study demonstrating a normalizing effect of carbamazepine on $[Ca^{2+}]_i$ similar to that of lithium will be reported and the possible influence of altered $[Ca^{2+}]_i$ on mood regulation will be discussed.

Elevated $[Ca^{2+}]_i$ in bipolar illness

Evidence of an elevated Ca^{2+} signal in various blood cells of patients with bipolar illness has accumulated over the past decade. Compared with controls, greater activity of the membrane Ca^{2+} extrusion pump (Ca^{2+}-ATPase) was found in red blood cells of patients with mania and bipolar depression (Linnoila et al. 1983; Bowden et al. 1988), as was greater inter-individual variability of the red cell Ca^{2+}ATPase in affectively ill patients (MacDonald et al. 1984). Increased activity of the Ca^{2+} extrusion pump could be a means of compensating for increased $[Ca^{2+}]_i$, or it could be a primary change that would be expected to lower $[Ca^{2+}]_i$.

Direct observations of $[Ca^{2+}]_i$ in using the intracellular Ca^{2+} chelating fluorescent dyes Fura 2 and Quin2 have repeatedly demonstrated increased resting ($[Ca^{2+}]_B$) and stimulated ($[Ca^{2+}]_S$) in blood platelets and lymphocytes of patients with bipolar affective disorders compared with controls (Table 1). In two studies both $[Ca^{2+}]_i$ measures were also significantly greater in manic and bipolar depressed patients than in unipolar depressed patients (Dubovsky et al. 1989, 1991a) but not in a third sample with a much smaller bipolar sample size (Eckert et al. 1992). While a number of reports indicate that elevated $[Ca^{2+}]_i$ is a state variable that normalizes with normalization of mood (Dubovsky et al. 1989, 1991a, b), others have suggested that it is a trait variable that persists with euthymia (Tan et al. 1990; Eckert et al. 1992).

To investigate the possibility that elevated $[Ca^{2+}]_i$ in peripheral cells in bipolar patients could be caused by a circulating substance, (perhaps a stress hormone that might be associated with any acute illness), a modification of the method of Lindner et al. (1987) was used to determine whether an ultrafiltrate of plasma obtained from bipolar patients would raise platelet $[Ca^{2+}]_i$ in normal controls. In a second experiment, we determined the in vitro effect of carbamazepine, a medication with antimanic properties, on $[Ca^{2+}]_i$ in lymphocytes of patients with bipolar disorder and controls.

Materials and methods

For experiment 1, informed consent was obtained from nine acutely ill unmedicated inpatients with a DSM-III-R diagnosis of bipolar ($n = 7$) or schizoaffective disorder, primarily manic ($n = 2$), and from nine age and sex matched controls without a past history or family history of psychiatric illness. Experiment 2 utilized 26 acutely ill inpatients with a diagnosis of mania or bipolar depression and 7 normal subjects with no psychiatric history in themselves or their families. All subjects were normotensive.

In the first experiment, each pair of a patient and a control was studied in a two day experiment to control for possible diurnal variations in circulating substances or platelet function. On day 1, 60 ml of blood from a patient was drawn into acid citrate dextrose anticoagulant (ACD) (2.5 g sodium citrate, 1.5 g citric acid, 2 g D-glucose in 100 ml water) with 100 μ*M* aspirin. Platelet-rich plasma (PRP) was prepared by centrifugation of blood at 210 *g* for 30 min. PRP was then centrifuged for 15 minutes at 900 *g* and the supernatant was filtered with a Nalgene 115 ml filter. Of the resulting filtrate, 15 ml was spun for 50 min at 500 *g* on an Amicon Centripep 100 filtration unit, after which the ultrafiltrate was centrifuged for 15 min at 500 *g* and was refrigerated overnight.

The next morning, blood was drawn from control subjects into ACD with aspirin as previously described (Dubovsky et al. 1989). PRP was centrifuged for 15 min at 900 *g*, and the resulting platelet pellet was resuspended in 1 m*M* HEPES plus EGTA buffer (10 m*M* HEPES, 145 m*M* NaCl, 5 m*M* KCl, 1 m*M* $MgSO_4$, 6 m*M* glucose, 1 m*M* EGTA), repellet and resuspended in HEPES plus EGTA and $CaCl_2$ (final Ca^{2+} concentration = 1.0 m*M*). This platelet suspension was divided in half, and each sample was centrifuged for 5 min at 900 *g*. One of the resulting platelet pellets was

Table 2. Effect of an ultrafiltrate from bipolar patients on control platelet intracellular calcium ion concentration ($[Ca^{2+}]_i$), nM (Mean ± SD)

$[Ca^{2+}]_i$ Measure	Plain sample	With ultrafiltrate	t	P by paired t-test
$[Ca^{2+}]_B$	87 ± 13.1	87 ± 10.1	0.11	0.92
$[Ca^{2+}]_S$	320 ± 72.4	315 ± 59.4	0.21	0.84

$[Ca^{2+}]_B$ resting $[Ca^{2+}]_i$; $[Ca^{2+}]_S$ agonist stimulated $[Ca^{2+}]_i$

resuspended in the ultrafiltrate collected the previous day mixed with HEPES with 1.0 m*M* final $[Ca^{2+}]$, and the other was resuspended only in the HEPES plus Ca^{2+} medium. Both sampels were incubated for 45 min at 37° C.

Following incubation, platelets were recovered by centrifugation for 5 min at 900 *g*. Platelet were resuspended in HEPES plus EGTA and $CaCl_2$ with final $[Ca^{2+}] = 0.5$ m*M* and the platelet count was adjusted to about 500,000/ml $[Ca^{2+}]_i$ was measured using Fura 2 as previously described (Dubovsky et al. 1989, 1991a). Briefly, 1.2 ml platelet suspensions were incubated for 30 min at 37°C with either 0.42 μ*M* fura 2-acetoxymethyl ester (Fura 2-AM) or an equivalent volume of DMSO (blank sample). Platelets were then re-pelleted by centrifugation at 900 *g* for 5 min to remove external dye and were resuspended in HEPES medium with 0.5 m*M* final $[Ca^{2+}]$. Fluorescence at excitation wavelengths of 340 and 380 n*M* was collected at 498 n*M* using a Perkin-Elmer model LS-5B spectrofluorometer. Fluorescence ratios at high (R_{max}) and low (R_{MIN}) Ca^{2+} concentrations were determined by lysing cells with SDS detergent and adding first excessive $CaCl_2$ and then excessive EGTA. Cell autofluorescence (blank sample) and quench by 200 μ*M* $MnCl_2$ of dye leaking out of cells were subtracted before calculating $[Ca^{2+}]_i$ according to the method of Grynkyewicz et al. (1985) and Rink (1988).

After resting ($[Ca^{2+}]_B$) levels of $[Ca^{2+}]_i$ were calculated, platelets were stimulated with 0.4 U/ml of thrombin plus 10 ng/ml of platelet-activating factor to determine the level of $[Ca^{2+}]_i$ that occurs with celluar activation ($[Ca^{2+}]_S$). Statistical analysis was by paired *t*-test.

In lymphocyte studies (experiment 2), blood was drawn into ACD anticoagulant and RPR was removed. Platelet-poor plasma was diluted back to the original volume with phosphate buffered saline (7.25 g monobasic NaH_2PO_4 plus 28.75 g dibasic NaH_2PO_4 in 250 ml H_2O, overlaid onto Ficoll paque and centrifuged for 30 min at 425 *g* to isolate the lymphocytes. Lymphocyte layers were removed from the Ficoll, suspended in HPES-EGTA buffer with a final $[Ca^{2+}]$ of 1.0 m*M*, and centrifuged for 20 min at 900 *g* at 5°C. The resulting lymphocyte pellet was resuspended in the same HEPES medium and centrifuged at 700 *g* for 10 min at 5°C. Red blood cells in the pellet were lysed with 5 ml of 0.2% NaCl followed by 5 ml of 1.6% NaCl in 0.2% glucose solution. The suspension was mixed, centrifuged and washed again with HEPES with Ca^{2+}. The pellet was resuspended in the HEPES medium and placed in petri dishes at room temperature to separate lymphocytes from monocytes, which adhere to the dish surface.

After removal from the dish the lymphocyte solution was centrifuged for 5 min at 700*g*, and the lymphocyte pellet was resuspended in HEPES-EGTA with 0.5 m*M* Ca^{2+}. Lymphocyte suspensions were divided in half and centrifuged for 5 min at 700 *g*, after which each of the two pellets was suspended in RPMI medium (Gibco) supplemented with 5% fetal calf serum, 1% L-glutamine and 1% penicillen/streptomycin. Lymphocyte presence was verified with a Diff Quik stain.

Approximately 2.5×10^6 lymphocytes per ml of supplemented RPMI were incubated for 18 hours 37° with or without 10 μg carbamazepine/ml of cell suspension. The next day, each lymphocyte suspension was centrifuged for 8 min at 210 *g* and resuspended in HEPES medium with $[Ca^{2+}] = 0.5$ m*M*. Cell viability was determined to by > 95% with Eosin Y dye and a hemacytometer, after which cell concentrations were adjusted to about 10×10^6/ml. Incubation with Fura 2-AM and calculation of $[Ca^{2+}]_i$ were performed as described above except that lmyphocytes were stimulated for determination of $[Ca^{2+}]_S$ with a combination of 1 μg/ml phytohemagultinin, 7.5 μg/ml concanavalin A and 20 μg/ml pokeweed mitogen in order to provide maximal rises in $[Ca^{2+}]_i$. The effect of carbamazepine incubation was determined by comparing samples from each patient incubated in RPMI alone with samples incubated in RPMI plus carbamazepine.

Inter-individual variability of $[Ca^{2+}]_i$ was assessed statistically with Bartlett's test for variance of standard deviations within each group. The significance of between-group differences in $[Ca^{2+}]_b$ and $[Ca^{2+}]_S$ was tested by *F*-tests followed by *t*-tests for unequal samples. Changes in $[Ca^{2+}]_i$ after carbamazepine incubation were assessed with paired *t*-tests.

Results and discussion

Table 2 demonstrates that in the first experiment there were no significant differences in $[Ca^{2+}]_B$ ($t = 0.11$; $P = 0.92$) or $[Ca^{2+}]_S$ ($t = 0.21$; $P = 0.84$) between platelets incubated in Ca^{2+}-HEPES alone and platelets incubated in HEPES with the same $[Ca^{2+}]$ plus an ultrafiltrate obtained from patients with bipolar affective disorders. This finding suggests that elevated $[Ca^{2+}]_i$ in bipolar disorder is not the result of a circulating factor. An animal study (Dubovsky et al., unpublished data) in which pigtail and bonnet Macaque monkeys demonstrated reversible elevated lymphocyte $[Ca^{2+}]_B$ and $[Ca^{2+}]_S$ during separation depression but not during stressful experiences such as a change ob habitat or excess competition for a limited water supply suggested that increased $[Ca^{2+}]_i$ is not a nonspecific manifestation of the stress response:

An ultrafiltrate from hypertensive patients has been found to elevate $[Ca^{2+}]_B$ in platelets of normal controls, indicating that a circulating substance, possibly an inhibitor of the sodium-potassium pump, alters intracellular Ca^{2+} homeostasis in hypertension (Lindner et al. 1987). This phenomenon seems specific for platelets, since elevated $[Ca^{2+}]_i$ has not been found in lymphocytes of hypertensive patients (Ashley et al. 1986). In contrast, the present findings suggest that bipolar disorder is associated with a more widespread alteration of cellular Ca^{2+} hemostasis intrinsic to each of the blood cell types that has been studied, increasing the possibility that the change in cellular function may extend to cells in the brain as well.

Another characteristic of increased $[Ca^{2+}]_i$ in peripheral cells in bipolar disorder is that it can be observed in both mania and depression. If hyperactivity of intracellular Ca^{2+} signalling is also present in the brain, the same change in different systems and at different times could activate or inhibit affective, behavioral and physiologic states, accounting for the mixtures and alternations of symptoms that are common in bipolar illness.

If the bidirectional effect of a hyperactive intracelluar Ca^{2+} signal contributed both to elevation and depression

Table 3. Mean intracellular calcium ion concentration ($[Ca^{2+}]_i$), n*M* (Mean ± SD) in lymphocytes from bipolar patients and controls incubated with and without carbamazepine

Subjects	Measure	Plain	Carbamazepine	*t*	*P* by paired t-test
Bipolar patients	$[Ca^{2+}]_B$	117 ± 18.2	110 ± 16.3	–3.45	0.002
	$[Ca^{2+}]_S$	652 ± 175.2	535 ± 93.0	– 4.08	0.0004
Controls	$[Ca^{2+}]_B$	97 ± 5.3	101 ± 8.3	1.51	0.18
	$[Ca^{2+}]_S$	543 ± 149.6	521 ± 70.4	–0.28	0.79

$[Ca^{2+}]_B$ resting $[Ca^{2+}]_i$; $[Ca^{2+}]_S$ agonist stimulated $[Ca^{2+}]_i$

of mood and related states, normalization of this signal could account for the bidirectional action of mood stabilizing drugs such as lithium and carbamazepine on both mania and depression. One indication that this may be an action of such drugs is the finding in two studies that platelet $[Ca^{2+}]_B$ and $[Ca^{2+}]_S$ were equivalent to control values in euthymic bipolar patients treated with a variety of medications (Dubovsky et al. 1989, 1991a). Eckert et al. (1992) found that serotonin-stimulated platelet $[Ca^{2+}]_S$ was increased in depressed patients receiving active treatment, but none of the patients were euthymic. Euthymic lithium-treated patients were noted by Tan et al. (1990) to have elevated levels of platelet $[Ca^{2+}]_B$ and $[Ca^{2+}]_S$, but as $[Ca^{2+}]_i$ in patients was three times the value reported in other Fura 2 studies using platelets from controls or ill patients (Lindner et al. 1987; Rao et al. 1985; Pollock and Rink 1986; Sang et al. 1987; Dubovsky et al. 1989, 1991 a, b), the finding could have been an artifact.

Tan et al. (1990) also reported that incubation of platelets from lithium-treated euthymic patients did not have a significant effect on $[Ca^{2+}]_i$, which might be expected if any effect of lithium had already developed when the patients began taking the drug. In addition, toxic (10 m*M*) lithium concentrations were utilized, and levels this high may paradoxically increase mobilization of intracellular Ca^{2+} whereas lower levels have the opposite effect (Batty and Nahorsky 1985; Whitworth and Kendall 1988). Conversely, Dubovsky et al. (1991b) found that incubation of platelets for 1 hour in a medium containing therapeutic (1 m*M*) levels of lithium significantly lowered $[Ca^{2+}]_B$ and $[Ca^{2+}]_S$ in untreated bipolar patients but not controls. This effect may have been limited to symptomatic patients because these patients have one or more hyperactive mechanisms for elevating $[Ca^{2+}]_i$ that are more sensitive to lithium, or it may have been easier to demonstrate a decrease in $[Ca^{2+}]_i$ in patients because their initial $[Ca^{2+}]_i$ was higher, a possibility contradicted by the very high platelet $[Ca^{2+}]_i$ levels in the Tan et al. (1990) study.

To determine whether carbamazepine might have an effect on $[Ca^{2+}]_i$ similar to that we observed with lithium, we incubated lymphocytes of bipolar patients and controls with therapeutic concentrations of carbamazepine. We utilized lymphocytes in order to test the hypothesis that an action of different antimanic drugs on $[Ca^{2+}]_i$ could be demonstrated in different peripheral cells. We chose a longer incubation period (18 h versus 1 h for platelets), which is technically possible with lymphocytes but not platelets, to ascertain whether the effect on $[Ca^{2+}]_i$ is persistent or transient.

As noted in Table 3, mean lymphocyte resting $[Ca^{2+}]_i$ (i.e., $[Ca^{2+}]_B$) prior to carbamazepine incubation was significantly higher in patients than in controls ($t = 4.8$; $P = 0.001$), supporting previous observations of differences in $[Ca^{2+}]_B$ between bipolar and normal individuals. However, while mean pre-carbamazepine lymphocyte $[Ca^{2+}]_S$ was higher in patients than controls, the difference was not significant ($t = 1.5$; $P = 0.1$), probably because of the substantial inter-individual variability of $[Ca^{2+}]_S$ and the relatively small number of controls. Inter-individual variability as indicated by standard deviations of the results was significantly greater in bipolars than controls for $[Ca^{2+}]_B$ ($F = 11.9$; $P = 0.005$) but not $[Ca^{2+}]_S$ ($F = 1.37$; $P = 0.7$). The statistically greater variability of $[Ca^{2+}]_B$ in bipolar patients than in controls, which is consistent with similar observations in platelets (Dubovsky et al. 1989), may reflect the biological heterogeneity of the disorder.

Table 3 also indicated that incubation of lymphocytes from bipolar patients resulted in a significant reduction both of $[Ca^{2+}]_B$ ($t = -3.45$; $P = 0.002$) and $[Ca^{2+}]_S$ ($t = -4.08$; $P = 0.004$) in patients but not controls. While statistically significant, the absolute reduction of lymphocyte $[Ca^{2+}]_i$ was not very impressive. However, the importance of the result is strengthened by an *F*-test on the ratio of variances followed by a two-sample *t*-test, which demonstrated that the direction of change of $[Ca^{2+}]_B$ with carbamazepine incubation differed significantly between patients and controls, more patients demonstrating a decrease and more controls demonstrating an increase ($t = -2.6$; $P = 0.01$). While small, the direction of decrease of lymphocyte $[Ca^{2+}]_i$ therefore was more often in the predicted direction in patients than in controls. That the $[Ca^{2+}]_i$-lowering action of carbamazepine that does exist depends on an intrinsic action of the drug on hyperactive cells and not on an initially higher level of $[Ca^{2+}]_i$ in bipolar patients is suggested by reduction of lymphocyte $[Ca^{2+}]_S$ in bipolar patients but not controls, even though patient and control levels of $[Ca^{2+}]_S$ were not significantly different before incubation.

The observation that 1-h incubation with lithium (Dubovsky et al. 1991b) lowered platelet $[Ca^{2+}]_B$ and $[Ca^{2+}]_S$ more than an 18-h carbamazepine incubation reduced these $[Ca^{2+}]_i$ measures in lymphocytes of acutely ill bipolar patients could reflect differences in intracellular calcium dynamics in the two cell types. Lymphocyte $[Ca^{2+}]_i$ would be expected to increase over the 18 hours incubation, perhaps making an effect of carbamazepine incubation less obvious, especially if an intermediate-term metabolic process compensated for an earlier decrease of $[Ca^{2+}]_i$. Alternatively, the impact of carmabazepine on intracellular Ca^{2+} dynamics may be less important than that of lithium or the two drugs may have different mechanisms of action, perhaps with a common final pathway.

Carbamazepine has been found to inhibit Ca^{2+} influx in a number of cellular models (Post 1987; Messing et al. 1985; Winkel and Lux 1985), and its antikindling effect has been linked to antagonism of increased Ca^{2+}-dependent phosphorylation of brain calmodulin kinase II (Taft et al. 1987). Walden et al. (1992) recently reported that carbamazepine had actions similar to those of calcium channel antagonists, as well as an additive effect with the calcium channel antagonist verapamil, in reducing Ca^{2+} dependent paroxysmal depolarizations in an animal model of hyperactive neurons. The calcium antagonist effect of carbamazepine did not appear to be mediated by the *N*-methyl-D-aspartate (NMDA) receptor.

Reduction of $[Ca^{2+}]_i$ in platelets by lithium and in lymphocytes by carbamazepine raises the possibility that the two drugs could have a common intracellular locus of action. One point of interest is the coupling of receptors to G-proteins (Avissar et al. 1990; Avissar and Schreiber 1992). G-proteins may be particularly important because of their linkage to the adenosine 3′,5′-cyclic monophosphate (cAMP) as well as the PI/Ca^{2+} second messenger systems. Each of these second messengers influences the other, so that an action on one would be expected to alter the other (Rasmussen 1986), but a common action on G-proteins would also explain why lithium and carbamazepine have been found to blunt the cAMP as well as the Ca^{2+} response to receptor stimulation.

Schreiber et al. (1991) reported increased mononuclear leukocyte G-protein responsiveness in acutely ill bipolar patients, with normalization of G-protein activity with euthymia after treatment with lithium. Lithium attenuated coupling of G-proteins to receptors linked both to cAMP and the PI/CA^{2+} system, possible by competing with Mg^{2+}, which facilitates G-protein hydrolysis necessary for its activation (Avissar and Schreiber 1989, 1992). These observations led Avissar and Schreiber (1992) to hypothesize that primary hyperfunction of G-proteins corrected by lithium leads to unstable oscillations of activation of G-protein dependent protein kinase A (linked to cAMP) and protein kinase C (linked to PI/Ca^{2+}) and destabilization of biological processes linked to affective states that depend on protein phosphorylation by these kinases.

If G-protein hyperfunction is deranged in bipolar illness, the effector for cellular dysregulation could be the bidirectional action of intracellular Ca^{2+}. Normalization of G-protein function by antimanic drugs could contribute to mood stabilization by reducing $[Ca^{2+}]_i$ to a range that normalizes both excessive activation and inhibition of cellular processes. Alternatively, increased $[Ca^{2+}]_i$ could be one of several consequences of a primary change in G-proteins or some other regulator of intracellular homeostasis that is not related to the pathopyhsiology of the illness, and normalization of intracellular Ca^{2+} might not be relevant to the therapeutic effect of a mood stabilizing medication. It is also possible that changes in G-proteins, intracellular $Ca^{2+}$and other cellular processes during illness and their normalization with successful treatment are all secondary to a more basic change in membrane structure or function (Pettegrew et al. 1982; Goodwin and Jamison 1990). However, the observations that both lithium and to some extent carbamazepine lower $[Ca^{2+}]_i$ in some affectively ill patients and that normalization of $[Ca^{2+}]_i$ is associated with a return to euthymia suggests that if an action on the Ca^{2+} second messenger system is not the therapeutically relevant effect, it is at least one marker of that effect.

Preliminary studies of the Ca^{2+} second messenger system in bipolar disorder have several implications. First, increased $[Ca^{2+}]_i$ during active illness and normalization of $[Ca^{2+}]_i$ with successful treatment are more evident in peripheral cells of bipolar patients than in other subjects. It may therefore only be possible to observe the relevant action of antimanic drugs on second messengers at therapeutic levels in actively ill patients. In the absence of an animal model of bipolar illness, animal studies antimanic drug effects should at least involve hyperactive cellular systems such as those involving induced epileptic activity (Walden et al. 1992; Jope et al. 1992) if they are to be considered relevant to drug actions in human illness.

The substantial variability of $[Ca^{2+}]_i$ in bipolar patients is consistent with the heterogeneity of these patients with respect to symptoms, course and treatment response. Further clinical investigations should take advantage of the heterogeneity of the Ca^{2+}-marker by examining whether different ranges of $[Ca^{2+}]_i$ in various peripheral cells are associated with different clinical syndromes and with responses to different treatments. Finally, the calcium channel antagonists, which appear promising in the treatment of bipolar illness (Dubovsky et al. 1989), differ in affinity for binding sites, types of calcium channel affected, and associated properties such as anticonvulsant effects. It may now be appropriate to begin to determine whether different bipolar subtypes defined by second messenger markers respond to different calcium antagonists and whether in vitro modification of peripheral cell $[Ca^{2+}]_i$ by a given drug predicts response to classes of medications with calcium antagonist properties.

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