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Short communication

Mood stabilizers have differential effects on endogenous ADP ribosylation in C6 glioma cells

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Abstract

Bipolar disorder is associated with increased levels and function of the G-protein, $G_s\alpha$ which may be normalized by treatment with mood stabilizing medications (i.e. lithium salts and the anticonvulsants, valproic acid and carbamazepine). In C6 glioma cells, endogenous ADP ribosylation was markedly increased by lithium chloride (+83%, $P < 0.005$), decreased by valproic acid (−48%, $P = 0.07$) whereas carbamazepine had no effect. Since ADP ribosylation of $G_s\alpha$ has been shown to increase turnover of this protein these results suggest a possible mechanism of action for lithium chloride. These results also suggest that lithium salts and the anticonvulsant mood stabilizers may have distinct mechanisms of action.

Keywords: Lithium chloride; Valproic acid; G-protein; Bipolar disorder; ADP ribosylation

1. Introduction

Lithium salts and the anticonvulsants, valproic acid and carbamazepine, are highly effective in the treatment of bipolar disorder (also known as manic depressive illness) (Bowden, 1995). All three of these mood stabilizing agents treat the acute symptoms of bipolar disorder (mania and depression) and prevent relapses if administered chronically to patients (Bowden, 1995). Although the precise mechanism of action of any of these agents in bipolar disorder has not been clearly identified, a growing body of evidence suggests that these agents have specific actions on brain signal transduction pathways (Hudson et al., 1993; Chen and Manji, 1995; Manji et al., 1995a). As reviewed elsewhere, these agents have effects on guanine nucleotide binding proteins (G-proteins), adenylyl cyclase and protein kinase C (Hudson et al., 1993; Chen and Manji, 1995; Manji et al., 1995a). It is not known whether all these agents act at a single site or whether they target different points in brain signal transduction pathways in the treatment of bipolar disorder.

Bipolar disorder has been shown to be associated with hyperfunctionality of G-proteins in blood cells (Schreiber et al., 1991) and higher levels of the stimulatory G-protein α-subunit ($G_s\alpha$) in postmortem brain (Young et al., 1993) and blood cells from patients with the disorder (Young et al., 1994; Manji et al., 1995b). Thus, it has been suggested that bipolar disorder is associated with increased signalling through $G_s\alpha$ and that blunting of signalling through this pathway by mood stabilizers may be important in its treatment (Hudson et al., 1993; Manji et al., 1995a). Lithium chloride has been shown to decrease gene expression of $G_s\alpha$ and $G_i\alpha$ in rat brain (Li et al., 1993), and to reduce $G_s\alpha$ and $G_i\alpha$ levels in PC12 cells which have been stimulated to differentiate in the presence of nerve growth factor (Li and Jope, 1995). Very recently, Nestler et al. (1995) showed differential effects of acute and chronic lithium chloride treatment on endogenous ADP ribosylation in rat brain, decreasing or increasing this process, respectively. Since ADP ribosylation of $G_s\alpha$ has been shown to increase the turnover of this protein and is critical to regulation of levels of G-protein α-subunits (Milligan et al., 1989; Milligan, 1993), this latter finding (Nestler et al., 1995) suggests a novel mechanism by which lithium chloride might decrease $G_s\alpha$ levels and thus decrease signalling through $G_s\alpha$-coupled pathways. This latter finding also points to a novel site of action of these agents, i.e. the processes and enzymes which regulate endogenous ADP ribosylation which are currently under

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investigation (Okazaki and Moss, 1994; Nestler et al., 1995).

The aim of the present study was to explore in a cellular model whether any or all of the mood stabilizers had effects on the level of endogenous ADP ribosylation. We report here that although lithium chloride markedly increased endogenous ADP ribosylation in C6 glioma, valproic acid had an opposite effect whereas carbamazepine had none.

2. Materials and methods

2.1. Cell culture

Rat C6 glioma cells (ATCC) were grown in Dulbecco's modified Eagle's medium plus 15% horse serum and 2.5% fetal calf serum. The cells were incubated for 7 days in either 1 mM lithium chloride, 0.5 mM valproic acid or 0.05 mM carbamazepine which represent therapeutic blood levels in patients treated with these drugs (Bowden, 1995). The medium was changed and fresh drugs were added at 24 h intervals. For the withdrawal treatment, after 7 days incubation with lithium chloride, the medium was removed, the cells washed twice with phosphate-buffered saline, pH 7.0 and fresh medium with no drug present was added. The cells were incubated for an addtiional 48 h before harvesting the membranes.

After incubation with the various drugs, the cells were washed twice and resuspended in phosphate-buffered saline, pH 7.0. The cells were pelleted at $13000 \times g$ and sonicated (70% intensity for 10 s) in 20 volumes of 10 mM Tris-HCl, 2 mM EGTA, 0.1 U aprotinin, pH 7.4. Homogenates were then centrifuged ($13000 \times g$, 10 min, 4°C) and the resulting membrane pellet was resuspended in 50 mM Tris, 2 mM EGTA, 5 mM $MgCl_2$, 0.1 U aprotinin, pH 7.4 and frozen (−70°C).

2.2. Endogenous ADP ribosylation

Endogenous ADP ribosylation was carried out as previously described (Williams et al., 1992) using 60 μg of membrane proteins incubated at 32°C for 45 min in 100 mM Tris, pH 7.5, 10 mM thymidine, 1 mM EDTA, 0.1% Triton X-100, 0.5 mM $MgCl_2$, 0.1 mM GTP, 25 mM dithiothreitol in the presence of 2 μM NAD containing 5 μCi of ^{32}P-labeled NAD (NEN Dupont) per tube. The reaction was stopped by the addition of ice-cold 20% trichloroacetic acid or 50 mM Tris-HCl, pH 7.8 and centrifugation ($2000 \times g$, 20 min, 4°C). The supernatants were discarded and the pellets washed in ethyl ether or 50 mM Tris-HCl, pH 7.8. The dried pellets were resuspended in 50 μl of Laemmli buffer and run on a 12% sodium dodecyl sulfate-polyacrylamide gel. Gels were dried (1 h, 80°C) and exposed to Kodak X-omat film for 1–2 days. The intensities of the 39 and 52 kDa bands were determined by laser densitometry (LKB Brommer Enhanced Ultrscan II) and expressed as the areas under the peaks.

2.3. Statistical analysis

Differences across treatment conditions were determined by separate one-way analyses of variance for each densitized protein band followed by unpaired *t*-tests to determine specific differences between control cells and individual drug treatments.

3. Results

Fig. 1 illustrates that ^{32}P was incorporated into five major bands in membranes prepared from C6 glioma which migrate at 110, 85, 52, 39 and 33 kDa, respectively, under the present experimental conditions. There was an overall significant difference between groups in the intensity of the 52 kDa ($F(4,47) = 6.67$, $P < 0.001$) and 39 kDa bands ($F(4,52) = 4.37$, $P < 0.005$). Chronic treatment with lithium chloride resulted in a marked increase (Figs. 1 and 2) in the ^{32}P incorporation in both the 52 kDa (+83%, $P < 0.005$) and the 39 kDa bands (+80%, $P < 0.01$) compared with untreated cells. On the other hand, chronic treatment with valproic acid resulted in a trend towards a decrease in the 52 kDa band (−48%, $P = 0.07$) compared with untreated cells. Carbamazepine treatment had little effect on the level of endogenous ADP ribosylation. After

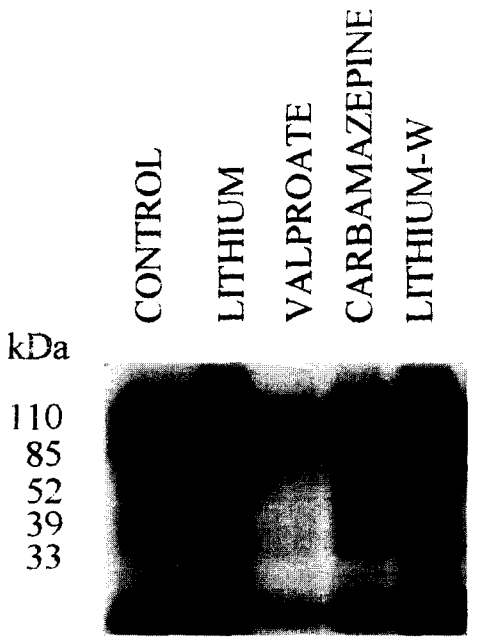

Fig. 1. A representative autoradiogram of ^{32}P incorporation into proteins separated by gel electrophoresis after endogenous ADP ribosylation as described in Materials and methods. Five major bands are seen migrating at the molecular weights shown. A marked increase after lithium chloride treatment is evident which decreases after withdrawal. LITHIUM-W, lithium chloride withdrawal.

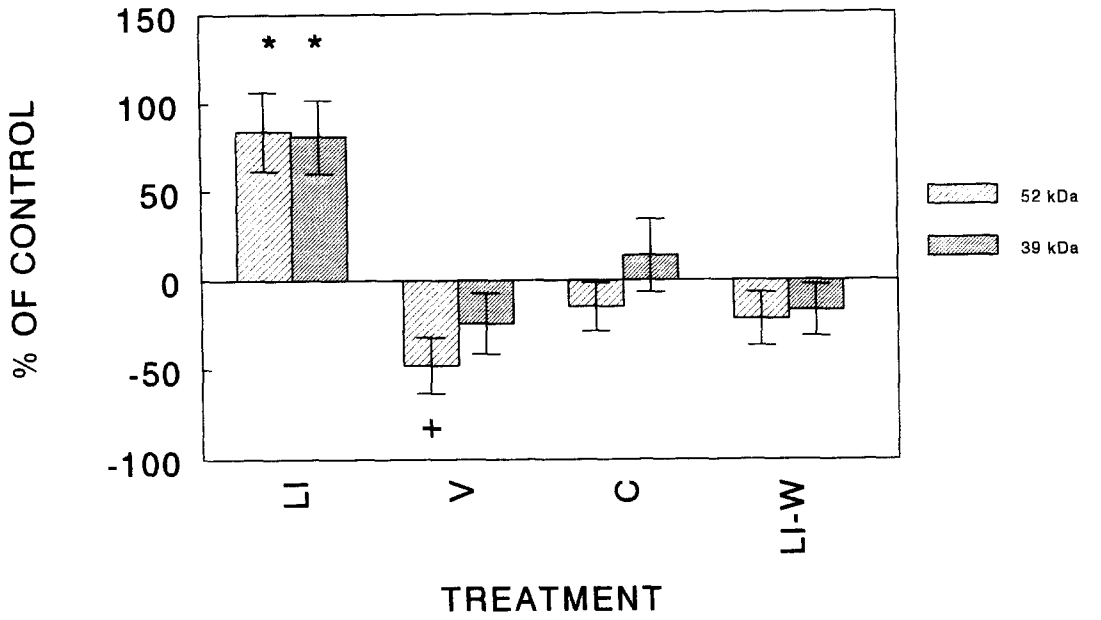

Fig. 2. Effect of mood stabilizers on ^{32}P incorporation into the 52 and 39 kDa bands as shown in Fig. 1. Each bar represents the mean ± S.E.M. of $n = 8$ to 12 plates in each condition. A significant increase is evident in both the 52 and 39 kDa bands after lithium chloride treatment. Valproic acid led to a reduction in the ^{32}P incorporation into the 52 kDa band whereas the other conditions had no significant effects. LI, lithium chloride; V, valproic acid; C, carbamazepine; LI-W, lithium chloride withdrawal. * $P < 0.01$ and + $P = 0.07$.

48 h of lithium chloride withdrawal, the intensities of the 39 and 52 kDa bands were not significantly different from untreated cells.

4. Discussion

The present study provides evidence for an action of lithium chloride at therapeutically relevant concentrations to increase endogenous ADP ribosylation of several substrates in C6 glioma cell membranes, including proteins which migrated at molecular weights known for $G_s\alpha$ and $G_i\alpha$. These findings are consistent with the recent report of Nestler et al. (1995). In contrast, the anticonvulsant mood stabilizers either had no effect (i.e. carbamazepine) or decreased (i.e. valproic acid) endogenous ADP ribosylation. These results support the hypothesis that lithium chloride may blunt $G_s\alpha$-coupled signalling by increasing $G_s\alpha$ turnover subsequent to increased ADP ribosylation (Milligan et al., 1989; Milligan, 1993) and are consistent with earlier findings of increased pertussis toxin-catalyzed ADP ribosylation and inactivation of $G_i\alpha$ after lithium chloride treatment in blood cells from patients and in rat brain (Manji et al., 1995b). These results also support the notion that the anticonvulsants may have different mechanisms of action than lithium chloride (Bowden, 1995).

The present study is limited in several respects. First, the study was carried out in C6 glioma cells which may not be generalizable to other cell types. Nonetheless, a similar effect for lithium chloride was shown under similar conditions in rat cerebral cortex suggesting that this effect may occur in other central nervous system cell types. Second, since little is currently known about the enzymes and other cellular components which are involved in brain endogenous ADP ribosylation, it is not possible to clarify the precise mechanism of action of lithium chloride on this process (Nestler et al., 1995). Obvious targets are the mono-ADP-ribosyltransferase enyzmes which have been characterized and cloned in other tissues (Okazaki and Moss, 1994). Furthermore, one or more of the ADP ribosylation factors which are critical, at least for the ADP ribosylation of $G_s\alpha$, may be involved in this process (Okazaki and Moss, 1994). Future experiments should address these specific mechanisms in central nervous system tissue.

In conclusion, lithium chloride but not anticonvulsants with mood stabilizing properties increased endogenous ADP ribosylation of substrates in brain consistent with the molecular weight of $G_s\alpha$ and $G_i\alpha$. This increase after lithium chloride treatment may in turn may lead to increased protein turnover (at least for $G_s\alpha$) as previously described (Milligan et al., 1989; Milligan, 1993) and downregulation of $G_s\alpha$-coupled signal transduction. Although lithium chloride does not alter $G_s\alpha$ levels in mature rat brain consistently (Li et al., 1993; Manji et al., 1995a), it has been shown to block the ability of nerve growth factor to markedly increase $G_s\alpha$ levels in cultured cells (Li and Jope, 1995) which may conceivably be due to increased turnover of $G_s\alpha$. Since the present findings are also consistent with increased pertussis-toxin catalyzed ADP ribosylation of $G_i\alpha$ in blood cells of lithium-treated patients with bipolar disorder (Manji et al., 1995a), future

studies should be directed to measurement of endogenous ADP ribosylation in tissues obtained from bipolar disorder patients before and after lithium treatment.

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