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Lithium, But Not Carbamazepine, Potentiates Hyperactivity Induced by Intra-accumbens Cholera Toxin

ORA KOFMAN, PETER P. LI AND JERRY J. WARSH

Biochemical Psychiatry, Clarke Institute of Psychiatry, Toronto, Ontario Canada M5T 1R8

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KOFMAN, O., P. P. LI AND J. J. WARSH. *Lithium, but not carbamazepine, potentiates hyperactivity induced by intra-accumbens cholera toxin.* PHARMACOL BIOCHEM BEHAV **59**(1) 191–200, 1998.—Elevated G protein abundance and/or function has been implicated in the pathophysiology and pharmacotherapy of bipolar affective disorder. To test the interactions between chronic lithium and carbamazepine on behavioral changes induced by cholera toxin (CTX), which catalyzes ADP-ribosylation and constitutively activates Gαs/olf, rats were given chronic dietary lithium, carbamazepine (CBZ), or regular food (REG) and injected bilaterally in the nucleus accumbens (nACC) with CTX (400 ng/ml/side) or vehicle. Locomotor activity was tested daily for 2 weeks after the injection. CTX increased locomotor activity, but a significant interaction between drug treatment and CTX reflected a two- to threefold increase of CTX-induced hyperactivity in the lithium-treated group. In contrast, on day 1, the CBZ-CTX group was significantly more active than the the LI-CTX and REG-CTX groups, both of which had suppressed locomotor activity. There was a significant reduction in CTX-catalyzed ADP ribosylation of Gαs (52 kDa and 45 kDa) in the nucleus accumbens in all three CTX-treated groups. The potentiation of the behavioral effect of CTX by lithium supports the hypothesis that lithium interacts with G proteins; however, the mechanism of interaction appears to be more complex than direct attenuation of Gαs function, as previously suggested. © 1998 Elsevier Science Inc.

Bipolar affective disorder Carbamazepine Cholera toxin G protein Lithium
Locomotor activity nucleus accumbens

ALTERATIONS in the abundance or function of the heterotrimeric guanine nucleotide binding (G) proteins have been reported in patients with bipolar affective disorder (BP) (18,46,56,57) and, conversely, G proteins have also been proposed to be a putative site of action for the two major mood stabilizers, lithium and carbamazepine (CBZ) (2,3). One line of evidence derives from comparisons of G protein abundance or function in blood cells of BP patients and control subjects. Unmedicated BP patients were found to have elevated levels of agonist-induced [^{3}H] 5′-guanylylimidodiphosphate (Gpp(NH)p, a guanosine triphosphate [GTP] analogue) binding to both cholera toxin (CTX)-sensitive and pertussis toxin (PTX)-sensitive G proteins in leukocytes (46). This increase was not observed in manic patients treated with lithium, suggesting that elevated [^{3}H] Gpp(NH)p binding was a state variable alleviated by lithium (46). Two studies have found elevations in the immunoreactivity levels of the stimulatory G protein α subunit (Gαs) in leukocytes of BP patients (31,57). In the study by Young et al. (57), patients were either lithium-naive or had been free of lithium for at least a year, thus permitting no inferences about possible effect of lithium on the level of Gαs immunolabeling; however, Manji et al. (31) found no difference between untreated and lithium-treated patients in Gαs immunolabeling, suggesting that the elevation of Gαs in peripheral tissue was not affected by lithium.

While G protein abnormalities in blood cells have also been found in patients with cardiac disease (26), the specificity and relevance of G protein changes in BP patients was strongly supported by evidence of elevated levels of the 52 kDa form of Gαs in the postmortem cerebral cortex of BP patients (56), but not in unipolar depressed (15) or schizophrenic patients (56). The most pronounced elevations were observed in the occipital and temporal lobes of the cortex and no changes in Gαs were observed in cerebellum, thalamus, or

Requests for reprints should be addressed to Ora Kofman, Ph.D., Department of Behavioral Sciences, Ben-Gurion University of the Negev, P.O.B. 653, Beer Sheva, IL-84105, Israel.

hippocampus. The 45 kDa form of Gαs was also significantly elevated in the occipital lobe, but not in other brain regions. Forskolin-induced adenylyl cyclase (AC) activity was also elevated, although no changes were found in basal AC activity or guanosine 5′-O-3-thiotriphosphate (GTPγS)-stimulated activity. Thus, while several studies have reported increased levels or function of Gαs proteins in BP patients, there is no conclusive clinical evidence that the therapeutic action of lithium is related to restoration of normal Gαs function.

The ability of lithium to block both agonist-induced stimulation and inhibition of adenosine 3′5′-cyclic monophosphatase cAMP formation (8,17,36,38,39) has been attributed to its inhibition of both stimulatory (Gs) and inhibitory G proteins (Gi) linked to AC. This was supported by the fact that GTP reversed the lithium-induced inhibition of agonist-stimulated cAMP formation in vitro (37). Preclinical studies have attempted to complement the clinical studies by examining the effects of chronic treatment with mood stabilizers on the function or abundance of G proteins in the brain. However, these studies yielded inconclusive results (32). Chronic lithium reduced muscarinic-, adrenergic- (1), and serotonergic-stimulated (54) GTP binding to G proteins. Carbamazepine or electroconvulsive shock (2) also disrupted agonist-induced GTP binding in rat brain.

Chronic dietary lithium in rats decreased $G\alpha i_1$, $G\alpha i_2$, and Gαs mRNA levels, but had no effect on the abundance of these G protein subunits in rat cerebral cortex (28,30). Colin et al. (14) found reduced $G\alpha i_{1/2}$ mRNA levels and reduced abundance of $G\alpha i_{1/2}$, but no change in immunoreactivity levels of Gαs and Gαo subtypes or Gβ. In rat hypothalamus and hippocampus, Gαi was elevated by chronic dietary lithium (28). Chronic lithium also had no effect on the phosphoinositide C-linked G protein α subunits, $G\alpha_{q/11}$, in rat brain, although there was evidence for translocation of protein kinase C (PKC) from the cytosolic to the membrane fraction (29). Chronic treatment with CBZ in rats reduced Gαs in the neostriatum and Gαi in the hypothalamus, neostriatum, and frontal cortex (28). In contrast, Li et al. (30) reported no effect of CBZ on G protein α subunit levels or mRNA in rat cerebral cortex.

It has also been suggested that lithium acts by stabilizing Gαi/o in its undissociated form, because (PTX)-stimulated adenosine diphosphate (ADP) ribosylation was enhanced by lithium in rats treated chronically with lithium (33), as well as in the platelets of normal volunteers treated with lithium for 14 days (20,44).

The investigation of the role of G proteins in BP disorder has been limited by the dearth of behavioral studies on animal models of BP disorder that involve direct perturbation of G proteins and their function. Because both lithium and CBZ have multiple effects on signal transduction, it is critical to show that the effects of mood stabilizers on G proteins can be correlated with the behavioral effects of these agents. A promising model in which to study the behavioral effects of Gs hyperfunction is the hyperactivity induced by injection of CTX into the nucleus accumbens (nACC) (16,34), striatum (24), or ventral tegmental area (VTA) (7) of rats. Because injection of CTX into the nACC enhanced cocaine-induced hyperactivity (16), and conditioned reward (23), this behavioral effect may involve postsynaptic dopamine (DA) receptors. Lithium has been shown to attenuate the behavioral manifestations of functional enhancement of DA receptor sensitivity following 6-hydroxydopamine (6-OHDA) lesions in the nACC (51) or following chronic haloperidol treatment (4,42,50), and delayed recovery from contralateral neglect following 6-OHDA lesions in the VTA (27). However, DA receptor binding (8, 27,42) was not altered by lithium, suggesting a postreceptor site of action. Lithium, but not CBZ, also attenuated the development of behavioral sensitization to repeated cocaine treatment (43). Accordingly, the present study examined the long-term effects of lithium on CTX-induced hyperactivity to determine if the interaction between lithium and Gαs is relevant to the therapeutic effect of lithium.

A further objective of the present study was to examine the ability of CBZ to modulate CTX-induced hyperactivity. Although several studies (2,11,28) have suggested similarities between lithium and CBZ in their mode of action on G proteins, other studies (30,43) suggest that these agents may have different mechanisms of action.

METHOD

Male Sprague–Dawley rats (Charles River Co., St. Constant, QC), weighing 220–300 g were divided into three treatment groups and fed either regular lab chow, lithium chow (0.22% lithium carbonate), or CBZ chow (0.25% CBZ for the first 5 days followed by 0.5% CBZ) (Bioserve Ltd., Frenchtown, NJ). The drug diets were maintained for 3 weeks prior to the surgery, and 2 weeks following the surgery (except where indicated below). Body weight was monitored daily, and rats on the lithium diet that lost more than 5 g/day were supplemented with 5 g regular food.

On the 21st day of the diet, each treatment group was subdivided into control or CTX groups and administered a bilateral injection of vehicle or CTX in the nACC. Rats were pretreated with 0.06 mg/kg atropine and anesthetized with 55 mg/kg sodium pentobarbital intraperitoneally (IP). Due to cross-tolerance between CBZ and pentobarbital, the dose of sodium pentobarbital was increased to 90 mg/kg for the CBZ-treated rats.

CTX (Sigma) was dissolved in a 50% solution of glycerol and deionized water to make a stock solution of 2 mg/ml. This solution was diluted with artificial CSF to a concentration of 400 ng/μl. The coordinates for the nACC injection were 1.6 mm rostral to bregma, 1.4 mm lateral from midline, and 7.5 mm ventral to dura, with bregma and lambda on the same plane (41). Injections were made using 28-gauge stainless steel tubing via a length of polyethylene tubing connected to a 1 μl Hamilton microsyringe. Injections were made manually over 5 min and the cannula was left in place for an additional minute at the end of the injection. Rats were treated with a long-acting antibiotic (Penlong), intramuscularly following the surgery. All procedures were approved by the institutional Animal Care Committee.

Rats injected with CTX generally lost weight and had to be supplemented with wet mash, 6 ml isotonic saline, and 6 ml isotonic sucrose until spontaneous feeding resumed (usually on the third day after the injection). During the period in which spontaneous feeding was impaired, the lithium-treated CTX rats received a single daily dose of 60 mg lithium chloride solution by gavage, the CBZ-treated CTX rats received a single daily dose of 150 mg CBZ, 25 mg carboxymethyl cellulose, and 0.125 ml glycerol in deionized water (total volume of suspension 5 ml), and the regular-diet rats received an equivalent volume of saline.

Behavioral Testing

The rats were tested for activity in automated activity monitors (Med Associates Inc.) constructed of transparent acrylic 41.9 × 41.9 cm, with a white PVC floor. Sixteen sensors facing 16 detectors, 2.54 cm apart were placed on opposite sides of the

box to measure locomotion and an additional array of sensors was placed 13.9 cm above the floor to measure rearing activity.

The rats were placed in the activity box 30 min once daily for 2 days prior to the surgery. These two sessions were averaged as the baseline activity measure for each rat. The day following the surgery, the rats were placed once daily for 14 days in the same box in which they had been habituated and activity was recorded for 30 min. All feeding procedures and injections mentioned above were done after the animals had been tested for locomotor activity and all testing took place between 0800–1400. Due to the large number of groups, the behavioral experiment was carried out in two replications of five to seven animals per group.

Histology and Drug Level Measurements

Rats were anesthetized with sodium pentobarbital and perfused with 20 ml physiological saline and 40 ml 10% formalin, and brains were removed and stored in a formalin/sucrose solution. Prior to the perfusion, cardiac blood from the lithium and CBZ rats was collected into heparinized test tubes for determination of plasma drug levels. Plasma lithium levels were determined by a standard clinical procedure using an ion-selective electrode (45). CBZ and its 10,11 epoxide metabolite were determined by reversed-phase liquid chromatography on a Partisil C_{18} column (Whatman, Clifton, NJ) eluted with acetonitrile/phosphate (36/64 by volume) buffer (10 mmol/l, pH 5.8) and detection by absorbance at 214 nm as previously described (49).

Brains were frozen and cut into 40 μm sections on a cryostat and then stained with cresyl violet. Sections were examined under a microscope while the experimenter was blind to the treatment group. Rats that did not have both cannulae in the accumbens were eliminated from the study.

CTX-Catalyzed ADP Ribosylation

The treatment conditions of the first experiment were replicated in a separate group of 41 rats. Rats were divided into three groups and treated for 24 days with regular, lithium, or CBZ chow. Baseline activity was tested on days 19 and 20 of the diet, as described above. On day 21, half the rats in each group were injected with CTX or vehicle bilaterally in the nACC as described above. Rats were tested for activity for 30 min daily for 3 days following the injection. On the third day they were decapitated, the brains were rapidly removed, and the nACC was dissected on a cold plate and frozen at −70°C until assayed. Blood from the cervical trunk was collected for analysis of serum lithium or CBZ levels, as described above. Two CBZ-treated rats were excluded due to toxic drug levels (59.8 and 79 μmol/l CBZ).

CTX-catalyzed ADP-ribosylation was assayed by a modification of previously described procedures (52,55). After thawing on ice, nACC samples (10–25 mg) were homogenized in 5 vol ADP-ribosylation buffer (RB) containing 10 mM thymidine, 20 mM isonicotinic acid hydrazide (INH), 1 mM 3-acetylpyridine adenine dinucleotide (APAD), 10 mM DTT, 0.1 mM, 5′-guanylylimidodiphosphate sodium salt (Gpp(NH)p), and 0.1% Triton X in 150 mM potassium phosphate, pH 7.0. The protein concentrations were determined by the Bradford method (6). CTX (2 mg/ml in 50% glycerol) was preactivated by incubation for 15 min at 37°C in 20 mM dithiothreitol (DTT) and 0.5% sodium dodecyl sulfate (SDS). [32P] ADP-ribosylation was performed in duplicate in 25 μl RB containing 2 μM [32P]NAD (3.0 μCi; New England Nuclear, Boston, MA), homogenate (40 μg protein), 20 mM adenosine 5′-diphosphoribose sodium salt, and activated CTX 40 mg/ml. The reaction mixtures were incubated at 30°C for 60 min and reactions terminated by the addition of 3 μl cold 100% trichloroacetic acid (TCA) (100% w/v). After incubation at 4°C for 15 min, samples were then centrifuged (15000 × g, 10 min) and supernatants discarded. Pellets were washed twice with 50 mM Tris (pH 8.0) buffer containing 3 mM benzamide, 1 mM DTT, 6 mM $MgCl_2$, 1 mM EDTA, 5% sucrose (w/v) and 1 μg/ml soybean trypsin inhibitor (SBTI), resuspended by homogenizing in 75 μl 50 mM Tris-HCl containing 5% SDS, 50 mM DTT, and heated [90°C, 5 min). Samples were then incubated with 7.5 μl gel loading buffer (4% SDS, 5 mM EGTA, 3 mM EDTA, 10% β-mercaptoethanol, 10% gylcerol, 250 mM Tris-HCl, (pH 6.8)]. The resulting mixture was heated at 100°C for 5 min. Proteins were resolved by SDS-polyacrylamide electrophoresis with 10% acrylamide/0.27% bisacrylamine gels as previously described (6). The gels were dried and autoradiographed with intensifying screens at −70°C and the bands corresponding to Gαs (52 kDa and 45 kDa) quantified by densitometry using an MCID (St. Catharines, Ont.) image analysis system as previously described (56). Linearity of detection relative to protein concentration was determined by assaying a range of sample protein concentrations (25–50 mg) processed in parallel with the experimental samples. Immunodetection and quantification of Gαs levels was performed using Gαs specific antiserum (RM/1, NEN-Dupont, Boston, MA) as previously described (56).

Statistics

Three-way ANCOVA was conducted for the effects of Diet (regular, lithium, or CBZ), CTX (vehicle or CTX), and days [1–14] for the two dependent variables, ambulatory activity and rearing, with daily body weight as a changing covariate. If significant interactions were found between the CTX and diet, further analysis of the interaction between mood stabilizers (diet) and CTX on a daily basis was done by two-way ANCOVAs and post hoc Scheffé tests. Statistical tests with p-values <0.05 were taken as statistically significant. The two presurgery sessions were averaged for each rat. A two-way ANOVA was conducted to determine if there were significant differences in baseline ambulatory or rearing activity prior to the CTX injections.

Drug levels were analyzed by t-tests between CTX and control groups for lithium- and CBZ-treated rats. CTX-ADP ribosylation was analyzed by two-way ANOVA for the effects of DIET and CTX for the 52 kDa and 45 kDa forms of Gαs.

RESULTS

CTX elicited an increase in activity, accompanied by initial weight loss, and occasionally, transient clonic seizures during the first 4–5 days after injection. Animals had to be maintained on a wet mash diet and supplemented with IP injections of saline during this period (see above). In some animals, there was a marked reduction of activity during the first day or two before the onset of hyperactivity, which was not related to change in body weight. Data were analyzed only for those animals that had bilateral placements in the nACC (Fig. 1).

Body Weight

Three-way ANOVA was conducted for the effect of DIET and CTX on body weight with days as a repeated measure. There was a main effect of diet, $F(2, 46) = 43.46$, $p < 0.000001$, CTX, $F(1, 46) = 15.24$, $p = 0.0003$, and days, $F(13, 598) =$

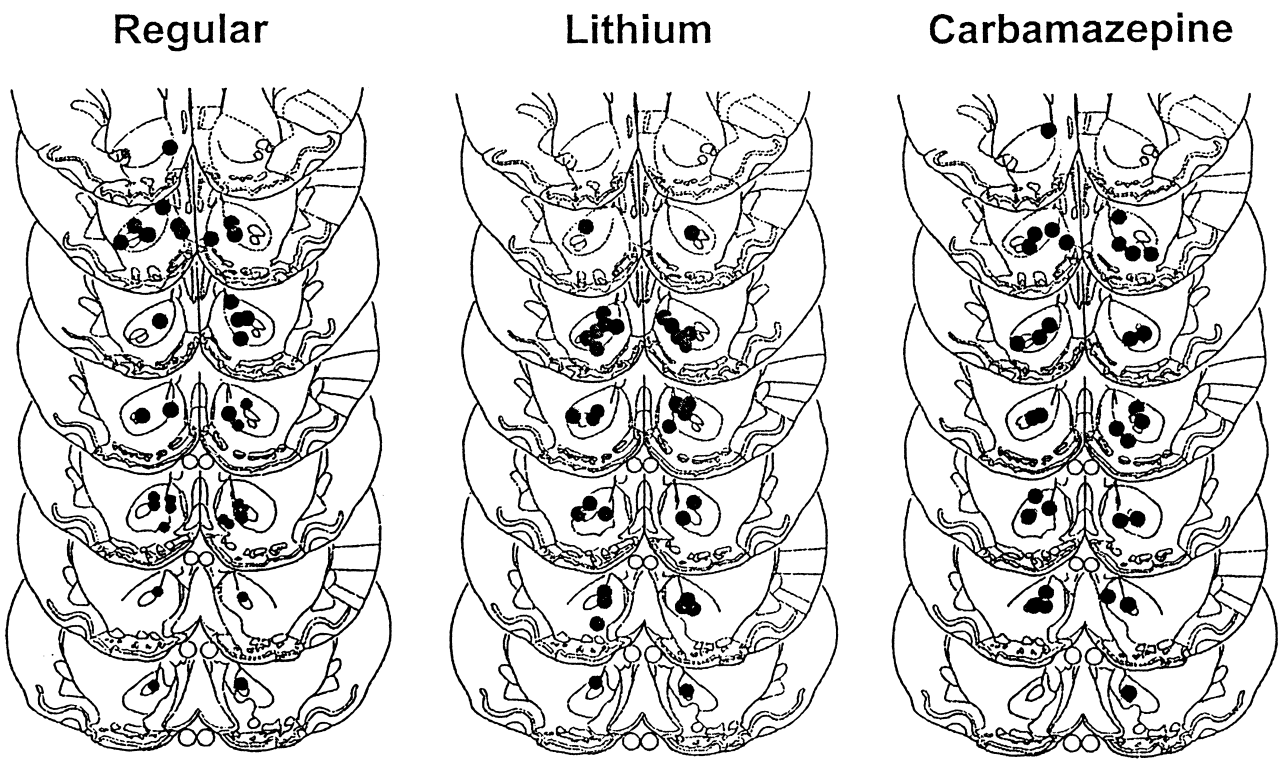

FIG. 1. Cannula placements of rats tested for locomotor activity. The gray and black circles represent sites for injections of the vehicle and CTX, respectively, for each of the pretreatment groups. Atlas sections are from the Paxinos and Watson atlas (41) from the top to bottom, as follows: 2.20 mm, 1.70 mm, 1.60 mm, 1.20 mm, 1.00 mm, 0.70 mm and 0.48 mm anterior to bregma.

109.22, $p < 0.000001$, and significant interactions between CTX × days, $F(13, 598) = 10.15$, $p < 0.000001$, and diet × days, $F(26, 598) = 1.77$, $p = 0.01$) (Fig. 2). Because of the significant changes in body weight, weight was used as a covariate in the subsequent analyses.

Ambulatory Activity

There were no main effects of either CTX or diet for baseline ambulatory activity and no interactions between these factors (inset Fig. 3). Three-way ANCOVA for ambulatory behavior, with weight as a covariate, showed a significant main effect of CTX, $F(1, 47) = 8.27$, $p < 0.007$, and significant interactions between diet and CTX, $F(2, 47) = 8.83$, $p < 0.006$, diet and days, $F(26, 611) = 4.06$, $p < 0.000001$, CTX and days, $F(13, 611) = 2.23$, $p < 0.01$. A three-way interaction was found for diet, CTX, and days, $F(26, 611) = 4.41$, $p < 0.000001$.

Further analysis was conducted using two-way ANCOVA (CTX × diet) for each day, with body weight as a covariate, followed by post hoc Scheffé tests to determine the differences between individual groups if the interaction was significant. The major finding was that there was a significant interaction between diet and CTX on days 3–10 and day 12 (Table 1). Post hoc analysis showed that the LI-CTX group had significantly elevated activity compared with the other CTX-treated and control groups on days 3–9 (Fig. 3).

Rearing

A three-way ANCOVA for the effects of diet, CTX, and days with weight as a covariate was conducted for the number of rears. There was a main effect of CTX, $F(1, 47) = 11.69$, $p < 0.002$, and a significant interaction between CTX and days, $F(13, 611) = 3.06$, $p < 0.0003$. The level of rearing fluctuated greatly between bouts of rearing and running with no rearing. No consistent interaction between the mood stabilizers and CTX was found; therefore, further statistical analysis was not conducted for rearing (Fig. 4). There was a significant diet effect for baseline rearing, $F(2, 48) = 5.59$, $p < 0.01$, which contrast analysis showed to be due to the fact that lithium-treated rats had lower levels of rearing ($p = 0.002$), as has been shown previously (25).

Drug Levels

Plasma lithium levels were 0.98 ± 0.42 mmol/l (mean ± SD) for the vehicle-injected group and 1.12 ± 0.62 mmol/l for the lithium-CTX group. There was no significant difference between the groups ($t = 0.53$). Levels of the CBZ 10,11 epoxide metabolite for the two CBZ groups were 38.89 ± 6.99 for the vehicle-injected group and 41.67 ± 9.26 for the CTX group. There was no significant difference between these groups ($t = 0.72$).

CTX-Catalyzed ADP Ribosylation

To confirm that CTX injection resulted in ADP-ribosylation of Gαs 52 and 45 kDa, CTX-catalyzed ADP ribosylation was tested 3 days after rats had been injected bilaterally with CTX. CTX-catalyzed ADP ribosylation in the nACC was significantly lower for the 52 kDa Gαs, $F(1, 33) = 17.9$, $p = 0.0002$, and for the 45 kDa Gαs, $F(1, 30) = 34.5$, $p = 0.000002$,

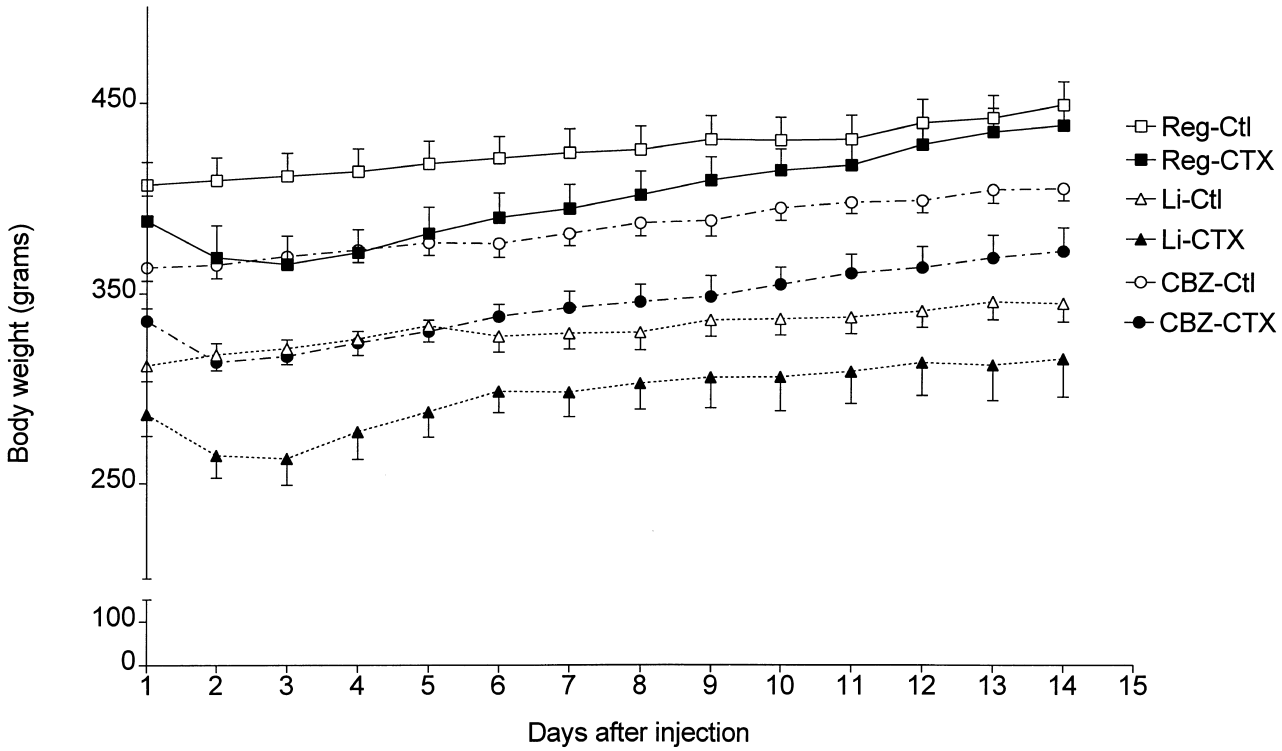

FIG. 2. Daily body weight (mean ± SEM) in grams for the treatment groups following the bilateral injection of vehicle (CTL) or cholera toxin (CTX). The X axis represents days following the injection. The number of Ss per group was: REG-CTL [9], REG-CTX [10], LI-CTL [10], LI-CTX [7], CBZ-CTL [9], and CBZ-CTX [9].

in rats injected with CTX (Tables 2 and 3). Gαs immunolabelling was reduced (25% ± 13.9 mean + SEM for the 52 kDa Gαs and 20.1% ± 13.2 for the 45 kDa), similar to findings in cultured cells following long-term treatment with CTX (9). However, a comparison of the percent reduction of CTX-catalyzed ADP-ribosylation for randomly matched pairs of control and CTX treated animals (regular diet) with the percent reduction in the abundance of Gαs, indicated that the decrement in ribosylation was significantly greater (*t*-tests for dependent samples, $t = 3.76$, $p = 0.002$ for the 52 kDa and $t = 3.19$, $p = 0.007$ for the 45 kDa). This confirms that CTX effectively ADP-ribosylated Gαs in this region. As in the previous group of animals, CTX significantly enhanced locomotor activity on the day the brains were removed (day 3 after the injection) $F(1, 33) = 12.2$, $p = 0.001$; however, in this experiment there was no interaction between the mood stabilizers and CTX on day 3. Plasma lithium levels were 0.85 ± 0.23 mmol/l (mean ± SD) for the vehicle-injected group and 0.73 ± 0.30 mmol/l for the lithium-CTX group. There was no significant difference between the groups ($t = 0.77$). Levels of the CBZ 10,11 epoxide metabolite for the two CBZ groups were 21.94 ± 7.96 for the vehicle injected group and 19.46 ± 13.46 for the CTX group. There was no significant difference between these groups ($t = 0.40$).

DISCUSSION

The major finding in the present study is that lithium prolonged and potentiated the hyperactivity induced by intra-nACC injection of CTX. CBZ neither attenuated nor enhanced the hyperactivity induced by CTX, but CBZ-treated animals did not show the the initial depression of locomotor activity on the first day after the injection that was observed in REG-CTX and LI-CTX groups. Locomotor activity was depressed below 50% of baseline in 8 of 10 rats of the REG-CTX group, 6 of 7 of the LI-CTX group, and only 1 of 9 of the CBZ-CTX group.

Because both lithium and CBZ diets resulted in slower weight gain in rats, the possibilty that lower body weight affected locomotor activity was controlled for by using weight as a covariate. Although the effect of injecting CTX in the accumbens on food consumption was not studied, the weight loss in the first 2 days was presumed to result from a preponderance of behaviors that may interfere with feeding, such as hyperactivity and forepaw padding. Electrolytic lesions of the accumbens have been found to disturb meal patterning, without significantly affecting body weight, except for an occasional and transitory loss of about 5% of body weight (13). Thus, the weight loss was more likely to be related to the enhancement of behaviors that were incompatible with feeding than to a nonspecific lesion effect.

The enhancement of CTX-induced hyperactivity in lithium-treated rats up to 4 days following injection was reported in a preliminary study (22); however, the present study found that the effect of lithium endured 2 weeks, and persisted after normal eating and grooming behavior had recovered. This finding does not support the hypothesis that the therapeutic effect of lithium is related to its ability to dampen hyperactivity mediated by enhanced Gαs levels or function, as was suggested in previous studies (1,56), and is unexpected in light of

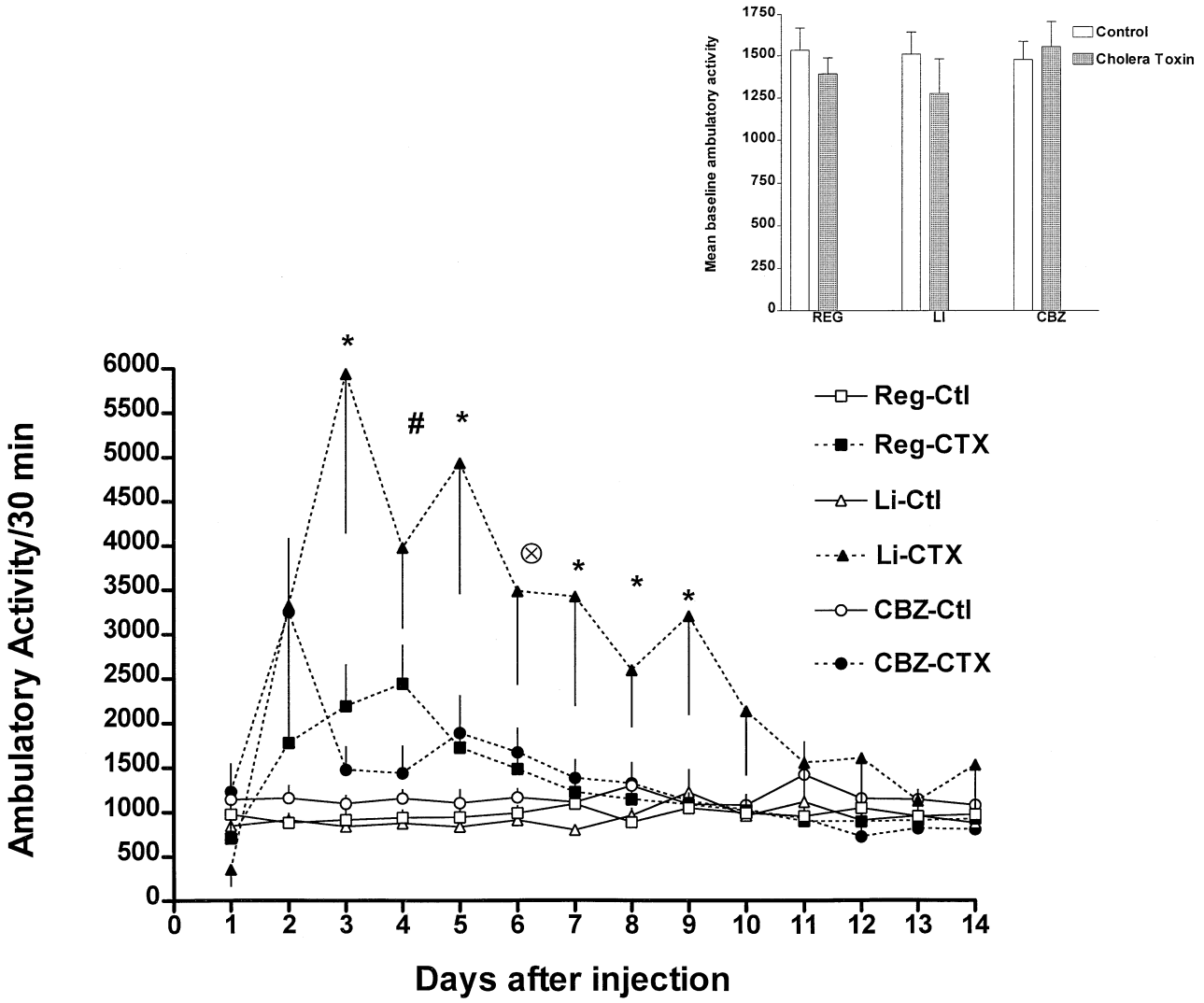

FIG. 3. Ambulatory activity (mean ± SEM) in 30 min as percent of baseline activity (Y axis) for 14 days following the injection of vehicle (CTL) or cholera toxin (CTX). The Ns are the same as in Fig. 2. The inset shows the mean + SEM of the baseline activity (average of two preinjection sessions). The individual group differences, determined by post hoc Scheffé tests, are shown as follows: *LI-CTX had higher activity than each of the other groups; #LI-CTX had higher activity than each of the other groups, except the REG-CTX group. Circled "x:" LI-CTX had higher activity than each of the other groups, except the CBZ-CTX group.

the antagonism between lithium and drugs such as forskolin and rolipram, which elevate cAMP levels (36,38,48). The effectiveness of the intra-accumbens injection of CTX on ADP ribosylation of Gαs was confirmed in a second experiment, in which the animals were sacrificed 3 days after the injection. All three CTX-treated groups showed behavioral hyperactivity and a concomitant decrease of CTX-catalyzed ADP ribosylation and immunolabelling for both forms of Gαs. The reduction in immunolabelling confirms, ex vivo in brain tissue, previous findings of marked reductions in Gαs immunoreactivity in cultured cells after application of CTX. However, in vitro, AC was maximally active even with a reduction of Gαs immunolabeling up to 90% following CTX (9).

The mechanism whereby lithium enhanced the CTX-induced locomotor hyperactivity is unclear. Lithium pretreatment does not enhance the substrate availability for CTX-catalyzed ADP ribosylation (14,28,30 and present study), and is known to reduce forskolin-induced stimulation of AC (36,38), suggesting that neither the catalytic subunit of AC or the Gαs protein are the sites at which the enhancement takes place. Alternatively, lithium could induce a compensatory increase in G protein-AC coupling, similar to that which was shown following chronic treatment with tricyclic antidepessants (12,40). The overall effect would then be a synergistic interaction between lithium and CTX on AC and cAMP production, similar to that reported by Masana et al. (33) in a microdialysis study from rat frontal cortex.

An alternative explanation is that lithium might enhance the CTX-induced hyperactivity by attenuating the action of inhibitory G proteins, rather than by acting directly on Gαs. Chronic lithium enhanced PTX-catalyzed ADP ribosylation in rat brain (33) and in platelets of normal volunteers (20,44).

TABLE 1

MAIN EFFECT OF CTX AND INTERACTIONS BETWEEN CTX AND DIET FOR EACH OF 14 DAYS FOLLOWING INJECTION OF CTX OR VEHICLE IN THE nACC (TWO-WAY ANCOVA FOR THE EFFECTS OF DIET AND CTX WITH WEIGHT AS A COVARIATE)

Day	Effect	*F*-score	*p*-Value
1	NS*		
2	CTX	$F(1, 47) = 6.18$	0.02
3	CTX	$F(1, 47) = 9.79$	0.005
	CTX × DIET	$F(2, 47) = 7.69$	0.002
4	CTX	$F(1, 47) = 14.35$	0.0005
	CTX × DIET	$F(2, 47) = 6.62$	0.003
5	CTX	$F(1, 47) = 7.96$	0.01
	CTX × DIET	$F(2, 47) = 6.73$	0.003
6	CTX	$F(1, 47) = 5.72$	0.03
	CTX × DIET	$F(2, 47) = 6.16$	0.005
7	CTX × DIET	$F(2, 47) = 6.74$	0.003
8	CTX × DIET	$F(2, 47) = 8.32$	0.001
9	CTX × DIET	$F(2, 47) = 4.58$	0.02
10	CTX × DIET	$F(2, 47) = 4.18$	0.03
11	NS		
12	CTX × DIET	$F(2, 47) = 5.16$	0.01
13	NS		
14	NS		

*No significant effects.

Both chronic in vivo lithium or in vitro lithium attenuated the 5-HT–induced reduction in PTX-catalyzed ADP ribosylation (54), and recently it was reported that 5-HT–induced reduction in PTX-catalyzed ADP ribosylation is enhanced in frontal cortex of postmortem brains of bipolar patients (18). Rats treated with chronic lithium also showed enhanced CTX-stimulated and reduced PTX-stimulated increments in the accumulation of cAMP in the dialysate from the frontal cortex of rats (33). These data led Manji et al. (32) to propose that lithium stabilizes Gi/o in the inactive heterotrimeric form. The potentiating effect of lithium may be mediated by attenuation of the increased sensitization of Gi/o, which has been reported to be one of the consequences of prolonged CTX exposure in vitro (5). It is conceivable that lithium acts both on Gs and Gi/o under physiological conditions, but that CTX-induced ADP-ribosylation may alter the conformation of the α subunit so that lithium can no longer act on it. If this were the case, then only the lithium inhibition of the function of Gi/o would manifest itself under conditions of constitutive activation of Gαs, resulting in potentiation of the behavioral effect of CTX.

The lithium potentiation of the behavioral effects of intra-accumbens CTX suggests that locomotor hyperactivity following intra-accumbens CTX is not a pharmacologically valid animal model for mania, even though CTX enhances conditioned reward (23) and increases locomotion (16). However, the effects of increased cAMP activity on locomotor behavior are region specific. The cAMP analogue, 8-bromo-cAMP, enhanced cocaine-induced behavioral sensitization in the accum-

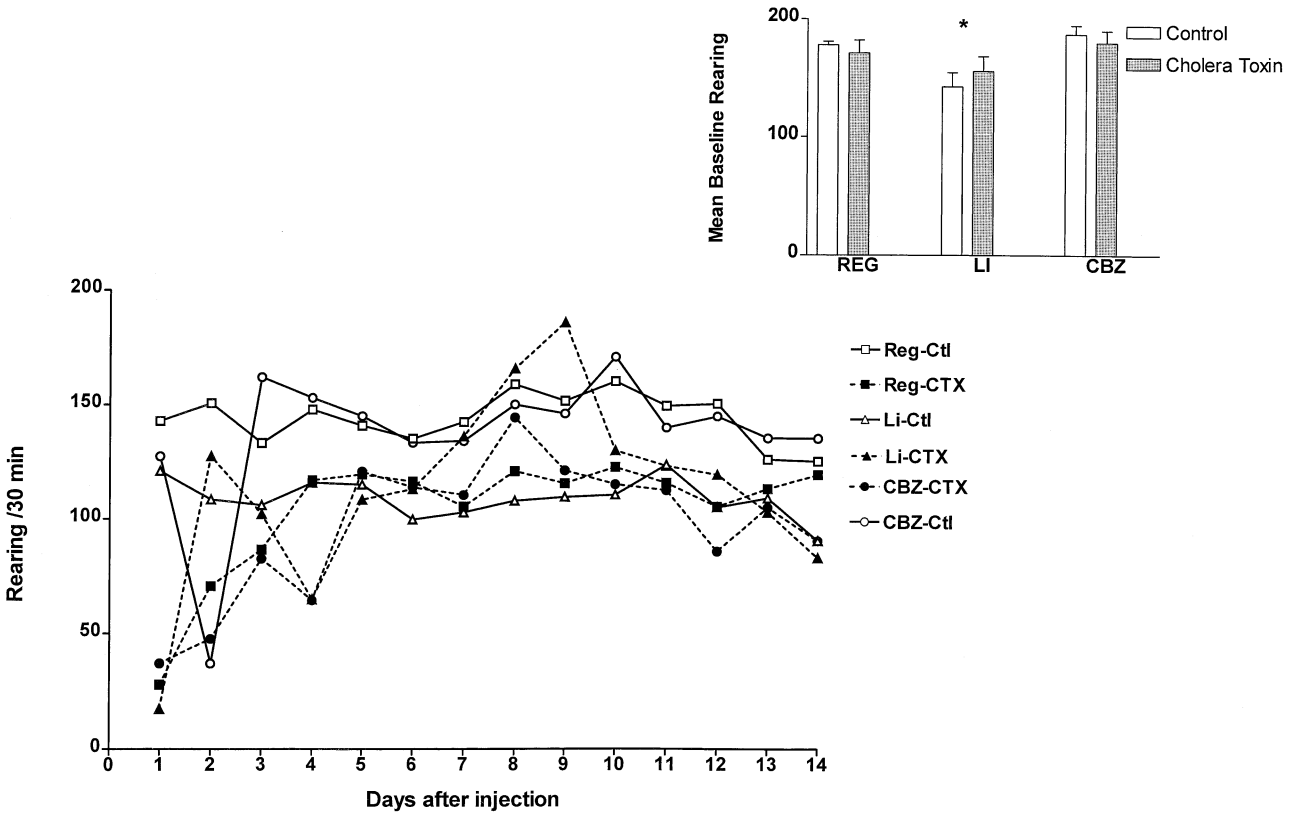

FIG. 4. Mean number of rears in 30 min for 14 days following the injection of vehicle (CTL) or cholera toxin (CTX). No significant interactions were found between CTX and DIET. The Ns are the same as in Fig. 2. The inset shows the mean + SEM of the baseline activity (average of two pre-injection sessions). *Main effect of DIET, $p < 0.01$.

TABLE 2

CTX-ADP RIBOSYLATION OF 52 KDA GαS (MEAN + SD NORMALIZED OPTICAL DENSITY) IN THE nACC FROM RATS TREATED CHRONICALLY WITH REGULAR, LITHIUM, OR CBZ CHOW FOR 24 DAYS

Pretreatment	Vehicle	CTX
Control	0.97 ± 0.3	0.58 ± 0.4
(*n*)	7	7
Lithium	1.23 ± 0.4	0.52 ± 0.3
(*n*)	6	5
CBZ	0.98 ± 0.2	0.71 ± 0.4
(*n*)	7	5

Effect of CTX: $F(1, 33) = 17.9$, $p < 0.0002$.

TABLE 3

CTX-ADP RIBOSYLATION OF 45 KDA GαS (MEAN ± SD NORMALIZED OPTICAL DENSITY) IN THE nACC FROM RATS TREATED CHRONICALLY WITH REGULAR, LITHIUM, OR CBZ CHOW FOR 24 DAYS

Pretreatment	Vehicle	CTX
Control	4.24 ± 0.7	1.63 ± 1.0
(*n*)	7	7
Lithium	4.26 ± 1.6	1.92 ± 1.0
(*n*)	5	6
CBZ	4.20 ± 1.2	2.16 ± 0.8
(*n*)	6	5

Effect of CTX: $F(1, 30) = 34.5$, $p < 0.000002$.

bens (35), and the cAMP phosphodiesterase inhibitor rolipram, in the nACC of rats, potentiated the locomotor activity induced by DA agonist, lisuride hydrogemaleate, although rolipram alone in the nACC decreased locomotor activity (53). In contrast, systemic rolipram attenuated the hyperactivity and locomotion induced by DA agonists (21). An inverse relation was found between noradrenaline-sensitive cAMP and locomotor activity in whole brain of inbred strains of mice (19). A correlation between locomotor activity and cAMP levels in striatum and midbrain was reported, but an inverse correlation was shown with cAMP levels in rat cortex (47). Conceivably, in regions other than the nACC, activation of Gi/o may actually induce "manic-like" symptoms, which would be attenuated by a drug that functionally stabilizes Gi/o in its inactive form.

The decreased activity observed on the first day following CTX administration had not been reported previously. Miller and Kelly (34) used a higher dose of CTX than that used in the present experiment, while Cunningham and Kelley (16) injected CTX and measured activity 2 h after the animals had been in the activity meter, which may have resulted in a "floor" effect of low baseline activity. In the present study, most animals in the lithium and control CTX-treated groups showed a marked decrease in activity, whereas those pretreated with CBZ did not. CBZ has been reported to reduce Gαs in the neostriatum (28), but is not likely that a similar effect in the nACC could account for the findings in the present study because there was no difference between the CBZ-CTX and other two CTX groups in the degree of CTX-ADP ribosylation in the second experiment. Moreover, CBZ did not prevent the hyperactivity induced by CTX. The dissociation between the effects of lithium and CBZ concur with the findings of Post and colleagues (43) who reported a dissociation between lithium and CBZ on cocaine-induced behavioral sensitization and on the induction of kindled seizures in the amygdala. The behavioral data obtained in this study also do not support the contention that lithium and CBZ share a common mechanism of therapeutic action via G proteins (3). However, both CBZ (11) and sodium valproate (10) have been found to attenuate forskolin-induced stimulation of cAMP in rat C6 glioma cell cultures, although in the case of sodium valproate, this attenuation was accompanied by a reduction of β-adrenergic receptors. The role of CBZ should be explored further in the first 24 h following the CTX injection, as rats treated with CBZ did not have suppressed locomotor activity on day 1, as was seen in the majority of the lithium and control groups injected with CTX. At present, it is not clear how this suppression is related to changes in cAMP levels.

Behavioral studies on the interaction between mood stabilizers and G proteins are critical in determining the relevance of the multifarious biochemical actions of mood stabilizers for their therapeutic mechanism and in testing novel substances. Behavioral antagonism has been reported between lithium and inositol (25), forskolin, dibutyryl cAMP (unpublished), and rolipram (48), all of which could be critical to the therapeutic effect of lithium. The data in the present study suggest that elevated Gαs immunoreactivity in the cortex of bipolar patients (31,56) may not be the direct site of lithium's therapeutic effect, but may be consequent to another pathophysiological process that is directly affected by lithium.

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REFERENCES

1. Avissar, S.; Schreiber, G.; Danon, A.; Belmaker, R. H.: Lithium inhibits adrenergic and cholinergic increases in GTP binding in rat cortex. Nature 331:440–442; 1988.
2. Avissar, S.; Schreiber, G.; Aulakh, C. S.; Murphy, D. L.: Carbamazepine and electroconvulsive shock attenuate β-adrenergic and muscarinic receptor coupling to G proteins in rat cortex. J. Pharmacol. (Mol. Pharmacol.) 189:99–103; 1990.
3. Avissar, S.; Schreiber, G.: The involvement of guanine-nucleotide binding proteins in the pathogenesis and treatment of affective disorders. Biol. Psychiatry 31:435–459; 1992.
4. Bloom, F. E.; Baetge, G.; Deyo, S.; Ettenberg, A.; Koda, L.; Magistretti, P. J.; Shoemaker, W. J.; Staunton, D. A.: Chemical and physiological aspects of the actions of lithium and antidepressant drugs. Neuropharmacology 22:359–365; 1983.
5. Boehm, S.; Huck, S.; Motejlek, A.; Drobny, H.; Singer, E. A.; Freissmuth, M.: Cholera toxin induces cAMP-independent down regulation of Gsα and sensitization of α_2 autoreceptors in chick sympathetic neurons. J. Neurochem. 66:1019–1026; 1996.
6. Bradford, M. M.: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–252; 1976.
7. Byrnes, J. J.; Weinstein, D. M.; Uretsky, N. J.; Wallace, L. J.:

Micro-injection of cholera toxin into the ventral tegmental area produces enhanced locomotor responses to subsequent amphetamine administration. Soc. Neurosci. Abstr. 21:971; 1995.
8. Carli, M.; Anand-Srivastava, M. B.; Molina-Holgado, E.; Dewar, K. M.; Reader, T. A.: Effects of chronic lithium treatments on central dopaminergic receptors systems: G proteins as possible targets. Neurochem. Int. 24:13–22; 1994.
9. Chang, F.-H.; Bourne, H. R.: Cholera toxin induces cAMP independent degradation of Gs. J. Biol. Chem. 264:5352–5357; 1989.
10. Chen, G.; Manji, H. K.; Wright, C. B.; Hawver, D. B.; Potter, W. Z.: Effects of valproic acid on β-adrenergic receptors, G proteins, and adenylyl cyclase in rat C6 glioma cells. Neuropsychopharmacology 15:271–280; 1996.
11. Chen, G.; Pan, B; Hawver, D. B.; Wright, C. B.; Potter, W. Z.; Manji, H. K.: Attenuation of cyclic AMP production by carbamazepine. J. Neurochem. 67:2079–2086; 1996.
12. Chen, J.; Rasenick, M. M.: Chronic treatment of C6 glioma cells with antidepressant drugs increases functional coupling between a G protein (Gs) and adenylyl cyclase. J. Neurochem. 64:724–732; 1995.
13. Clifton, P. J.; Somerville, E. M.: Disturbance of meal patterning following nucleus accumbens lesions in the rat. Brain Res. 667: 123–128; 1994.
14. Colin, S. F.; Chang, H. C.; Mollner, S.; Pfeuffer, T.; Reed, R. R.; Duman, R. S.; Nestler, E. J.: Chronic lithium regulates the expression of adenylate cyclase and Gi-protein α subunit in rat cerebral cortex. Proc. Natl. Acad. Sci. USA 88:10634–10637; 1991.
15. Cowburn, R. F.; Marcusson, J. O.; Eriksson, A.; Wiehager, B.; O'Neill, C.: Adenylyl cyclase activity and G protein subunit levels in postmortem frontal cortex of suicide victims. Brain Res. 623: 297–304; 1994.
16. Cunningham, S. T.; Kelley, A. E.: Hyperactivity and sensitization to psychostimulants following cholera infusion into the nucleus accumbens. J. Neurosci. 13:2342–2350; 1993.
17. Ebstein, R. P.; Hermoni, M.; Belmaker, R. H.: The effect of lithium on noradrenaline-induced cyclic AMP accumulation in rat brain: Inhibition after chronic treatment and absence of supersensitivity. J. Pharmacol. Exp. Ther. 213:161–167; 1980.
18. Friedman, E.; Wang, H.-Y.: Receptor-mediated activation of G proteins is increased in postmortem brains of bipolar affective disorder subjects. J. Neurochem. 64:1145–1152; 1996.
19. Hamburger-Bar, R.; Robert, M.; Newman, M.; Belmaker, R. H.: Interstrain correlation between behavioral effects of lithium and effects on cortical cyclic AMP. Pharmacol. Biochem. Behav. 24:9–13; 1986.
20. Hsiao, J. K.; Manji, H. K.; Chen, G.; Bitran, J. A.; Risby, E. D.; Potter, W. Z.: Lithium administration modulates platelet Gi in humans. Life Sci. 50:227–233 ;1992.
21. Iyo, M.;, Maeda, Y.; Inada, T; Kitao, Y.; Sasaki, H.; Fukui, S.: The effects of a selective cAMP phosphodiesterase inhibitor, rolipram, on methamphetamine-induced behavior. Neuropsychopharmacology 18:33–39; 1995.
22. Kelley, A. E.; Finn, M.; Cunningham, S. T.; Renshaw, P.; Sachs, G.: Lithium diet enhances the behavioural response to cholera toxin infusion into the nucleus accumbens. Soc. Neurosci. Abstr. 19:130.15; 1993.
23. Kelley, A. E.; Holahan, M.; Finn, M.: Cholera toxin infusion into nucleus accumbens: Interactions with motor activity, dopamine agonists and conditioned reinforcement. Soc. Neurosci. Abstr. 21:168; 1995.
24. Kelly, E.; Willcocks, A. L.; Nahorski, S. R.: Neurochemical and behavioural evidence that dopamine D_2 receptors in striatum couple to Ni regulatory protein and inhibition of cyclic AMP accumulation. Naunyn Schmiedebergs Arch. Pharmacol. 335: 618–623; 1987.
25. Kofman, O.; Belmaker, R. H.: Biochemical, behavioural and clinical studies of the role of inositol in lithium treatment and depression. Biol. Psychiatry 34:839–852; 1993.
26. Kots, A. Y.; Gumanova, N. G.; Akhmedzhanov, N. M.; Varentsov, S. I.; Gerasimova, C. I.; Bulargina, T. V.; Shakhov, Y. A.: The GTP-binding regulatory proteins, Gs and Gi are altered in erythrocyte membranes of patients with ischemic heart disease resulting from coronary atherosclerosis. Arterioscler. Thromb. 13: 1244–1251; 1993.
27. Kozlowski, M. R.; Neve, K. A.; Grisham, J. E.; Marshall, J. F.: Chronic lithium administration alters behavioral recovery from nigrostriatal injury: Effects of neostriatal [^{3}H] spiroperidol binding sites. Brain Res. 267:301–311; 1983.
28. Lesch, K. P.; Aulakh, C. S.; Tolliver, T. J.; Hill, J. L.; Wolozin, B. L.; Murphy, D. L.: Differential effects of long-term lithium and carbamazepine administration on Gs, Gi_1 and Gi_2 mRNA abundance in rat cerebral cortex. Eur. J. Pharmacol. (Mol. Pharmacol.) 207:355–359; 1991.
29. Li, P. P.; Sibony, D.; Green, M. A.; Warsh, J. J.: Lithium modulation of phosphoinositide signaling system in rat cortex: Selective effect on phorbol ester binding. J. Neurochem. 61:1722–1730; 1993.
30. Li, P. P.; Young, T.; Tam, Y. K.; Sibony, D.; Warsh, J. J.: Effects of chronic lithium and carbamazepine treatment on G-protein subunit expression in rat cerebral cortex. Biol. Psychiatry 34:162–170; 1993.
31. Manji, H. K.; Chen, G.; Shimon, H.; Hsiao, J. K.; Potter, W. Z.; Belmaker, R. H.: Guanine nucleotide-binding proteins in bipolar affective disorder—Effects of long term lithium treatment. Arch. Gen. Psychiatry 52:135–144; 1995.
32. Manji, H. K.; Potter, W. Z.; Lenox, R. H.: Signal transduction pathways. Molecular targets for lithium's actions. Arch. Gen. Psychiatry 52:531–543; 1995.
33. Masana, M. I.; Bitran, J. A.; Hsiao, J. K.; Potter, W. Z.: In vivo evidence that lithium inactivates Gi modulation of adenylate cyclase activity in brain. J. Neurochem. 59:200–205; 1992.
34. Miller, R. J.; Kelly, P. H.: Dopamine-like effects of cholera toxin in the central nervous system. Nature 255:163–166; 1975.
35. Miserenido, M. J. D.; Nestler, E. J.: Behavioral sensitization to cocaine: Modulation by the cyclic AMP system in the nucleus accumbens. Brain Res. 674:299–306; 1995.
36. Mørk, A.; Geisler, A.: Mode of action of lithium on the catalytic unit of adenylate cyclase from rat brain. Pharmacol. Toxicol. 60: 241–248; 1987.
37. Mørk, A.; Geisler, A.: Effects of GTP on hormone-stimulated adenylate cyclase activity in cerebral cortex, striatum and hippocampus from rats treated chronically with lithium. Biol. Psychiatry 26:279–288; 1989.
38. Newman, M. E.; Belmaker, R. H.: Effects of lithium in vitro and ex vivo on components of the adenylate cyclase system in membranes from the cerebral cortex of the rat. Neuropharmacology 26:211–217; 1987.
39. Newman, M. E.; Ben-Zeev, A.; Lerer, B.: Chloramphetamine did not prevent the effects of chronic antidepressants on 5-hydroxytryptamine inhibition of forskolin-stimulated adenylate cyclase in rat hippocampus. Eur. J. Pharmacol. 207:209–213; 1991.
40. Ozawa, H.; Rasenick, M. M.: Coupling of the stimulatory GTP-binding protein Gs to rat synaptic membrane adenylate cyclase is enhanced subsequent to chronic antidepressant treatment. Mol. Pharmacol. 36:803–808; 1989.
41. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates. Orlando FL: Academic Press; 1986.
42. Pittman, K. J.; Jakubovic, A.; Fibiger, H. C.: The effects of chronic lithium on behavioral and biochemical indices of dopamine receptor supersensitivity in the rat. Psychopharmacology (Berlin) 82:371–377; 1984.
43. Post, R. M.; Weiss, S. R.; Pert, A.: Differential effects of carbamazepine and lithium on sensitization and kindling. Prog. Neuropharmacol. Biol. Psychiatry 8:425–434; 1984.
44. Risby, E. D.; Hsiao, J. K.; Manji, H. K.; Bitran, J.; Moses, F.; Zhou, D. F.; Potter, W. Z.: The mechanism of action of lithium, II: Effects on adenylate cyclase activity and beta-adrenergic receptor binding in normal subjects. Arch. Gen. Psychiatry 48:513–524; 1991.
45. Sampson, M.; Ruddel, M.; Elin, R. J.: Lithium determinations evaluated in eight analyzers. Clin. Chem. 40:869–872; 1994.
46. Schreiber, G.; Avissar, S.; Danon, A.; Belmaker, R. H.: Hyperfunctional G proteins in mononuclear leukocytes of patients with mania. Biol. Psychiatry 29:273–280; 1991.

47. Skolnick, J.; Daly, J.: Norepinephrine-sensitive adenylate cyclases in rat brains: Relation to behavior and tyrosine hydroxylase. Science 184:174–176; 1974.
48. Smith, D. E.: Effects of lithium and rolipram enantiomers on locomotor activity in inbred mice. Pharmacol. Toxicol. 66:142–145; 1990.
49. Soldin, S. J.: Anticonvulsants. In: Wong, S. H. Y., ed. Therapeutic drug monitoring and toxicology by liquid chromatography. New York: Marcel Dekker; 1985:351–365.
50. Staunton, D. A.; Magistretti, P. J.; Shoemaker, W. J.; Bloom, F. E.: Effects of chronic lithium treatment on dopamine receptors in the corpus striatum II. No effect on denervation or neuroleptic-induced supersensitivity. Brain Res. 232:401–412; 1982.
51. Swerdlow, N. R.; Lee, D.; Koob, G. F.; Vaccarino, F. J.: Effects of chronic dietary lithium on behavioral indices of dopamine denervation supersensitivity in the rat. J. Pharmacol. Exp. Ther. 236: 324–329; 1985.
52. Tamir, A.; Gill, D. M.: ADP-ribosylation by cholera toxin of membrane protein. J. Neurochem. 50:1791–1797; 1988.
53. Wachtel, H.: Neurotropic effects of the optical isomers of the selective adenosine cyclic 3′,5′-monophosphate phosphodiesterase inhibitor rolipram in rats in vivo. J. Pharm. Pharmacol. 35:440–444; 1982.
54. Wang, H. Y.; Friedman, E.: Lithium interferes with coupling of serotonin receptors to their G proteins. ECNP Abstr. 317; 1994.
55. Williams, M. B.; Li, X.; Gu, X.; Jope, R. S.: Modulation of endogenous ADP-ribosylation in rat brain. Brain Res. 592:49–56; 1992.
56. Young, L. T.; Li, P. P.; Kish, S. J.; Siu, K. P.; Kamble, A.; Hornykiewicz, O.; Warsh, J. J.: Cerebral cortex Gsα protein levels and forskolin-stimulated cyclic AMP formation are increased in bipolar affective disorder. J. Neurochem. 61:890–898; 1993.
57. Young L. T.; Li, P. P.; Kamble, A.; Siu, K. P.; Warsh, J. J.: Mononuclear leukocyte levels of G proteins in depressed patients with bipolar affective disorder. Am. J. Psychiatry 151:594–596; 1994.