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Chronic lithium regulates the expression of adenylate cyclase and G_i-protein α subunit in rat cerebral cortex

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ABSTRACT **A possible role for adenylate cyclase and guanine nucleotide-binding proteins (G proteins) in contributing to the chronic actions of lithium on brain function was investigated in rat cerebral cortex. It was found that chronic treatment of rats with lithium (with therapeutically relevant serum levels of ≈1 mM) increased levels of mRNA and protein for the calmodulin-sensitive (type 1) and calmodulin-insensitive (type 2) forms of adenylate cyclase and decreased levels of mRNA and protein for the inhibitory G-protein subunits $G_i\alpha 1$ and $G_i\alpha 2$. Chronic lithium did not alter levels of other G-protein subunits, including $G_o\alpha$, $G_s\alpha$, and $G\beta$. Lithium regulation of adenylate cyclase and $G_i\alpha$ was not seen in response to short-term lithium treatment (with final serum levels of ≈1 mM) or in response to chronic treatment at a lower dose of lithium (with serum levels of ≈0.5 mM). The results suggest that up-regulation of adenylate cyclase and down-regulation of $G_i\alpha$ could represent part of the molecular mechanism by which lithium alters brain function and exerts its clinical actions in the treatment of affective disorders.**

In contrast to most psychotherapeutic agents, which acutely affect neurotransmitter receptor activation, lithium appears to act initially upon postreceptor components of signal-transduction pathways. Lithium acutely inhibits neurotransmitter (hormone)-, forskolin-, and guanine nucleotide-stimulated adenylate cyclase activity in both peripheral tissues and brain (1–3). Acute lithium also inhibits several phosphatases in the phosphatidylinositol cycle and interferes with neurotransmitter-induced phosphatidylinositol hydrolysis in several tissues, including brain (3–5). More recently, Avissar *et al.* (6) have demonstrated that acute lithium attenuates GTP binding by cholera toxin- and pertussis toxin-sensitive G proteins *in vitro* and *in vivo*, an effect that persists with chronic lithium treatment *in vivo*. However, despite numerous studies of the acute actions of lithium, there is relatively little information available concerning the long-term neuronal adaptations that chronic exposure to lithium induces in brain. Identification of such long-term adaptive changes is of particular importance, since the antimanic and antidepressant actions of lithium require its chronic administration.

Past studies of the long-term actions of other types of psychotropic drugs (e.g., antidepressants, antipsychotics, morphine, and cocaine) have demonstrated that these drugs produce chronic adaptations in some of the signal-transduction proteins that are regulated by the drugs acutely (see refs. 7–10). Although few effects of chronic lithium are established, there have been reports that chronic lithium produces a variety of effects on adenylate cyclase activity in brain (1–3). These findings are difficult to interpret mechanistically in that they reflect indirect measures of catalytic activity, which can be affected by many factors. To directly study lithium regulation of adenylate cyclase, we investigated the effects of chronic lithium on adenylate cyclase expression, studies made possible by the recent availability of cDNA clones (11) and monoclonal antibodies (12) to calmodulin-sensitive (type 1) and calmodulin-insensitive (type 2) forms of adenylate cyclase. We also studied lithium regulation of the expression of the heterotrimeric G proteins, which couple diverse types of neurotransmitter receptors to regulation of adenylate cyclase and other intracellular effectors (13). We report that chronic, but not acute, lithium treatment induces a marked increase in levels of mRNA and protein for both the type 1 and type 2 forms of adenylate cyclase and decreases levels of mRNA and protein for the inhibitory G-protein subunits $G_i\alpha 1$ and $G_i\alpha 2$. The possibility that these adaptations contribute to the therapeutic actions of lithium in bipolar and unipolar affective disorders is discussed.

METHODS

Lithium Administration. Male Sprague–Dawley rats (initial weight, 140–160 g) were fed pellets containing 0.24% (wt/wt) lithium carbonate (Teklad, Madison, WI) for either 6 days ("short-term lithium") or 4 weeks ("therapeutic lithium") according to established protocols (14). In some experiments, rats were fed pellets containing 0.17% lithium chloride for 4 weeks ("chronic low-dose lithium"). Serum lithium concentrations were measured by the clinical laboratories of Yale–New Haven Hospital. The average lithium levels from the short-term, therapeutic, and chronic low-dose feeding regimens were 0.98 ± 0.18, 1.1 ± 0.25, and 0.57 ± 0.14 mM, respectively. The 6-day treatment period for the short-term regimen was chosen as the time when serum lithium concentrations have reached steady-state (i.e., therapeutic) levels but when the animal has been exposed to such levels for a short time only. The short-term and chronic low-dose lithium treatments were utilized to assess the time and dose dependence of lithium regulation of adenylate cyclase and G proteins. Control animals were fed the identical diets without added lithium. Normal drinking water and hypertonic saline (1.5%) were available to all rats ad libitum; this has been shown to avoid dehydration in previous investigations (see ref. 14), and there were no signs of dehydration in the current study. Rats that received chronic lithium gained weight at a slower rate than control rats: from an initial weight of 150 g, control and lithium-treated rats weighed, at the end of the 4-week treatment period, 250 and 330 g, respectively. However, this lithium-induced retarded weight gain *per se* cannot account for the observed regulation of adenylate cyclase and

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G proteins, as chronic treatment of rats with a number of other psychotropic drugs (which lead to similar degrees of retarded weight gain) do not produce these effects (refs. 9, 10, and 15 and unpublished observations).

Isolation of RNA and Northern Blotting. At the end of the treatment protocols, cerebral cortex was removed rapidly from decapitated animals. Total RNA was isolated by guanidinium isothiocyanate extraction followed by ultracentrifugation in cesium chloride exactly as described (15). Northern blot analysis of adenylate cyclase type 1, adenylate cyclase type 2, and G-protein subunit mRNA was carried out using 20 μg, 10 μg, and 10 μg, respectively, of total RNA per lane in a 1% agarose gel. RNA in the gels was transferred to nitrocellulose filters, which were then hybridized to cDNA probes (labeled with ^{32}P by the random primer method; Amersham) for 16 hr at 42°C in the presence of 40% (vol/vol) formamide. The cDNA probes used for adenylate cyclase and G proteins are described elsewhere (refs. 11 and 16; R.R.R., unpublished observations). The probes for adenylate cyclase type 1 and type 2 each recognized single bands of approximately 11.5 and 4.1 kilobases (kb), respectively, as found in previous studies (11). The probes for G-protein subunits also recognized specific bands as reported previously ($G_i\alpha1$, 3.5 kb; $G_i\alpha2$, 2.4 kb; $G_i\alpha3$, 3.5 kb; $G_s\alpha$, 1.9 kb; $G_o\alpha$, 4.5 and 4.1 kb; and $G\beta$, 3.0 kb) (15, 16). All Northern blots were subsequently reprobed with a random-prime ^{32}P-labeled cDNA probe for cyclophilin kindly provided by J. G. Sutcliffe, Research Institute of Scripps Clinic (17). Resulting blots were dried and autoradiographed. Levels of adenylate cyclase and G-protein mRNA were quantified by computerized laser densitometry (Pharmacia) of autoradiograms or by Betagen (Waltham, MA) Betascope analysis of the original blots and normalized to cyclophilin mRNA levels by the same instrument.

Immunoblotting. For adenylate cyclase immunolabeling, cerebral cortex was homogenized (20 mg (wet weight) per ml) in ice-cold 20 mM Tris, pH 7.4/1 mM dithiothreitol/1 mM EGTA containing 50 kallikrein-inhibitor units of aprotinin (Sigma) and 10 μg of leupeptin (Sigma) per ml. Homogenates were centrifuged at 10,000 × *g* in a refrigerated microcentrifuge for 10 min and pellets were resuspended in 1% SDS. Protein content was assayed by the method of Lowry *et al.* (18). Aliquots of resuspended pellets (containing 150–300 μg of protein) were subjected to SDS/polyacrylamide gel electrophoresis with 5% acrylamide/0.13% *N,N'*-methylenebisacrylamide in the resolving gels. Proteins were transferred electrophoretically to nitrocellulose filters in the presence of 0.025% SDS, and resulting filters were immunolabeled for adenylate cyclase as described (9). All blotting buffers contained 150 mM NaCl, 20 mM sodium phosphate (pH 7.4), 0.05% Tween (Sigma), and 0.5% (wt/vol) nonfat dry milk. A monoclonal antibody (BBC-2; 1:5000 dilution) prepared against purified adenylate cyclase from bovine brain cerebral cortex (12) and ^{125}I-labeled goat anti-mouse immunoglobulin (1000 cpm/μl; New England Nuclear) were used in these studies. Resulting blots were dried and autoradiographed. These conditions recognized two major bands of about 150 and 130 kDa, plus a smear between the bands. The following lines of evidence support the view that this represented specific labeling of adenylate cyclase: (*i*) the labeling was enriched in membrane fractions compared with crude homogenates and was fully solubilized by 10 mM Lubrol (Sigma) as described previously for adenylate cyclase (12); (*ii*) the labeling was greatly enriched in fractions of neostriatum compared with cortex, as is the case for adenylate cyclase (12, 19); and (*iii*) the pattern of labeling resembled that reported previously for cerebral cortex (12).

For G-protein immunolabeling, crude homogenates of cerebral cortex were adjusted to contain 1% SDS and their protein content was determined. Aliquots (10–50 μg of protein) were subjected to SDS/polyacrylamide gel electrophoresis (with 9% acrylamide/0.24% *N,N'*-methylenebisacrylamide in the resolving gels) and to immunolabeling for G-protein subunits exactly as described (9, 15). Rabbit polyclonal antisera, either purchased from New England Nuclear ($G_i\alpha1/2$) or kindly provided by John Northrup of Yale University ($G\beta$), and ^{125}I-labeled goat anti-rabbit IgG (300 cpm/μl; New England Nuclear) were used in these studies. Levels of G-protein immunoreactivity were quantified by densitometry. These conditions specifically label G-protein subunits ($G_i\alpha1/2$, 40–41 kDa; $G\beta$, 35–36 kDa) and result in linear levels of immunoreactivity over a 5-fold range of tissue concentration (9, 15).

RESULTS

Lithium Regulation of Adenylate Cyclase. In initial experiments, lithium regulation of adenylate cyclase expression was studied by Northern blotting. Rats were treated chronically with lithium under therapeutically relevant conditions—that is, for 4 weeks with final serum levels of ≈1 mM. Such lithium treatment increased mRNA levels for both type 1 and type 2 adenylate cyclase by 50–60% in cerebral cortex (Fig. 1*A*). In contrast, treatment of rats with lithium for 6 days (with final lithium levels of ≈1 mM) or for 4 weeks but at a lower dose (with final lithium levels of ≈0.5 mM) failed to alter mRNA levels of either form of adenylate cyclase, although there was a tendency for the short-term treatment to increase expression of the type 1 enzyme (Fig. 2).

To determine whether lithium regulation of adenylate cyclase mRNA was associated with equivalent regulation of enzyme protein, levels of adenylate cyclase immunoreactivity were studied by immunoblotting. Chronic treatment of rats with lithium under therapeutic conditions produced a dramatic induction of adenylate cyclase immunoreactivity (Fig. 1*B*). The 150-kDa band has been identified in previous studies as a calmodulin-insensitive form of adenylate cyclase and most likely represents the type 2 enzyme. The 130-kDa band could represent the type 1 form of the enzyme, which has been reported to migrate between 115 and 135 kDa (12, 20, 21).

Lithium Regulation of G Proteins. Next, we studied lithium regulation of G-protein expression in cerebral cortex. Administration of lithium under therapeutic conditions (i.e., 4 weeks at ≈1 mM) decreased mRNA levels of $G_i\alpha1$ and $G_i\alpha2$

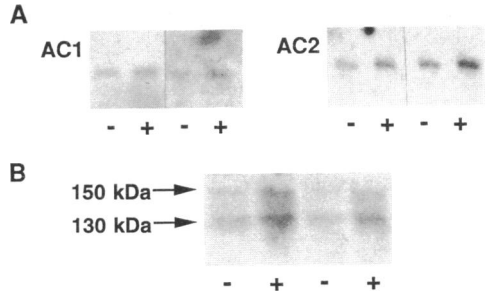

FIG. 1. Autoradiograms illustrating chronic lithium regulation of adenylate cyclase expression in rat cerebral cortex. Rats were treated chronically with lithium under therapeutic conditions, as described in *Methods*. Total RNA or membrane fractions, isolated from cerebral cortex of control (−) and drug-treated (+) animals, were then subjected to Northern blotting (*A*) or immunoblotting (*B*). Levels of mRNA for type 1 (AC1) and type 2 (AC2) adenylate cyclase were, respectively, 159 ± 4% and 150 ± 6% of control [mean ± SEM, $n = 6$; $P < 0.05$ (by χ^2 test) both for AC1 vs. control and for AC2 vs. control]. Adenylate cyclase immunoreactivity was difficult to quantify due to the low levels of the enzyme present in control cortex; however, the effect shown in the figure was consistent among six control and six treated animals.

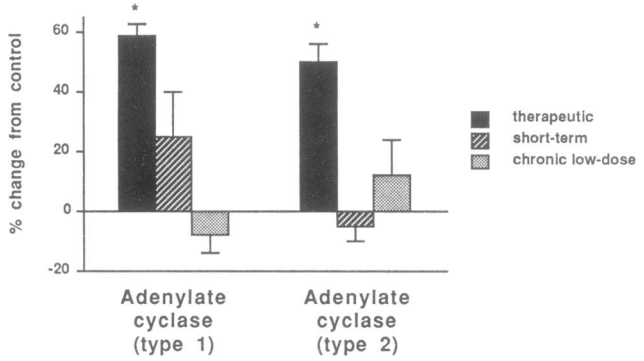

FIG. 2. Time and dose dependence of lithium regulation of adenylate cyclase mRNA. Rats were treated with lithium under three conditions: therapeutic, 4 weeks with final lithium levels of ≈1 mM (solid bars); short-term, 6 days with final lithium levels of ≈1 mM (hatched bars); or chronic low-dose, 4 weeks at a lower dose with final lithium levels of ≈0.5 mM (stippled bars). Total RNA, isolated from cerebral cortex of control and drug-treated animals, was then subjected to Northern blotting for type 1 and type 2 adenylate cyclase. Data are expressed as mean ± SEM and represent the results obtained from 6–12 control and lithium-treated rats. ★, $P < 0.05$ by χ^2 test.

by ≈20% (Fig. 3*A*). In contrast, such lithium treatment did not affect mRNA levels of the other G-protein subunits studied, which included $G_s\alpha$, $G_o\alpha$, and $G\beta$ (Fig. 3*A*). The effect of lithium on $G_i\alpha 3$ could not be assessed due to the very low resting levels of this G-protein subunit in cerebral cortex. Lithium down-regulation of $G_i\alpha$ mRNA was only found under therapeutic conditions; no effects were seen in response to short-term or chronic low-dose lithium treatment (Fig. 4).

To investigate whether chronic lithium-induced decreases in $G_i\alpha 1/2$ mRNA were associated with equivalent changes at the protein level, lithium regulation of $G_i\alpha$ immunoreactivity was investigated. It was found that chronic (therapeutic) lithium administration decreased levels of $G_i\alpha 1/2$ immunoreactivity by nearly 20% but had no effect on levels of G_β immunoreactivity in cerebral cortex (Fig. 3*B*).

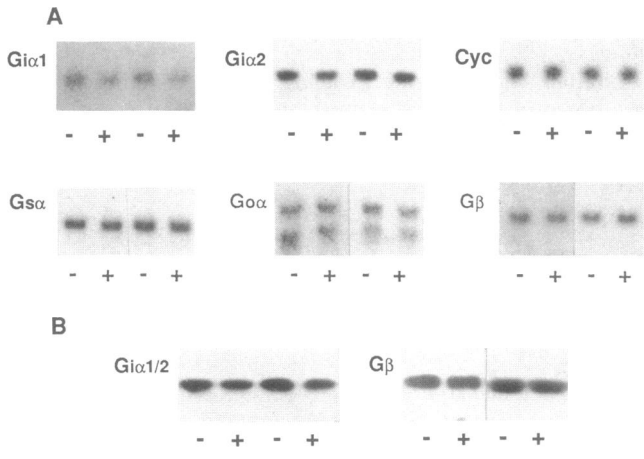

FIG. 3. Autoradiograms illustrating chronic lithium regulation of G-protein expression in rat cerebral cortex. Rats were treated chronically with lithium under therapeutic conditions. Total RNA or crude homogenates, isolated from cerebral cortex of control (−) and drug-treated (+) animals, were then subjected to Northern blotting (*A*) or immunoblotting (*B*). Levels of G-protein mRNA were normalized to levels of cyclophilin mRNA (Cyc), which were not altered by chronic lithium. Levels of G-protein mRNA (mean ± SEM, as % of control) were as follows: $G_i\alpha 1$, 76 ± 5% ($n = 12$; $P < 0.05$); $G_i\alpha 2$, 82 ± 2% ($n = 5$; $P < 0.05$) $G_o\alpha$, 108 ± 9% ($n = 6$); $G_s\alpha$, 103 ± 5% ($n = 6$); $G\beta$, 103 ± 9% ($n = 5$). Levels of G-protein immunoreactivity were as follows: $G_i\alpha 1/2$, 83 ± 3% ($n = 10$; $P < 0.05$); $G\beta$, 102 ± 7% ($n = 5$). *P* values were determined by χ^2 test. Lithium regulation of G-protein mRNA and immunoreactivity as illustrated here was replicated in two separate experiments.

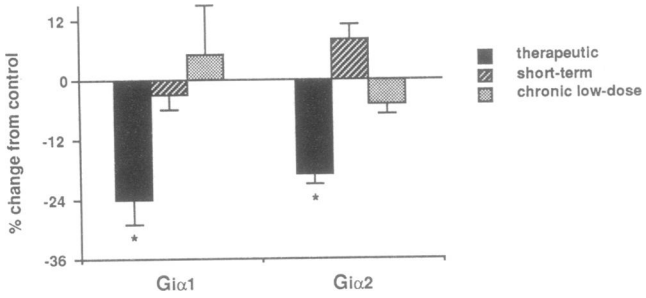

FIG. 4. Time and dose dependence of lithium regulation of $G_i\alpha$ mRNA. Rats were treated with lithium under three conditions, therapeutic, short-term, or chronic low-dose, as described in the legend to Fig. 2. Total RNA, isolated from cerebral cortex of control and drug-treated animals, was then subjected to Northern blotting for $G_i\alpha 1$ and $G_i\alpha 2$. Data are expressed as mean ± SEM and represent the results obtained from 6–12 control and lithium-treated rats. ★, $P < 0.05$ by χ^2 test.

DISCUSSION

The results demonstrate that chronic lithium treatment increases levels of adenylate cyclase mRNA and protein in rat cerebral cortex. Both the calmodulin-sensitive (type 1) and calmodulin-insensitive (type 2) forms of adenylate cyclase were regulated by chronic lithium administration. These effects were not seen with a shorter period of lithium treatment or with chronic treatment at a lower lithium dose. These findings demonstrate that adenylate cyclase expression, like that of other signal-transduction proteins, can be regulated in the nervous system. Moreover, the results indicate that lithium regulation of adenylate cyclase expression may contribute to its clinical efficacy, which also requires chronic exposure to therapeutic concentrations.

Previous reports have focused on inhibition of adenylate cyclase by acute and chronic lithium. Acute lithium is thought to inhibit adenylate cyclase activity at least in part through a direct action on the catalytic unit of the enzyme. One possibility is that lithium competes for Mg^{2+}-binding sites on the enzyme, which are required for its catalytic activity (1, 3, 22). Studies on chronic lithium have reported decreased levels of neurotransmitter-, guanine nucleotide-, and forskolin-stimulated adenylate cyclase activity in membranes or slices of cerebral cortex (1, 2). These inhibitory effects appear to represent persistence of the acute inhibitory actions of lithium on adenylate cyclase. However, it was also noted in these studies that chronic lithium *increased* basal levels of cAMP production ≈2-fold in both membranes and slices of cerebral cortex (1, 2), and we have corroborated these observations by demonstrating that, under our treatment conditions, chronic lithium increases basal levels of adenylate cyclase activity in cerebral cortical membranes (unpublished observations). Increased levels of adenylate cyclase mRNA and protein reported here are consistent with these observations and indicate that increased basal levels of cAMP production and of adenylate cyclase catalytic activity could be due to an increase in enzyme expression. In this context, up-regulation of adenylate cyclase expression by chronic lithium can be viewed as a compensatory, homeostatic response to persistent acute inhibition of the enzyme. The increases observed in adenylate cyclase expression and basal activity appear to predominate over the persistent acute inhibition of the enzyme in the chronic lithium-treated state: recent findings indicate that chronic lithium produces a >2-fold increase in basal extracellular levels of cAMP in cerebral cortex as measured by *in vivo* microdialysis (23).

Previously, lithium has also been shown to interfere with G-protein function acutely (6). Acute incubation of isolated cerebral cortical membranes with lithium *in vitro* decreases GTP-binding by pertussis toxin- and cholera toxin-sensitive G-proteins. This effect is also seen after acute *in vivo* administration of lithium and persists after chronic lithium treatment (6). Lithium inhibition of GTP binding may be associated with an attenuation in the ability of neurotransmitters to regulate adenylate cyclase activity. In the present study, we have demonstrated that chronic lithium decreases $G_i\alpha$ expression in cerebral cortex, with no effect observed on other G-protein subunits. This decreased expression of $G_i\alpha$ represents an additional action of lithium on G proteins, as this effect was observed in response to chronic lithium only. The observed decrease in levels of $G_i\alpha$, without a change in $G_s\alpha$ or $G\beta$, would be expected to decrease the ability of inhibitory neurotransmitters to regulate adenylate cyclase activity. This action, along with increased adenylate cyclase expression, would produce a concerted, and possibly synergistic, up-regulation of the adenylate cyclase system in the chronic lithium-treated state.

Although the effect of chronic lithium on $G_i\alpha$ expression was relatively small (about 20%), a reduction of this magnitude would be expected to exert significant functional consequences. It has been shown that inhibition of $G_i\alpha/G_o\alpha$ by 10–15% (by pertussis toxin administration) decreases by 40–50% the ability of neurotransmitters to produce their electrophysiological effects on specific neuronal cell types, whereas inhibition of the G proteins by 40–50% leads to an almost complete blockade of the electrophysiological responses (24).

Lithium regulation of adenylate cyclase and $G_i\alpha$ mRNA and protein is consistent with the possibility that such regulation occurs at the level of gene expression, although alternative mechanisms, such as regulation of mRNA stability or translation, cannot be ruled out. As adenylate cyclase and G proteins are regulated by lithium acutely, it will be interesting to determine whether other acute targets of lithium (for example, components of the phosphatidylinositol system) are also regulated by chronic lithium treatment. The results of the present study indicate that some of the chronic effects of lithium on brain function may be mediated by alterations in adenylate cyclase and G-protein expression and support the view that through the study of long-term lithium regulation of signal-transduction proteins a more complete understanding will emerge concerning the mechanisms by which lithium produces its clinical antimanic and antidepressant actions.

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