The effect of lithium on the adrenoceptor-mediated second messenger system in the rat brain

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Objective: Lithium remains the most widely used treatment for bipolar disorder; however, the molecular mechanisms underlying its therapeutic actions have not been fully elucidated. We studied the in-vivo effect of lithium on the density of α-adrenoceptor (α-AR) and β-AR subtypes and linked second messenger systems in the rat brain. Methods: The densities of α-ARs, α1-ARs, and β-ARs and β2-ARs in the cortex and cerebellum of rats treated with lithium (0.4%), orally, for 30 days were measured using [3H]prazosin, [3H]clonidine and [3H]CGP-12177, respectively. The activity of adenylyl cyclase (AC) and levels of inositol trisphosphate (IP3), both second messengers linked to these receptors, were estimated using [3H]ATP and [3H]myoinositol, respectively. Results: A significant decrease in the densities of cortical α-ARs (85%, p < 0.0001), α2-ARs (50%, p < 0.0001), β1-ARs (26%, p < 0.0001) and β2-ARs (25%, p < 0.0001) was observed after lithium treatment. However, only the density of α-ARs was significantly decreased (25%, p < 0.0001) in the cerebellum. The affinity of [3H]prazosin for cerebellar α1-ARs was increased. A small, but statistically significant, increase (19%, p < 0.0001) in the density of total β-ARs was seen in the cerebellum, without altering the affinity of the radioligand for these receptors. Basal AC activity was not altered in the lithium-treated rat cortex. However, the norepinephrine-stimulated AC activity, which represents α1-AR-linked and β2-AR-linked AC, was significantly increased (66%, p < 0.0001). Both basal IP3 formation and norepinephrine-stimulated IP3, which represents α1-AR-linked phospholipase C activity, were significantly decreased (50%, p < 0.0001) in the lithium-treated rat cortex. Conclusion: Our results suggest that long-term administration of lithium treatment downregulates the cortical, but not cerebellar, α-ARs, α1-ARs, β1-ARs and β2-ARs. Thus, it may be concluded that lithium induces region-specific and differential functional downregulation of α-AR and β-AR subtypes in the rat brain.

Objective : Le lithium demeure le traitement le plus répandu du trouble bipolaire, mais on n’a pas éluclidé entièrement les mécanismes moléculaires qui en sous-tendent les effets thérapeutiques. Nous avons étudié l’effet in vivo du lithium sur la densité des sous-types α-adrénorecepteur (α-AR) et β-AR et des systèmes de second messager reliés dans le cerveau du rat. Méthodes : On a mesuré les densités des récepteurs α-AR, α1-AR, β1-AR et β2-AR dans le cortex et le cervelet de rats traités au lithium (0,4 %) par voie orale pendant 30 jours en utilisant la [3H]prazosine, la [3H]clonidine et le [3H]CGP-12177, respectivement. On a estimé l’activité de l’adényly cyclase (AC) et les concentrations d’inositol trisphosphate (IP3), tous deux seconds messagers reliés à ces récepteurs, en utilisant [3H]ATP et [3H]myoinositol respectivement. Résultats : On a observé, après le traitement au lithium, une diminution importante des densités d’α-AR (85 %, p < 0.0001), d’α2-AR (50 %, p < 0.0001), de β1-AR (26 %, p < 0.0001) et de β2-AR (25 %, p < 0.0001) dans le cervelet. Dans le cervelet, toutefois, seule la densité de α-AR a diminué considérablement (25 %, p < 0.0001). L’affinité de la [3H]prazosine pour les α1-AR du cervelet a augmenté. On a constaté une augmentation modeste mais statistiquement significative (19 %, p < 0.0001) de la densité du total des β-AR dans le cervelet sans qu’il y ait modification de l’affinité des radioligands pour ces récepteurs. L’activité basale de l’AC n’a pas changé dans le cortex des rats traités au lithium. L’activité de l’AC stimulée par la norépinéphrine, qui représente l’AC reliée aux récepteurs α-AR et au β2-AR, a toutefois augmenté considérablement (66 %, p < 0.0001). La formation d’IP3 basal et d’IP3 stimulé par la norépinéphrine, qui représente l’activité de la phospholipase C reliée aux α-AR, a toutefois diminué considérablement (50 %, p < 0.0001).
Introduction

Adrenoceptors (ARs) are members of the family of 7-transmembrane-domain guanine nucleotide protein–coupled receptors (GPCRs) and are a significant pharmacological target in clinical medicine. ARs have been classified into several specific subtypes on the basis of pharmacological distinctions. The ARs were first classified into \( \alpha \) and \( \beta \) subtypes\(^1\) and later into \( \alpha_1 \)-ARs and \( \alpha_2 \)-ARs\(^2\) and \( \beta_1 \)-ARs and \( \beta_2 \)-ARs.\(^3\) Noradrenaline (NE) at physiological concentrations primarily binds to \( \alpha \)-ARs, whereas epinephrine binds to and activates both \( \alpha \)-ARs and \( \beta \)-ARs.\(^4\) \( \alpha \)-ARs couple predominantly with the pertussis-toxin-insensitive G-protein \( G_q \), resulting in the hydrolysis of membrane phospholipids; subsequent activation of phospholipase C-b (PLC-b) generates the major second messengers inositol (1,4,5)-trisphosphate (IP\( _3 \)) and diacylglycerol.\(^4\) Activation of \( \alpha \)-ARs by agonists inhibits adenylyl cyclase (AC), resulting in a decrease in the cyclic adenosine monophosphate (cAMP) content of the cell. \( \beta \)-ARs are pharmacologically classified into \( \beta_1 \), \( \beta_2 \), \( \beta_3 \) and \( \beta_4 \)-subtypes, and activation of \( \beta \)-ARs will activate AC and increase the cAMP content of the cell. The \( \alpha \)-ARs and \( \beta \)-ARs have been well characterized pharmacologically and, more recently, the use of molecular biology techniques has provided new information about the structure and functional domains of these receptors.

The central noradrenergic system is implicated in the hypothesised origin of depression, and the known therapeutic action of antidepressants is ascribed to their inhibitory action at presynaptic transporters of serotonin (5-hydroxytryptamine [5-HT]) and NE.\(^5\) Lithium remains the most widely used treatment for bipolar disorder, and this monovalent cation represents one of psychiatry’s most important treatments for more than 50 years. Manji et al\(^6\) have reviewed progress in the identification of key components of signal transduction pathways as targets for lithium action and have suggested that regulation of signal transduction in regions of the brain by lithium affects the function of multiple neurotransmitter systems and may thus explain lithium’s efficacy in manic–depressive psychosis.

Growing evidence suggests dysfunction of \( \alpha \)-ARs in depression.\(^7\) A significant decrease in the density of \( \alpha \)-ARs is reported in the cortex and hippocampus of people who have committed suicide and have a retrospective diagnosis of depression.\(^8\) In contrast, increased \( \alpha \)-AR–agonist binding in the cortex and hippocampus of depressed individuals who have committed suicide has also been reported.\(^9\) There is evidence for dysregulation of the locus coeruleus (LC)-NE system in depression. It has been suggested that long-term activation of the LC, resulting in depletion of synaptic NE concentrations and compensatory changes in ARs, is the primary cause of suicide attempts in depressed patients.\(^{10}\) Downregulation of the central adrenergic system as the principal action of antidepressant treatment has been suggested. A significant reduction in the density of \( \alpha_2 \)-ARs in the rat brain\(^{11,12}\) and also in platelets of depressed patients\(^{13}\) has been reported with long-term treatment with antidepressants, thus suggesting that \( \alpha \)-AR antagonists are good antidepressant agents. Long-term treatment with antidepressants has been found to reduce \( \alpha \)-adrenergic sensitivity, while enhancing responses to serotonergic and \( \alpha \)-adrenergic stimulation.

Despite the demonstrated efficacy of lithium in reducing both the frequency and severity of recurrent affective episodes and after decades of clinical use, the molecular mechanisms underlying its therapeutic actions have not been fully elucidated. One of the most established mechanisms of lithium action is the modulation of phosphoinositide hydrolysis, by inhibiting the enzyme inositol phosphomonoesterase.\(^{14}\) The involvement of the 5-HT\(_2\)-receptor–mediated\(^{15}\) and the 5-HT\(_1\)-receptor–mediated\(^{16}\) second messenger system in the mechanism of action of lithium has also been suggested. Alterations in the \( \alpha \)-AR-mediated system by various antidepressants have been reported.\(^{17}\) Previous studies have reported downregulation of \( \beta \)-ARs and 5-HT receptors and subsensitization of \( \beta \)-AR-coupled AC activity in the rat cortex after lithium treatment.\(^{18}\) However, the effect of lithium on the AR-mediated second messenger system has not been studied so extensively. We report the effect of oral lithium administration on the \( \alpha \)-AR-linked and \( \beta \)-AR-linked second messengers in regions of the rat brain.

Methods

This study was approved by our institute’s Animal Ethics Committee (IAEC). Adult male Sprague–Dawley rats, weighing 200–250 g, were used for all the experiments. Animals (\( n = 18 \)) were housed in cages (3–4 rats per cage) and exposed to a regular day–night period with free access to food and water. Li\( _2\)CO\( _3 \) (0.1%) was administered orally (0.5 mL) once daily in the morning for 30 days. Control rats received 0.5 mL of saline by the same route for the same period. Animals were weighed before and after exposure. The weight gain (9%) after lithium exposure was lower in experimental animals than in control rats (16%). Blood levels of lithium were measured by an Ion Selective Electrolyte Analyzer (Medica, Bedford, Mass.) with a lithium electrode. Levels of 0.6–0.8 mEq/L were obtained within 4 weeks of treatment. All the animals were killed by decapitation 24 hours after the last dose. Brains were removed on an ice-cold petri dish; the cerebral cortex and cerebellum.
were taken out and used for membrane preparation. Tissues obtained from 3 rats were pooled for receptor-binding assay, each assay being done in duplicate. A total of 3 experiments were carried out for each region, thus obtaining 6 data points for each receptor-binding study.

Crude membrane pellet was obtained from brain tissue, homogenized in 20 volumes of Tris-HCl buffer (50 mmol/L, pH 7.4) containing 0.32 mol/L sucrose, following the procedure described by Creese and Snyder\(^{23}\) and as described earlier.\(^{17}\) The pellet was resuspended in 50 mmol/L sodium potassium phosphate buffer (pH 7.4) for \[^{3}H\]prazosin binding, in 20 mmol/L sodium HEPES buffer (pH 7.4) for \[^{3}H\]clonidine binding and in Tris assay buffer (50 mmol/L, pH 7.5) containing 2.5 mmol/L MgCl\(_2\), 0.5 mmol/L ascorbate and 150 mmol/L NaCl for \[^{3}H\]CGP-12177 binding. The protein concentration was estimated using the method described by Lowry et al\(^{24}\) and made up to 1 mg/mL using respective buffers.

Membranes obtained from the cortex and cerebellum of both experimental and control rat brains were used for binding studies. The density of \(\beta\)-ARs was estimated by using \[^{3}H\]prazosin (0.05–0.6 nmol/L; specific activity [s.a.] 27 Ci/mmol [1 Ci = 3.7 \times 10^{10} \text{ Bq}], Amersham, Little Chalfont, Bucks, UK), essentially following the procedure described by Hyttel et al\(^{21}\) and as described in detail earlier.\(^{17}\) Nonspecific binding was defined by using phentolamine (10 μmol/L). The density of \(\alpha\)-ARs was estimated only in the cortex by using \[^{3}H\]clonidine (0.06–0.80 nmol/L; s.a. 74.6 Ci/mmol, Amersham), essentially following the procedure described by Kalaria and Harik\(^{22}\) and as described in detail earlier.\(^{17}\) Nonspecific binding was defined by using 1.0 μmol/L clonidine.

The total \(\beta\)-ARs were labelled using \[^{3}H\]CGP-12177 (s.a. 55.0 Ci/mmol, Amersham) (\(\beta\)-AR antagonist), essentially following the method described by Kalaria et al\(^{25}\) with minor modifications. For the binding studies, an aliquot of membrane proteins obtained from the cortex (250 μg) and cerebellum (200 μg) was incubated with different concentrations of \[^{3}H\]CGP-12177 (0.08–0.90 nmol/L for cortex and 0.30–2.0 nmol/L for cerebellum) in the presence and absence of 10 μmol/L propranolol (\(\beta\)-AR antagonist) to determine nonspecific binding. The reaction volume was made up to 500 μL with Tris assay buffer (50 mmol/L, pH 7.5) and incubated at 37°C for 30 minutes. Clenbuterol (β-AR agonist), 10 μmol/L, was used in the assay to block β-ARs and estimate the density of β-ARs. Atenolol (β-AR antagonist), 10 μmol/L, was used in the assay to block β-ARs and estimate the density of β-ARs.

The reactions for all the binding experiments were stopped by the addition of ice-cold respective assay buffers, and the reaction mixtures were rapidly filtered through GF/B filters under vacuum. The filters were transferred to vials containing scintillation fluid, 5 mL, and allowed to equilibrate overnight. Radioactivity was measured in a liquid scintillation counter (Tri-Carb 2001TR, Packard, US) at 65% efficiency.

Both basal AC activity and AC activity stimulated by NE (10 μmol/L) were estimated in homogenates obtained from the cortex of both control and experimental rats using \[^{3}H\]ATP (s.a. 43.0 Ci/mmol, Amersham), following the procedure described by Malnoe et al\(^{26}\) and described in detail earlier.\(^{15}\) AC activity was expressed as picomoles of \[^{3}H\]cAMP formed per milligram of protein.

IP3 levels were estimated using \[^{3}H\]myoinositol (s.a. 16.5 Ci/mmol, Amersham), following the procedure described by Kendall and Naharoski\(^{27}\) and as described in detail earlier.\(^{28}\) The radioactivity of \[^{3}H\]IP3 formed was measured in a liquid scintillation counter at 65% efficiency. The levels of IP3 were estimated as picomoles of \[^{3}H\]IP3 formed per milligram of protein.

The data from the binding experiments were analyzed using the LIGAND software program.\(^{25}\) Scatchard graphs were constructed, and maximal binding (Bmax) of \[^{3}H\]prazosin, \[^{3}H\]clonidine and \[^{3}H\]CGP-12177 and their affinity (Kd) were determined by linear regression. The Bmax and Kd values were expressed in femtomoles per milligram of protein and nanomoles per litre, respectively. All the data are expressed as means (standard deviation [SD]). The statistical analysis was done using the Student's t test. Differences were considered to be significant at \(p < 0.05\).

**Results**

The density of cortical \(\alpha\)-ARs was significantly decreased (85%; \(t_{10} = 40.8, p < 0.0001\)) when compared with the density in the control cortex (mean 73.2 [standard deviation [SD] 3.3] fmol/mg protein), without any change in Kd values (Table 1, Fig. 1A). However, the density of cerebellar \(\alpha\)-ARs was decreased only by 25% (50.7 [SD 4.0] fmol/mg protein; \(t_{10} = 7.19, p < 0.0001\)) when compared with the density in control rat cerebellum (mean 66.7 [SD 3.7] fmol/mg protein). Interestingly, the affinity of \[^{3}H\]prazosin for \(\alpha\)-ARs in the cerebellum was increased, as seen by the decrease in Kd values (mean 0.08 [SD 0.01] nmol/L; \(t_{10} = 14.3, p < 0.0001\)).

The density of \(\alpha\)-ARs was estimated only in the cortex of control and lithium-treated rat brain, because the binding of \[^{3}H\]clonidine, in the concentrations used, did not give substantial binding in cerebellum tissue. After lithium treatment, there was a significant decrease in the density of cortical \(\alpha\)-ARs in the cortex (50%; \(t_{10} = 22.3, p < 0.0001\)) when compared with control levels. However, there was no significant change in the affinity of \[^{3}H\]clonidine for \(\alpha\)-ARs (Table 1, Fig. 1B).

The oral administration of lithium significantly decreased the density of cortical total \(\beta\)-ARs (46%; \(t_{10} = 18.8, p < 0.0001\)) when compared with control values. However, there was no significant change in the affinity of \[^{3}H\]CGP for these receptors after lithium treatment (Table 1, Fig. 2).

When differentiated pharmacologically, the density of \(\beta\) and \(\beta\)-ARs was equally decreased after lithium treatment. The mean density of cortical \(\beta\)-ARs was decreased by 26% (18.8 [SD 2.5] fmol/mg protein; \(t_{10} = 5.92, p < 0.0001\)) and the mean density of \(\beta\)-ARs was decreased by 25% (14.4 [SD 1.0] fmol/mg protein; \(t_{10} = 6.83, p < 0.0001\)) when compared with the density in control cortex. However, no significant change in the affinities was observed (Table 1). The ratio of \(\beta\)-ARs to \(\beta\)-ARs in cortex was found to be 1:31 (control = 1:33) after lithium treatment, suggesting that downregulation in both \(\beta\)-
ARs and β2-ARs contributes equally to the decrease in total β-ARs.

Interestingly, in the cerebellum, a small, but statistically significant, increase in the density of total β-ARs (19%; $t_{19} = -6.0, p < 0.0001$) was observed after lithium treatment without change in the affinity of [3H]CGP for β-ARs. There was, however, a change (or increase) in the mean density of β2-ARs (8.4 [SD 1.2] fmol/mg protein; $t_{19} = -3.96, p = 0.003$) but not in the mean density of β1-ARs (20.2 [SD 3.2] fmol/mg protein; $t_{19} = -1.1, p = 0.36$) in this region when compared with control values (Table 1). The affinity of [3H]CGP for both subtypes was also not altered after lithium treatment.

The basal AC activity in the cortex of lithium-treated rats (mean 4.22 [SD 0.53] pmol/mg protein) was not significantly different from the levels in control rat cortex (mean 3.76 [SD 0.43] pmol/mg protein; $t = -3.96, p = 0.003$) but not in the mean density of β2-ARs (20.2 [SD 3.2] fmol/mg protein; $t = -1.1, p = 0.36$) in this region when compared with control values (Table 1). The affinity of [3H]CGP for both subtypes was also not altered after lithium treatment.

Table 1: In-vivo effect of lithium on α1-adrenoceptors and β-adrenoceptors in regions of the rat brain

<table>
<thead>
<tr>
<th>ARs; rat group</th>
<th>Brain region; density (Bmax) and affinity (Kd); mean (and standard deviation)</th>
<th>Cortex</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-ARs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>73.2 (3.3)</td>
<td>0.10 (0.02)</td>
</tr>
<tr>
<td>Lithium</td>
<td></td>
<td>10.6 (1.8)†</td>
<td>0.10 (0.01)</td>
</tr>
<tr>
<td>$t = 40.8$</td>
<td></td>
<td>(p = 1.00)</td>
<td>7.19</td>
</tr>
<tr>
<td>β1-ARs</td>
<td></td>
<td>48.3 (2.0)</td>
<td>0.35 (0.02)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>23.8 (1.8)†</td>
<td>0.32 (0.02)</td>
</tr>
<tr>
<td>$t = 22.3$</td>
<td></td>
<td>(p = 0.03)</td>
<td></td>
</tr>
<tr>
<td>Total β-ARs</td>
<td></td>
<td>61.5 (1.6)</td>
<td>0.19 (0.02)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>33.4 (3.3)†</td>
<td>0.19 (0.03)</td>
</tr>
<tr>
<td>$t = 18.8$</td>
<td></td>
<td>(p = 1.00)</td>
<td>-6.0</td>
</tr>
<tr>
<td>β2-ARs</td>
<td></td>
<td>25.5 (1.2)</td>
<td>0.01 (0.02)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>18.8 (2.5)†</td>
<td>0.09 (0.02)</td>
</tr>
<tr>
<td>$t = 5.92$</td>
<td></td>
<td>(p = 0.41)</td>
<td>-3.96</td>
</tr>
<tr>
<td>β2-ARs</td>
<td></td>
<td>19.2 (1.4)</td>
<td>0.08 (0.02)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>14.4 (1.0)†</td>
<td>0.10 (0.01)</td>
</tr>
<tr>
<td>$t = 6.83$</td>
<td></td>
<td>(p = 0.053)</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

AR = adrenoceptor; ND = not detected; NS = nonsignificant.

The densities of α1-, α2-, and β1- and β2-adrenoceptors were estimated using [3H]prazosin, [3H]clonidine and [3H]CGP:12177, respectively, as described in the Methods section, in the cortex and cerebellum of rats exposed to lithium for 30 days and in control rats. Values are the mean and standard deviation of 3 experiments, each assayed in duplicate. Degrees of freedom for each parameter are 10.

Discussion

Despite lack of knowledge of its exact mode of action, lithium has become a widely accepted antimanic drug for the last 4 decades in curing the symptoms of manic-depressive psychosis. The therapeutic effect of lithium on patients with mania appears within 3–5 days. However, the long-term administration of lithium is necessary for the prophylactic effect on recurrent manic-depressive psychosis. A significant loss of noradrenergic innervations has been shown to cause behavioural manifestations.26 It seems possible that such a condition also exists in manic-depressive psychosis and that lithium treatment reduces the symptoms by altering various neurotransmitters, particularly the monoaminergic system. Lithium has been shown to affect serotonergic and nonserotonergic receptors. The differential effect of lithium on adrenergic receptor-binding and AC activity has been reported, suggesting that these effects are specific with respect to tissue and brain regions.31 However, it is not clear whether the action of lithium on these receptors is primary or secondary and whether it is related to the clinical effects. Central postsynaptic α1-ARs and β2-ARs are also involved in the action of antidepressants.32 However, the lack of increase in the density of α1-ARs after repeated administration of antidepressants was also demonstrated.33 Significantly elevated Bmax for high-affinity α1-ARs was reported in platelets of depressive patients when compared with controls, suggesting dysfunction of α1-ARs in depression, which is corrected by lithium treatment.34 These findings suggest that the modulation of α1-ARs following antidepressant treatment is not a general phenomenon and that the effect may be dose and duration dependent. In the current study, a significant (85%) downregulation of only cortical α1-ARs was observed after lithium treatment. When compared with this, the downregulation in the cerebellum was only 25%. This reduced downregulation in the cerebellum can be attributed to the fact that the affinity of [3H]prazosin for α1-ARs was increased after lithium treatment, as seen from decreased Kd values. The change in affinity shows that the direct binding of lithium to the cerebellar α1-ARs is decreased, reflecting a decreased downregulation.

Initial studies of both α1- and β2-ARs have demonstrated no change in the density of these receptors in the cerebellum.35 Similarly, no changes in the α1-ARs have been found with age in brain regions including the cerebellum and brain stem.34 However, significant binding for α1-ARs has been re-
ported in the cerebellum of healthy rats when compared with reduced binding in agranular weaver cerebellum. mRNA localization of \(\alpha\)-AR subtypes has revealed that mRNA predominates in the cerebellum and cortex in humans, in contrast to its restricted distribution in rats. Significant expression of \(\alpha\)-AR subtypes has also been reported in the cerebellum along with the cortex and hippocampus in the rat brain. The highest level of expression of all the 3 \(\alpha\)-AR subtypes has also been reported in the cerebellum and striatum.

Our experiments with the sensitivities of \(\alpha\)-ARs to inactivation by chloroethyl clonidine (CEC) in rat brain regions showed the highest proportions of CEC-sensitive sites in the cerebellum and cortex (unpublished data). Wilson and Minneman have also reported similar findings. Various studies of the effect of \(\alpha\)-ARs agonists and antagonists on the release of various neurotransmitters in the brain have used the cerebellum.

On the basis of our previous findings and the reported literature on the regional distribution of adrenergic receptors in the rat brain and the differential sensitivity for various agonists and antagonists, we studied the effect of lithium on \(\alpha\)-ARs in the cerebellum as well as the cortex.

\(\alpha\)-ARs are linked positively to PLC, and downregulation of these receptors will lead to a decrease in PLC activity, thus decreasing the IP3 levels. In this study, both basal and NE-stimulated IP3 formation were studied in cortical tissues. It was observed that after lithium treatment, concomitant with the downregulation in \(\alpha\)-ARs, both the basal and NE-stimulated IP3 formation were significantly decreased (50%) compared with the control levels. Trejo et al. in their study, have suggested that NE-induced inositol phosphate formation in brain slices is mainly mediated by the activation of \(\alpha\)-ARs.

A significant downregulation of cortical \(\alpha\)-ARs was observed in the experimental group. The effect of lithium on \(\alpha\)-ARs was significantly greater than the effect on \(\alpha\)-ARs. Downregulation of \(\alpha\)-ARs leads to an increase in cAMP accumulation because of the activation of AC that is negatively linked to these receptors. In this study, NE-stimulated, but not the basal, AC activity was significantly increased in the cortex after lithium treatment. Increased 5-HT-stimulated AC activity with a decrease in 5-HT, receptor density after lithium treatment has been reported. Lithium has been shown to enhance presynaptic 5-HT transmission and interact with intracellular second messenger systems coupled to these receptors.

Apart from estimating the total \(\beta\)-ARs, these receptors were differentiated into \(\beta_1\)-ARs and \(\beta_2\)-ARs by using specific blockers, and the density of the subtypes was measured in both control and lithium-treated rats. We observed that the downregulation in total \(\beta\)-ARs was contributed to equally by the downregulation of \(\beta_1\)-ARs and \(\beta_2\)-ARs. When compared with the extent of downregulation (46%) in total \(\beta\)-ARs, there was a concomitant increase in NE-stimulated AC activity as seen from an increase in cAMP formation in the lithium-treated cortex. Lithium at a concentration that exerts prophylactic effects in affective disorders is known to alter NE turnover and the \(\beta\)-AR-dependent cAMP accumulation. It has been suggested that long-term lithium treatment induces

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**Fig. 1:** Scatchard graph of \([^{3}H]\)prazosin (A) and \([^{3}H]\)clonidine (B) binding to \(\alpha\)-adrenoceptors (\(\alpha\)-ARs) and \(\alpha\)-ARs, respectively, in the cortex of rats treated with lithium (Li) (0.4%), orally (0.5 mL), for 30 days. Binding experiments were done using \([^{3}H]\)prazosin (0.05–0.6 nmol/L) and \([^{3}H]\)clonidine (0.06–0.8 nmol/L) in cortical membranes, as described in the Methods section. Data points are the means of 3 experiments, each assayed in duplicate. The inset represents saturation curves for both binding experiments. Data points are the means (and standard deviation) of 3 experiments, each assayed in duplicate.
sub sensi tiv ity in the β-AR–AC system, for which downregulation of β-ARs is chiefly responsible. Studies have also shown a region-specific alteration of G-protein-induced activation of the phosphoinositide signal transduction system and G-protein γ-subunits that are involved in cAMP formation in the prefrontal cortex of individuals with major depression who committed suicide.

In the present study, the in-vivo effect of long-term administration of lithium on α-AR and β-AR subtypes was assessed to explore the involvement of these receptors in the mechanism of action of lithium. The results showed a significant downregulation of cortical α-AR and β-AR subtypes without any significant downregulation of these receptors in the cerebellum. The effect of lithium was more pronounced on cortical α-ARs than on β-ARs. However, the effect is equally distributed between β1- and β2- subtypes of cortical β-ARs. The increased affinity of the radioligands for cerebellar receptors after lithium treatment might be the result of decreased direct interaction of lithium with these receptors. However, in the cortex there was no change in affinity after lithium treatment. It is evident from the present study that cortical α-ARs and β-ARs, with equal involvement of β1- and β2- subtypes, are involved in the therapeutic action of lithium, regardless of other mechanisms. Thus, one mode of action of lithium may be attenuation of the cellular response by downregulating adrenergic receptor subtypes, as seen in our study. Therefore, it is possible to postulate that long-term treatment with lithium, which contributes to the prophylactic effect seen in manic–depressive psychosis, is mediated by cortical α-ARs and β-ARs.

Lithium is considered to be the first-choice mood stabilizer in recurrent mood disorders. In spite of this fact, patients have been observed to show a variable response to lithium treatment. In some cases, it is completely effective in the prevention of manic or depressive relapses, whereas in other cases it appears to show no influence on the disease course. As lithium therapy needs at least 6 months to be effective in stabilizing mood disorders, it is necessary to study the alterations in receptor-mediated signal transduction, especially adrenergic receptors, as a possible mechanism for its efficacy. Subsequent to the monoamine hypothesis of depression, it has become evident that it is not the increase in intrasynaptic monoamine availability that accounts for the effectiveness of antidepressants but, rather, the changes observed in ARs in specific regions. The possible definition of a genetic liability profile for the efficacy of lithium therapy has also been suggested, apart from its association with noradrenergic and serotonergic neurotransmission and IP3 metabolism. Thus, even if some positive results have been reported with these parameters, no unequivocal candidate for lithium efficacy has been identified. Although the data from our experiments may not currently allow a meaningful prediction of lithium response and the regional specificity of its efficacy, further research aimed at the development of individualized treatment of mood disorders, including the possibility of subtype-specific and region-specific α-AR agonists and/or antagonists, is a possibility.

It could be concluded from this study that lithium induces region-specific and differential functional downregulation of α-AR and β-AR subtypes in the rat brain. Reports have also demonstrated significant effects of lithium on the regulation of gene expression in the central nervous system, effects that may play a major role in the long-term stabilization of mood.

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**Table 2: In-vivo effect of lithium on adenylyl cyclase (AC) activity and IP3 levels in the cortex of the rat brain**

<table>
<thead>
<tr>
<th>Effect; rat group; t value</th>
<th>Group; mean (and standard deviation)</th>
<th>Stimulation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC activity*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.76 (0.43)</td>
<td>5.05 (0.18)</td>
</tr>
<tr>
<td>Lithium</td>
<td>4.22 (0.53)</td>
<td>8.42 (0.26)†</td>
</tr>
<tr>
<td>t = 0.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| IP3 levels*               |                                        |                |
| Control                   | 0.33 (0.05)                            | 0.83 (0.08)    | 150 |
| Lithium                   | 0.17 (0.05)†                           | 0.44 (0.07)†   | 160 |
| t = 0.89                  |                                        |                |

IP3 = inositol (1,4,5)-trisphosphate; NE = norepinephrine.
*AC activity and [3H]IP3 formation were estimated in the cortex of control and lithium-treated (0.4% Li2CO3, orally) rats, as described in the Methods section. The values are mean and standard deviation of 3 experiments, each assayed in duplicate. AC activity was expressed as picomoles of [3H]cAMP formed per milligrams of protein and IP3 levels as picomoles of [3H]IP3 formed per milligram of tissue. Degrees of freedom for each parameter are 10.
†p < 0.0001

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Fig. 2: Scatchard graph of [3H]CGP-12177 binding to β-adrenoceptors (β-ARs) in the cortex of rats treated with lithium (0.4%), orally (0.5 mL) for 30 days. Binding experiments were done using [3H]CGP-12177 (0.08–0.9 nmol/L) in cortical membranes, as described in the Methods section. Data points are the means of 3 experiments, each assayed in duplicate. The inset represents saturation curves for the [3H]CGP binding experiment. Data points are the means (and standard deviation) of 3 experiments, each assayed in duplicate.
Further exploring the effects of lithium on these intracellular targets will be helpful to our understanding of the mechanism of action of lithium, to differentiate responders from nonresponders and also to understand the biological factors that predispose individuals to manic–depressive psychosis.

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Contributors: Mr. Devaki and Dr. Nadgir designed the study. Mr. Devaki and Dr. Shankar Rao acquired the data. All authors analyzed the data and wrote the article. Drs. Shankar Rao and Nadgir critically reviewed the article. All authors gave final approval for its publication.

References