Lithium preferentially inhibits adenylyl cyclase V and VII isoforms

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Abstract

Lithium ions’ inhibition of adenylyl cyclase (AC) has not been previously studied for the newly discovered AC isoforms. COS7 cells were transfected with each of the nine membrane-bound AC isoforms cDNAs with or without D1- or D2-dopamine receptor cDNA. AC activity was measured as [3H]cAMP accumulation in cells pre-incubated with [3H]adenine followed by incubation with phosphodiesterase inhibitors together with either the D1 agonist SKF-82958 alone, or forskolin, in the presence or absence of the D2 agonist quinpirole. At 1 mM or 2 mM lithium inhibited only AC-V activity when the enzyme was stimulated by forskolin, a direct activator of AC. Lithium inhibited AC-V (by 50%), AC-VII (by 40%) and AC-II (by 25%) when stimulated via the D1 receptors, but did not affect the Ca2+-activated isoforms when stimulated by the Ca2+ ionophore A23187. Quinpirole inhibits AC via the Gi protein. Lithium did not affect quinpirole-inhibited FSK-activated AC-V activity nor did it affect superactivated AC-V or AC-I following the removal of quinpirole. The data suggest interference of lithium with transduction pathways mediated via AC-V or AC-VII; only the active conformation of these AC isoforms is inhibited by lithium; the inhibitory effect of lithium is abolished when the enzyme is superactivated. The marked inhibition of AC-V and AC-VII by lithium suggests that these two isoforms may be involved in mediating the mood-stabilizing effect of lithium.

Key words: Adenylyl cyclase, dopamine, isoforms, lithium.

Introduction

Lithium salts (lithium) have a mood-stabilizing effect making lithium a first-line treatment of bipolar disorder for over 50 yr. However, the mechanism underlying the therapeutic action of lithium is not understood (Jope, 1999; Manji et al., 1995; Montezinho et al., 2006; Frickaerts et al., 2006). Among a plethora of effects, lithium has been shown to have a significant effect on a major receptor-coupled second-messenger system by inhibiting the generation of cAMP (Dousa and Hechter, 1970; Ebstein et al., 1976, 1980; Mork and Geisler, 1989; Newman and Belmaker, 1987). cAMP production, induced by various neurotransmitters and hormones, is inhibited by lithium at therapeutic concentrations in vivo, ex vivo and in vitro (Dousa and Hechter, 1970; Ebstein et al., 1976, 1980; Mork and Geisler, 1989; Newman and Belmaker, 1987). Competition between lithium and Mg2+ has been suggested to be responsible, at least in part, for the inhibitory effect of lithium on adenylyl cyclase (AC, EC 4.6.1.1) activity (Mork and Geisler, 1989; Newman and Belmaker, 1987). Lithium may also alter the activity of guanine nucleotide-binding proteins (G proteins) which regulate AC activity (Avisar et al., 1988; Masana et al., 1992). The relevance of AC for the mechanism of the action of lithium is emphasized by the fact that AC from human brain tissue, removed during surgical operation, is more sensitive to lithium inhibition than rodent brain AC (Klein et al., 1985).

The inhibitory effect of lithium on AC received little attention in recent years with respect to the mechanism of mood stabilization, because AC is a ubiquitous enzyme which is widespread in all body tissues. Recently, ten isoforms of AC, denominated AC-I to
AC-X, have been identified and cloned (Hanoune and Defer, 2001). Each of these isoforms is the product of a different gene and they vary in their structure, functional attributes and tissue distribution (Cooper, 2003; Hanoune and Defer, 2001; Hurley, 1998; Sunahara and Taussig, 2002). We hypothesized that lithium may have differential effects on AC isoforms which are involved in transduction pathways associated with the control of mood, thus explaining lithium’s specificity as a mood-stabilizing agent. In the present investigation we have studied the effect of lithium on AC activity in cell cultures expressing each of the AC isoforms.

Methods

Plasmids

AC-containing plasmids (pXMD1-AC-I, pXMD1-AC-II, pXMD1-AC-III, pXMD1-AC-IV, pXMD1-AC-V, pCMV5-neo-AC-VI, pXMD1-AC-VII, pCMV5-neo-AC-VIII) described previously by Nevo et al. (1998) were obtained from Dr Z. Vogel (The Weizmann Institute, Rehovot, Israel). pcDNA3-AC-IX, was obtained from Dr F. Antoni (University of Edinburgh, Scotland, UK). Other plasmids were D₃L and D₃ dopaminergic receptor cDNAs in pcDNAI Amp described previously by Nevo et al. (1998) obtained from Dr Z. Vogel.

Transient cell transfection

Twenty-four hours before transfection a confluent 75 cm flask of COS7 cells was trypsinized and split into 10 cm diameter tissue culture plates. When plate confluency reached 50–70% the cells were transfected into 10 cm diameter tissue culture plates. When plate confluency reached 50–70% the cells were transfected into 10 cm diameter tissue culture plates. Twenty-four hours before transfection a confluent COS7 cell monolayer was trypsinized and split into a 75 cm flask. After 24 h the cells were trypsinized and re-cultured in 24-well plates.

cAMP accumulation

COS7 cells, transfected with each of the AC isoforms with or without D₃ or D₃ dopaminergic receptors and grown in 24-well plates were incubated for 2 h with growth medium containing 5 µCi/ml [³²P]adenine (both for the acute and the chronic experiments), in the presence or absence of 1 mM or 2 mM lithium. For AC stimulation, the medium was replaced with Dulbecco’s Modified Eagle’s Medium (DMEM) containing either 1 µM forskolin (FSK) or 0.1 mM of the D₃ receptor agonist, SKF-82958. Cells expressing the D₃ dopaminergic receptors were stimulated by both 1 µM FSK and 1 µM quinpirole, a D₂ receptor agonist. Stimulation of the cells was done in the presence of the phosphodiesterase inhibitors IBMX (0.5 mM) and RO-20-1724 (0.5 mM) to prevent cAMP degradation. To obtain AC superactivation, cells expressing the AC-V or AC-I isoform and the D₂ dopaminergic receptors were treated for 18 h with 1 µM quinpirole, in absence or presence of 1 mM or 2 mM lithium. Agonist withdrawal was achieved by a quick wash with DMEM and replacement of the agonist-containing medium with DMEM containing 10 µM sulpiride and either 1 µM FSK (AC-V) or 10 µM A23187 (AC-I) and the incubation proceeded for 10 min at 37°C. For the quantification of [³²P]cAMP formation, the incubation was terminated by removal of the incubation medium and addition of 2.5% perchloric acid containing 0.1 mM unlabelled cAMP, followed by neutralization with 4.2 M KOH and 1.85 M K₂CO₃. Intracellular [³²P]cAMP was separated from other labelled nucleotides by two-step column chromatography, using ion exchange resin and alumina (Avidor-Reiss et al., 1996) and [³²P]cAMP level was determined by measuring the radioactivity in the eluates.

Statistical analysis

Student’s t test and ANOVA were used to analyse significance of differences as indicated under each experiment.

Results

Transfection of each of the AC isoforms I-IX with or without the D₃ receptor into COS7 cells resulted in overexpression of the enzyme and in a several-fold increase in cAMP generation compared with non-transfected cells (Figure 1). The basal AC activity of non-transfected cells is 3.5 x 10⁻⁵ cAMP pmol/min (5 x 10⁹) cells. All isoforms were several-fold stimulated by FSK and by the D₃ receptor agonist (SKF-82958) excluding AC-IX which lacks a FSK-binding site in its catalytic domain (Hurley, 1999). Non-transfected cells expressed a low level of endogenous AC activity and did not respond to the activating agents (Figure 1). Cells transfected with the AC-V isoform exhibited nearly twice higher FSK-activated AC activity than cells transfected with any other isoform, suggesting high FSK sensitivity of this isoform.

In COS7 cells exposed to 1 mM lithium for 2 h the basal activity of the AC isoforms was not affected (data not shown). However, under the same conditions, lithium significantly inhibited FSK-stimulated AC-V
by 25% whereas other AC isoforms were not affected (Figure 2). Lithium (2 mM) for 2 h showed a similar pattern and an overnight (18 h) 1 mM lithium treatment of the cells produced similar inhibition levels (data not shown). COS7 cells expressing AC-II, AC-V or AC-VII and D_1 dopaminergic receptors and activated by an acute (10 min) exposure to the D_1 agonist SKF-82958 exhibited significant inhibition of AC activity of 25% (AC-II), 50% (AC-V) and 40% (AC-VII) induced by 1 mM lithium (Figure 3). Other isoforms were not affected.

To investigate the specificity of lithium’s effect on FSK and D_1 receptor-activated AC activity in the various isoforms, lithium’s effect on Ca^{2+}-activated AC-I, AC-III and AC-VIII was studied using the Ca^{2+} ionophore A23187. As shown in Figure 4, 10-min exposure of COS7 cells expressing AC-I or AC-VIII to 10 μM A23187 significantly increased cAMP accumulation. The increase in cAMP levels in A23187-activated AC-I and AC-VIII was not affected by 1 mM lithium (Figure 3). Other isoforms were not affected.

Discussion

Multiple studies of the effect of lithium on AC have been carried out (Ebstein et al., 1976, 1980; Mork and Geisler, 1989; Newman and Belmaker, 1987) when the existence of ten AC isoforms was still unknown. The molecular cloning of these isoforms 10 yr later (Sunahara et al., 1996) revealed a differential tissue distribution and a complex and unique regulation of each isoform (Cooper, 2003; Sunahara and Taussig, 2002). These findings return interest to the effect of lithium on AC and raise the question whether lithium’s effect on AC is selective to one or more of the AC isoforms. The present study is the first to examine the effect of lithium on the various AC isoforms. In agreement with previous studies (Ebstein et al., 1980) we did not find an effect of lithium on basal (non-stimulated) AC activity of any isoform. On the
other hand, when AC was stimulated by the direct activator FSK, 1 mM lithium, which is within the therapeutic concentration range, inhibited AC-V activity by 25%. This inhibition of FSK-stimulated AC-V may imply a direct effect of lithium at therapeutically relevant concentrations on the FSK-stimulated conformation of AC-V, an isoform which is abundant in brain (Hanoune et al., 1997).

The inhibitory effect of lithium on AC activated via D1 dopaminergic receptors also showed isoform specificity. The AC-V isoform showed about 50% lithium-inhibitory activity, AC-VII was 40% inhibited and AC-II was only 25% inhibited by lithium. Lithium did not inhibit A23187-activated AC-I and AC-VIII, known to be activated by increased Ca$^{2+}$ levels. Although AC-III is reported in some studies to be a Ca$^{2+}$-activated isoform (Cooper, 2003), the results of the present study do not support this claim.

The lack of inhibition of the basal activity of the enzyme and the pronounced inhibition of the stimulated enzyme both directly by FSK and via Gs by D1 receptor stimulation may suggest that lithium affects only the stimulated conformation of the enzyme. Moreover, the inhibition of AC-V, AC-VII and AC-II when stimulated by a D1 agonist suggests an involvement of the Gs protein interaction site on AC in lithium’s inhibition (Avissar et al., 1988). The lack of an inhibitory effect of lithium on superactivated AC-V and AC-I suggests that lithium selectively affects a specific conformation of the enzyme that is different from the conformation of the superactivated enzyme. The lack of an additive effect of the D2 dopaminergic agonist quinpirole and lithium could be due to a floor effect. Namely, it may not be ruled out that an additive/synergistic effect could be potentially observed at lower concentration of quinpirole, and thus lithium could potentially modulate the quinpirole potency at inhibiting FSK-stimulated AC-V. Other possibilities could be that quinpirole converts the enzyme into its basal conformation which is not affected by lithium or that lithium interacts with AC-V on the same enzyme site as quinpirole-activated Gi protein.

The AC-V isoform is highly enriched in the brain where it is localized to regions involved in dopaminergic transmission such as the striatum, nucleus accumbens and the olfactory tubercle (Glatt and Snyder, 1993; Matsuoka et al., 1997; Mons and Cooper, 1994; Pieroni et al., 1993). The marked inhibition of AC-V by lithium in comparison with other AC isoforms suggests a possible dopaminergic region-specific mechanism for the mood-stabilizing effect of lithium. This finding is in line with evidence indicating that bipolar disorder is associated with dopaminergic dysregulation (Brugue and Vieta, 2007) and with the findings that drugs that inhibit dopamine transmission exert an anti-manic effect, while stimulation of dopamine transmission exerts an antidepressant effect (Schatzberg et al., 1985). Our results showing that lithium may be associated with dopaminergic-specific
mechanisms are also consistent with the finding that lithium and carbamazepine suppress Fos protein expression induced by amphetamine only in the striatum (Lee et al., 1999). Since the AC-V isoform is also expressed in the glomerulus and the collecting tubule in the kidney (Chabardes et al., 1999; Helies-Toussaint et al., 2000), lithium’s inhibition of this isoform may be related to lithium-induced polyuria and polydypsia.

It is noteworthy that AC-V knockout mice have been generated (Iwamoto et al., 2003; Lee et al., 2002). Iwamoto et al. (2003) reported that these mice are motorically impaired but Lee et al. (2002) did not find this dysfunction. The mice were also characterized for G protein expression (Iwamoto et al., 2004), D_2 dopamine receptor function (Lee et al., 2002), longevity (Yan et al., 2007), stress (Yan et al., 2007), pain response (Kim et al., 2007) and cardiac function (Okumura et al., 2003a,b) but mood-related phenotype has not been reported. The results of the present study warrant such characterization.

It is puzzling how to compare previous findings (Montezinho et al., 2007; Mork and Geisler, 1989; Newman and Belmaker, 1987) showing lithium’s inhibition of total AC activity in the brain with our findings showing that only AC-V-, AC-VII- and AC-II-activated isoforms are lithium sensitive. The issue of the abundance of the various AC isoforms in different brain regions is not yet fully resolved. It is well established that AC-V is the dominant isoform in the striatum (Glatt and Snyder, 1993; Matsuoka et al., 1997; Mons and Cooper, 1994). Multiple studies show abundance of all nine membranal AC isoforms in different brain regions (Hanoune et al., 1997; Hanoune and Defer, 2001; Mons et al., 1998; Sunahara and Taussig, 2002; Visel et al., 2006) although a considerable inconsistency is found among the reports. From the data available it is not possible to deduce what portion of total AC activity is derived from AC-V, AC-VII or AC-II in brain regions other than the striatum.

Although the AC-VII isoform is more widespread in the nervous system (Chern, 2000) and therefore
less region-specific than AC-V, lithium’s inhibitory effect on Dₑ agonist-stimulated AC-VII is also intriguing. Its gene is localized in a region of mouse chromosome 8 which is a quantitative trait locus (QTL) for two depression-related animal models (Yoshikawa et al., 2002). In the human genome its chromosomal site (16q12–13) is in proximity to the haptoglobin gene suggested to be associated with major depression (Maes et al., 1994). An association has recently been found between a tetranucleotide repeat polymorphism of human AC-VII and familial depression by Hines et al. (2006). These authors also found increased depression-like behaviour in female mice overexpressing AC-VII and decreased depression-like behaviour in AC-VII heterozygote knockout female mice, suggesting that increased brain AC-VII expression is a risk factor for depression.

D₈ agonist-stimulated AC-II demonstrated mild, yet significant, lithium inhibitability. It is of interest to note that AC-II and AC-VII belong to the same group of AC isoforms. They are stimulated by the βγ subunit of the Gₛ proteins and by protein kinase C (PKC) (Sunahara and Taussig, 2002), characteristics that may be related to their lithium inhibitability.

To summarize, FSK-stimulated AC-V and D₈ dopamine agonist-stimulated AC-V and AC-VII are the most lithium-inhibitable isoforms but the basal activity or the superactivated enzyme, are not affected by the drug. No additive effect was observed between the inhibitory effects of quinpirole (via the Gi protein) and lithium on AC-V. Since AC-V is involved in dopaminergic transmission in the brain, our results suggest a dopaminergic region-specific mechanism for the mood-stabilizing effect of lithium. The inhibition of AC-VII could also account for the antidepressant effect of the drug. The specificity of inhibition of these brain-abundant isoforms could open a new arena of mood-stabilizing drug development based on inhibition of AC-V and AC-VII.

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Statement of Interest
None.

References


Iwamoto T, Okumura S, Iwatsubo K, Kawabe J, Ohtsu K, Sakai I, Hashimoto Y, Izumitani A, Sango K, Ajiki K,