Effect of centrally active drugs on the regulation of Adenylyl cyclase (AC) isoforms

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Abstract

The last year was devoted to several aspects related to the regulation of AC isoforms:
1. The effect of carbamazepine and valproate on the various AC isoforms was studied *in vitro* in cell cultures; 2. An AC5 knockout mice colony was established and behavioral parameters in these mice were assessed to determine if these mice demonstrate lithium-like behavior (since lithium has been shown to inhibit AC5); 3. The effect of various protein kinases on AC5 activity was investigated; 4. The effect of chronic and intermittent dopaminergic stimulation on AC activity in a mouse model of Parkinson's disease was evaluated. Understanding the modes of AC regulation may help improving the therapeutic regimens of drugs that affect the cAMP cascade.

AC and mood stabilization; relevance to Bipolar Disorder

The effect of mood stabilizers on AC activity

In our previous work we found that lithium (Li), a mood stabilizer used in the treatment of bipolar disorder, specifically inhibits forskolin (FSK)-stimulated AC5 activity and D1 agonist-stimulated AC5, AC7 and AC2. During the last year we studied the effect of two additional mood stabilizers, carbamezapine (CBZ) and valproate (VPA) on AC isoforms activity. VPA did not significantly inhibit FSK-stimulated nor did it inhibit ACs stimulated by a D1 agonist. CBZ, on the other hand, significantly inhibited FSK-stimulated AC5 and AC1 and all the membrane bound ACs when stimulated by a D1 agonist.

Do AC5 knockout mice demonstrate a Li-like behavior?

Since both Li and CBZ inhibit AC5, we hypothesized that AC5 knockout mice will show changes in behavior similar to those that occur following lithium administration. To test our

hypothesis we established an AC5 knockout mice colony (breading pairs were kindly donated by Dr. Y. Ishikawa, University of Medicine and Dentistry of New Jersey, Newark, New Jersy) and examined the performance of the wild type strain versus that of the homozygote AC5 knockout mice in the Porsolt forced swim test (a behavioral model for antidepressant effects). The test included a single, six minutes exposure to a water tank containing 10 cm of water maintained at 23–25°C. The duration of immobility was manually recorded during the last 4 min of the session. A mouse was considered to be immobile when it floated or made only small movements necessary to keep its head above the water. Immobility time is significantly shorter when antidepressants or lithium are administered. We expected that the homozygotes AC5 knockout mice will exhibits behavior similar to that of animals treated with Li, which was shown to inhibit AC5. Indeed, the homozygotes AC5 knockout mice showed a significantly shorter immobility time in the porsolt forced swim test (Figure 1) suggesting that inhibition of AC5 by lithium is related to its antidepressant effect.

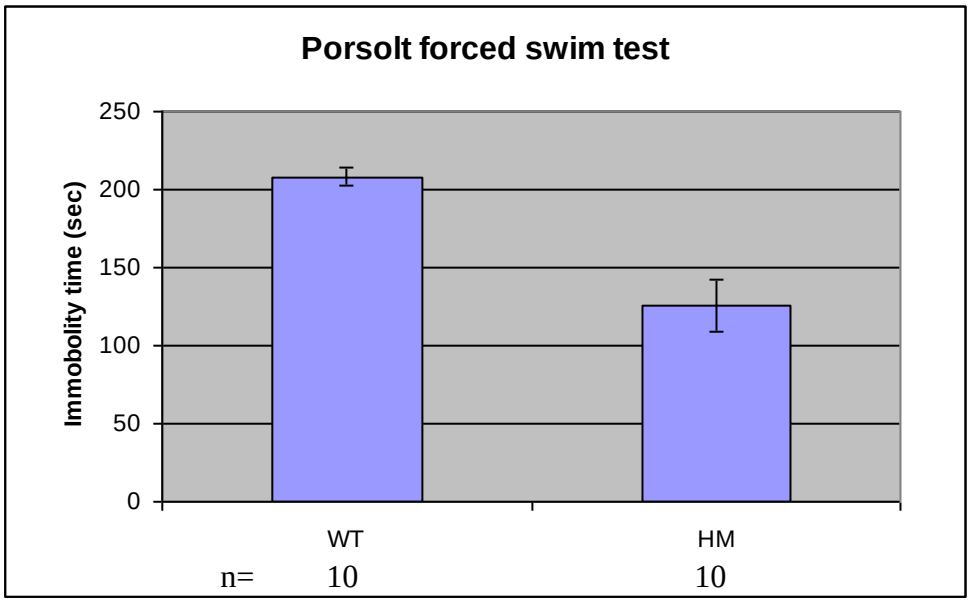

Figure 1: Performance of AC5 knockout mice in the forced swimming test
The test included a single, six min exposure to a water tank containing 10 cm of water maintained at 23–25°C. The duration of immobility was manually recorded during the last 4 min of the session. A mouse was considered to be immobile when it floated or made only small movements necessary to keep its head above water.
Results are mean of $\pm$ S.E.M

The amphetamine-induces hyperactivity test is a behavioral model for mania. Since it is well known that Li reduces hyperactivity induces by amphetamine we are currently testing

AC5 knockout mice in this model. Our hypothesis is that AC5 homozygotes knockout mice will be less hyperactive than the wild type mice following amphetamine injection.

The effect of PKC inhibitor on AC activation and inhibition

In the previous study we demonstrated significant alterations of AC activity following intermittent stimulation of the D2 dopaminergic receptor compared with changes in the enzyme activity that occur after continuous stimulation of the receptor. The mechanisms mediating these alterations are not clear as yet. In order to identify factors that influence the modulation of AC activity after continuous and intermittent stimulation of the D2 receptor, we added the Protein Kinase C (PKC) inhibitor, GF109, to a cell culture expressing both D2 dopaminergic receptors and AC5 and measured AC activity during continuous and intermittent stimulation of the dopaminergic receptor. Inhibition of PKC significantly inhibits AC superactivation following continuous stimulation (Fig 2) while adding the PKC inhibitor during the intermittent stimulation did not cause a significant effect compared with control.

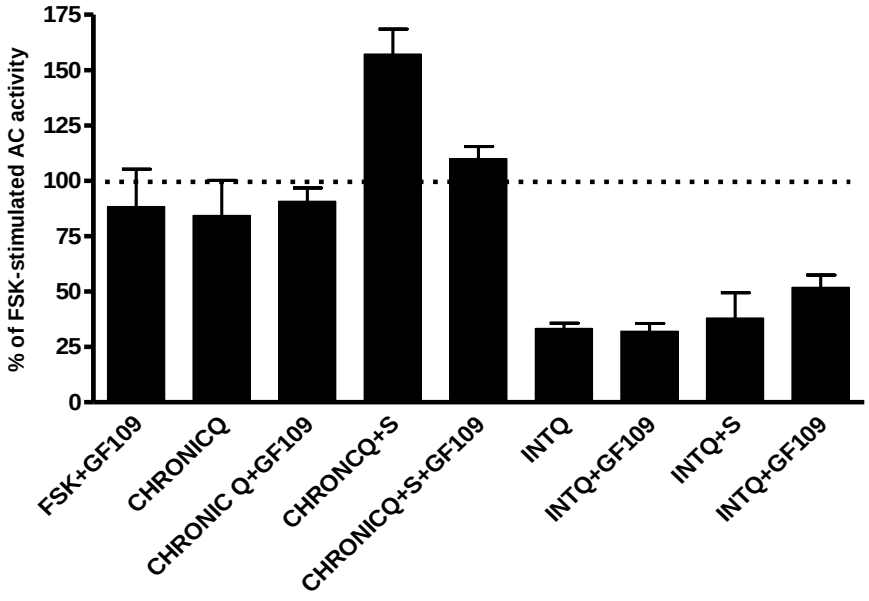

Figure 2: The effect of a PKC inhibitor on AC5 activity following continuous and intermittent dopaminergic stimulation.
COS7 cells expressing AC5 and D2 dopaminergic receptors were exposed to quinpirol, D2 agonist,for 18 h continuously (Chronic Q) or intermittently by interrupting the stimulation three times (each time for 15 min) during 18 h of stimulation (Int Q). These experiments were carried out with or without the PKC inhibitor GF109. Under both conditions forskolin (FSK), an direct activator of AC, was added to the cells in the last 10 min of the incubation and cAMP levels were determined before and after removal of the agonist by addition of sulpiride (S)
FSK: forskolin
GF109: PKC inhibitor
S: addition of sulpiride, a D2 dopaminergic receptor antagonist in the last 10 min of the treatment

AC activity after continuous and intermittent dopaminergic stimulation in MPTP-treated mice

Based on our results with cell cultures, where we demonstrated pronounced differences in AC modulation following continuous and intermittent doipaminergic stimulation, we are now investigating whether similar alternations in AC activity occur also *in vivo* in an animal model of Parkinson's disease. As a model for Parkinson's disease, we used mice treated with MPTP, a neurotoxin that specifically causes the death of dopaminergic neurons in the substantia nigra. MPTP-injected mice (Parkinsonian mice) and saline-injected mice (control) were treated with continuous L-DOPA regimen (by subderally implanted pellets that release L-DOPA continuously in a controlled manner) or with intermittent L-dopa regimen by oral administration of the drug three times a day. After 21 days of treatment the striatum was removed and we are now determining AC activity in samples collected from the striata of the treated mice

Publications of the last year

1. **Mann L**, Heldman E, Shaltiel G, Belmaker RH, Agam G, Lithium Preferentially Inhibits Adenylyl Cyclase V and VII Isoforms. Int J Neuropsychopharmacology 2007.

2. Amar S, Shaltiel G, Shamir A, **Mann L**, Belmaker RH, Agam G, The possible involvement of the dopamine D2-receptor pathway components in schizophrenia. Int J Neuropsychopharmacology 2007.

3. **Mann L**, Vogel Z, Heldman E, Intermittent stimulation of the D_2 dopaminergic receptors leads to receptor hypersensitivity and a loss in AC superactivation. Submitted.

Presentations in conferences

a) L. Mann, E. Heldman, RH. Belmaker and G. Agam. The effect of lithium on various isoforms of adenylyl cyclase. 17th international conference on bipolar disorder, June. 2007, Pittsburgh, US.

b) L. Mann, E. Heldman, RH. Belmaker and G. Agam. The Inhibitory Effect of Mood Stabilizers on Adenylyl Cyclase Isoforms. 16th meeting of the Israel Society for Neuroscience, Dec. 2007, Eilat, Israel.

c) L. Mann, Y. Bersudsky, RH. Belmaker and G. Agam. Do adenylyl cyclase knockout mice have lithium like behavior? 11th annual meeting of the Israel Society for biological Psychiatry, March 2007, Kfar-Giladi, Israel.

d) L. Mann, E. Heldman, RH. Belmaker and G .Agam. Lithium Preferentially Inhibits Adenylyl Cyclase V and VII Isoforms to be presented at the XXVI CINP Congress, July 2008, Munich, Germany.