KAWASAKI MED. J. VOL. 14 : NO. 2, 75-81 (1988)

The Effects of Long-Term Treatment with Lithium on Adenylate Cyclase Coupled with Catecholamine Receptors

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Accepted for publication on April 2, 1988

ABSTRACT. Increment of cyclic AMP in tissue slices in response to noradrenaline and dopamine stimulation was studied in all part of the rat brain. Chronic lithium administration significantly inhibited the sensitivity of noradrenaline-sensitive adenylate cyclase in the frontal cortex, whereas the sensitivity of dopamine-sensitive adenylate cyclase was significantly inhibited in bulbus olfactorius and hippocampus. A possible relationship between the antidepressant action of lithium and its significant inhibition of the sensitivity of noradrenaline-sensitive adenylate cyclase was therefore suggested. The anti-manic effect, on the other hand, may be related to the inhibitory action on the sensitivity of dopamine-sensitive adenylate cyclase.

Key words : lithium — adenylate cyclase — cyclic AMP — noradrenaline — dopamine

Manic-depressive psychosis is a disease with a cyclic occurrence of emotional disturbance. Disposition for the occurrence of this disease shows a tendency of hereditary transmission. Along with schizophrenia, manic-depressive psychosis represents two major endogenous psychoses. The emotional disturbance manifests itself into two opposite directions, manic and depressive psychosis. In manic psychosis, a highly elevated mood, disturbance of thinking such as flights of ideas and psychomotor excitation are noted. In depressive psychosis, symptoms such as a depressive mood and inhibited thinking are noted.

While the pathogenic mechanism of manic-depressive psychosis has not yet been fully elucidated, appearance and clinical application of tricyclic antidepressants, monoamine oxidase inhibitors and lithium between the latter half of the 1950s to the 1960s has promoted a gradual progress towards understanding of the nature of this disease. In recent psychopharmacological studies, attention has been focused on the relationship between manic-depressive psychosis and pathological changes in receptors of the central nervous system.

At present, depression is treated with tricyclic antidepressants, monoamine oxidase inhibitors, electric shock and lithium. Manic psychosis is treated with lithium, carbamazepine and psychotropic agents. Only lithium is effective against both diseases. Elucidation of the mechanism of action of lithium therefore appears to be a key to understanding the pathogenesis of manic-depressive psychosis.

Manic-depressive psychosis is thought to be the result of a change in

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the receptors of the central nervous system, especially noradrenaline and dopamine receptors. Manic psychosis is believed to be especially related to dopamine receptors and depressive psychosis to noradrenaline receptors.[1] In the present study, the effects of chronic lithium administration on adenylate cyclase coupled with these receptors were studied.

Fig. 1 shows a diagram for cell membrane receptors. No receptor —N-protein— adenylate cyclase complexes are formed when agonists are not bound to receptors, but when they are bound to receptors the complexes are formed to activate adenylate cyclase and intracellular ATP is transformed into cyclic AMP. This cyclic AMP is considered to be a second messenger through which the differentiating function of each cell is manifested. Consequently, by measuring the storage of cyclic AMP in the cell in response to agonist stimulation, it is possible to assess the sensitivity of receptor-coupled adenylate cyclase.

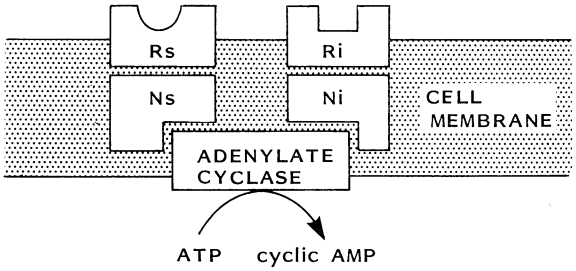

Fig. 1. Diagram for cell membrane receptors

MATERIALS AND METHODS

Male Wistar rats weighing 55–65 g at 4 weeks of age were divided into control and lithium groups. The former group was maintained on ordinary powder rations alone; whereas the latter received the same diet containing 0.1% lithium carbonate. Body weight was measured weekly. The plasma lithium concentration was measured by atomic absorption spectrophotometry.

After 5 weeks, the animals were sacrificed by decapitation without anesthesia. Brains were quickly removed and the olfactory bulb, frontal cortex, corpus striatum, hippocampus, thalamus, hypothalamus, midbrain and cerebellum were taken out according to the method of Glowinski and Iversen,[2] and their wet weight was determined. All portion was cut into slices approximately 0.5 mm thickness. After which the slices were divided into agonist loading and agonist non-loading groups. Slices of the former group were incubated from the beginning in the presence of 10^{-8}–10^{-2} M noradrenaline or dopamine. The reaction was allowed to proceed for 10 minutes in the presence of 20 volumes of Krebs-Ringer solution at 37°C, and stopped by boiling at 100°C for 5 minutes. After the reaction was stopped, the tissue was homogenized, and an aliquot was used for the measurement of protein concentration by the Lowry method. The remaining homogenate was centrifuged at 2500×g for 15 minutes at 4°C, and the cyclic AMP content of the supernatant was determined.

The composition of Krebs-Ringer solution is shown in Table 1. The solution

TABLE 1. Composition of Krebs-Ringer solution (pH 7.4)

NaCl	117.0 mM
KCl	4.7 mM
$CaCl_2$	2.5 mM
KH_2PO_4	1.2 mM
$MgSO_4$	1.2 mM
HEPES	10.0 mM
ATP	1.0 mM
IBMX	0.5 mM
Glucose	11.5 mM

was adjusted to pH 7.4, and saturated with 95% O_2–5% CO_2 beforehand. This buffer contained 3-isobutyl-1-methylxanthine (IBMX), an inhibitor of cyclic AMP phosphodiesterase to prevent degradation of cyclic AMP.

Cyclic AMP was measured by using the kit for cyclic AMP measurement produced by Amersham. The method consists of addition of certain amount of ^3H-labelled cyclic AMP to a sample containing an unknown amount of unlabelled cyclic AMP inducing competitive binding with protein. Free cyclic AMP not bound to protein forming a complex was absorbed to active charcoal and B/F separation was accomplished by centrifugation. The protein-bound radioactivity in the supernatant was measured in a liquid scintillation counter. The count was thus inversely proportional to the amount of cyclic AMP in the sample. Standard curves were constructed to estimate the amount of cyclic AMP in the sample.

RESULTS

1) Body weight

Changes in body weight are shown in Fig. 2. As measured by the student's t-test, body weight in the lithium group was significantly less in the first week. After the second week, no significant difference was found between the two groups.

2) Plasma lithium concentration

Plasma lithium concentrations are shown in Fig. 2. A steady state was already reached in the first week. The range of the therapeutic concentration of lithium in man is rather narrow, between 0.3–1.0 mEq/1. In the present study, in order to maintain agreement with the therapeutic concentration in man, the powder ration was adjusted so that it contained 0.1% lithium carbonate.

3) Time course of cyclic AMP production

The time course of cyclic AMP is shown in Fig. 3. In both the agonist loaded and non-loaded group, the peak was reached after 10 minutes followed by a gradual decrease. Consequently, the reaction time of 10 minutes was employed in subsequent experiments.

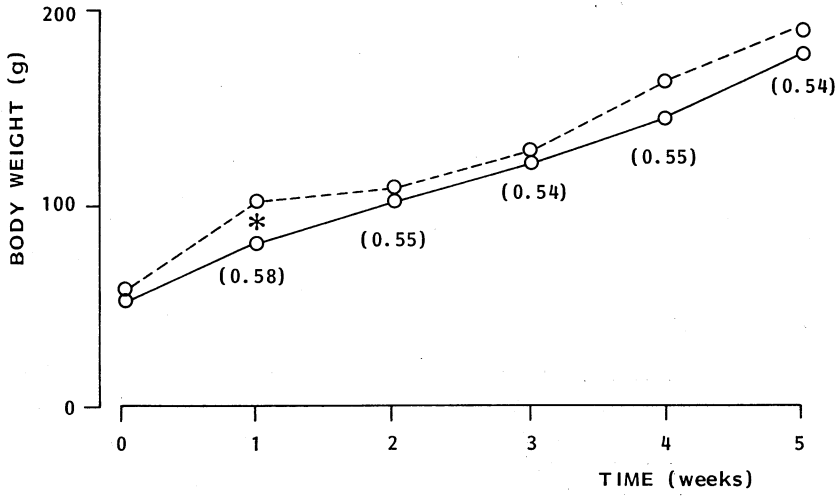

Fig. 2. Body weight and plasma lithium concentration
- - - : control
——— : lithium
* : $p<0.001$
( ) : plasma lithium concentration (mEq/l)
n=8

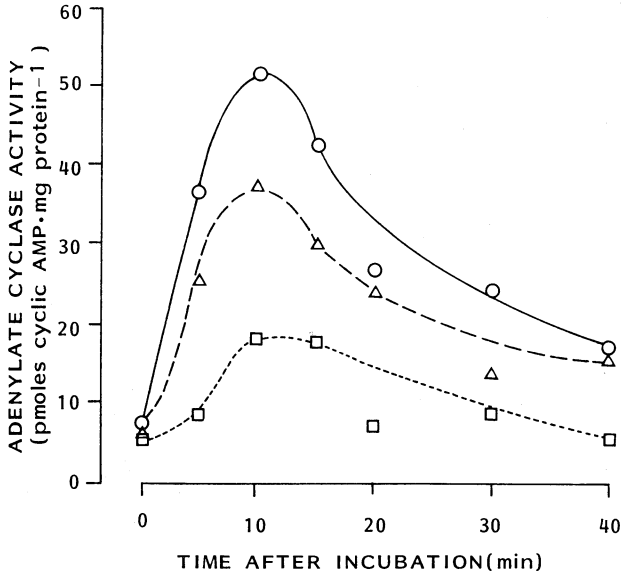

Fig. 3. Time course of cyclic AMP production in the frontal cortex
○ : noradrenaline loaded
△ : dopamine loaded
□ : non-loaded
n=4

4) Dose-response curve for cyclic AMP production

The dose-response curve for cyclic AMP production is shown in Fig. 4. After both noradrenaline loading and dopamine loading, the peak was reached at 10^{-4} M. Therefore, 10^{-4} noradrenaline and dopamine were employed in all subsequent experiments.

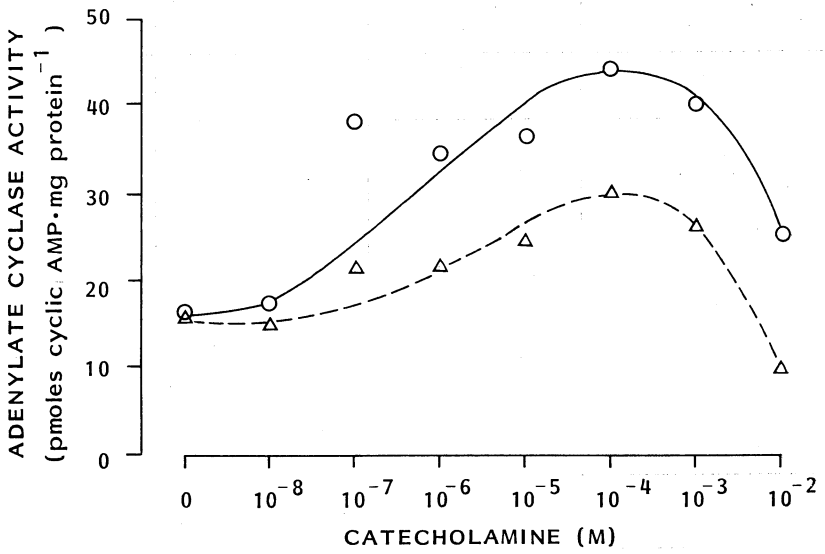

Fig. 4. Dose-response curve for cyclic AMP production in the frontal cortex
○ : noradrenaline loaded
△ : dopamine loaded
n=4

5) Amounts of cyclic AMP stored at various parts of the brain

Table 2 summarizes the amount of cyclic AMP in various parts of the brain in the control and lithium groups. The amount of stored cyclic AMP was calculated by subtracting the amount of cyclic AMP found in the agonist non-loaded group from that in the agonist loaded group. In other words, the amount of stored cyclic AMP reflects the sensitivity of noradrenaline-sensitive adenylate cyclase or dopamine-sensitive adenylate cyclase.

As measured by the student's t-test, chronic lithium administration significantly inhibited noradrenaline-sensitive adenylate cyclase in the frontal cortex. Dopamine-sensitive adenylate cyclase was significantly inhibited in the olfactory bulb and hippocampus.

Lithium concentrations in the frontal cortex and hippocampus are 1.18 ± 0.25 mEg/kg and 1.51 ± 0.38 mEg/kg respectively.

These corresponded to about 2-3 times the concentration in blood. No significant difference was found between the two groups as measured by the student's t-test.

TABLE 2. Amount of cyclic AMP
(pmol/mg protein, mean ±S.D.)

	control		
	noradrenaline non-loaded	noradrenaline loaded	sensitivity of adenylate cyclase
frontal cortex	18.7 ± 3.2	56.5 ± 5.9	37.8 ± 5.9
hippocampus	8.0 ± 2.3	23.6 ± 3.2	15.6 ± 3.2
	dopamine non-loaded	dopamine loaded	sensitivity of adenylate cyclase
olfactory bulb	7.2 ± 2.3	18.6 ± 2.5	11.5 ± 2.5
frontal cortex	18.7 ± 3.2	36.5 ± 7.5	17.7 ± 7.5
striatum	12.0 ± 3.2	21.6 ± 4.1	9.6 ± 4.1
hippocampus	8.0 ± 2.3	20.4 ± 2.9	12.4 ± 2.9
thalamus	7.6 ± 2.2	10.6 ± 2.5	3.3 ± 2.5
hypothalamus	12.8 ± 3.0	22.2 ± 5.1	9.4 ± 5.1
midbrain	12.2 ± 4.1	17.6 ± 3.0	5.4 ± 3.0
cerebellum	25.3 ± 4.0	33.8 ± 5.4	8.5 ± 5.4
	lithium		
	noradrenaline non-loaded	noradrenaline loaded	sensitivity of adenylate cyclase
frontal cortex	16.2 ± 2.4	46.3 ± 3.7	30.1 ± 3.7 **
hippocampus	7.4 ± 2.6	22.0 ± 3.4	14.6 ± 3.4
	dopamine non-loaded	dopamine loaded	sensitivity of adenylate cyclase
olfactory bulb	8.8 ± 2.0	17.7 ± 2.8	8.9 ± 2.8 *
frontal cortex	16.2 ± 2.4	33.3 ± 6.2	17.1 ± 6.5
striatum	11.2 ± 3.8	23.0 ± 5.3	11.8 ± 5.3
hippocampus	7.4 ± 2.6	17.5 ± 1.7	10.1 ± 1.7 *
thalamus	8.0 ± 2.4	11.4 ± 3.1	3.4 ± 3.1
hypothalamus	13.0 ± 3.8	21.8 ± 4.1	8.8 ± 4.1
midbrain	10.3 ± 3.1	17.1 ± 3.3	6.8 ± 3.3
cerebellum	22.0 ± 4.3	31.5 ± 4.9	9.5 ± 4.9

* : $p<0.05$, ** : $p<0.001$ (control vs lithium)
n=10

DISCUSSION

Changes of the theories of depression from the psychopharmacological viewpoint began in 1965, when the catecholamine hypothesis was proposed by Schildkraut.[3] According to this hypothesis, increase of catecholamine in the synaptic interspace leads to mania and decrease of catecholamine in this space leads to depression. Mania and depression were explained as mirror image-like changes. In fact, antidepressants such as tricyclic compounds and monoamine oxidase inhibitors increase monoamines, especially noradrenaline, in the synaptic interspace. This hypothesis thus appeared to be capable of explaining the disease picture of manic-depressive psychosis quite clearly. It, however, was unable to explain the presence of antidepressants not influencing the metabolic turnover

of catecholamines and a marked delay in the appearance of the antidepressant effects from the time of arrival of the drug at the synaptic interspace. Subsequently, in 1978, Sulser *et al.*[4] reported a decrease in the sensitivity of noradrenaline receptors in response to most treatments for depression, such as tricyclic antidepressants, non-tricyclic antidepressants, monoamine oxidase inhibitors and electric shock. For such a decrease in sensitivity to occur, chronic treatment is required. Such a fall in sensitivity and the appearance of an antidepressant effect showed an agreement in time. This was the basis for his hypothesis on the augmentation of the sensitivity of noradrenaline receptors in depression. The paradoxes described above were solved by this hypothesis.

One of the purposes of the present study was to test whether or not this hypothesis of augmentation of noradrenaline receptors in depression is valid for lithium therapy as well. Our results showed that the sensitivity of adenylate cyclase coupled with noradrenaline receptors of the frontal cortex was inhibited by chronic lithium administration. Lithium was thus found to act like other antidepressants.

In 1983, Bunney and Garland,[5] based on clinical studies using dopamine agonists and antagonists, proposed the hypothesis that the development of manic psychosis was due to augmentation of the sensitivity of dopamine receptors.

Another goal of this study was to test whether this hypothesis for mania is applicable for lithium therapy. Our results showed that chronic lithium therapy inhibited the sensitivity of adenylate cyclase coupled with dopamine receptors in the olfactory bulb and hippocampus. Therefore, the mechanism of treatment of manic psychosis with lithium may be mediated by the inhibition of the sensitivity of adenylate cyclase coupled to dopamine receptors.

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