A COMPARISON OF LITHIUM EFFECTS ON HUMAN BRAIN AND RAT BRAIN NORADRENALINE-SENSITIVE ADENYLATE CYCLASE

BY

Ehud Klein, R.H. Belmaker, Michael Newman & Jan Gruszkiewicz

Abstract: Lithium (Li) is a drug with numerous biochemical effects. Many of these biochemical effects occur only at high Li concentrations, or disappear after chronic Li treatment. Such effects are probably not related to Li's mechanism of therapeutic action. Inhibition of noradrenaline (NE)-sensitive adenylate cyclase has been proposed as a possible therapeutic mechanism of action for Li. Tolerance does not develop to this effect, which has been reported to occur reliably beginning at 2mM Li in rat cortex. Since 2mM Li is at the upper limit of therapeutic levels in humans, controversy has continued as to whether Li inhibition of NE-sensitive adenylate cyclase is a mechanism of Li's therapeutic action or a mechanism of Li toxicity. We hypothesized that human brain NE-sensitive adenylate cyclase may be more sensitive to Li inhibition than rat brain NE-sensitive adenylate cyclase. Fresh cortical human grey matter was obtained from the edges of surgically removed brain tumors in seven patients. Results showed significant inhibition of NE-sensitive adenylate cyclase at 1mM Li. These results support the possibility that inhibition of NE-sensitive adenylate cyclase is a mechanism of Li's therapeutic action.

Key-words: Lithium - cyclic AMP - cortex - human brain - noradrenaline

Inhibition of adenylate cyclase has been an attractive theory of lithium (Li) action since Doussa and Hechter (1970) first reported that Li can inhibit noradrenaline (NE)-sensitive adenylate cyclase in rat brain homogenates. When adenylate cyclase was proposed to be the second messenger for NE neurotransmission (Robinson et al. 1971), Li inhibition of adenylate cyclase was easily related to an overall theory of affective disorder psychopharmacology: Schildkraut (1965) proposed that NE neurotransmission is excessive in mania, Li is antimanic (Schou et al. 1954), and Li could be shown to inhibit second messenger function distal to the NE receptor (Forn and Valdecasas 1971).

However, this theory received a severe setback with the realization that Doussa and Hechter (1970) and other early workers, had used concentrations of Li between 25-50mM to achieve adenylate cyclase inhibition. These concentrations could most likely be related to mechanisms of Li toxicity, rather than mechanism of Li action (Belmaker and Ebstein 1979). Moreover, adenylate cyclase is a widely-spread enzyme in the body and in itself is nonspecific, the tissue specificity being determined by the receptor to which it is attached (Nathanson 1977). Inhibition of adenylate cyclase therefore seemed to be a more likely mechanism for the widespread symptoms of Li toxicity at high Li concentrations, rather than the highly specific therapeutic effects of Li at clinical concentrations. The concept that Li inhibition of adenylate cyclase could explain Li's antimanic action was put aside as a misleading oversimplification almost as quickly as it had previously become a leading theory.
In 1976 Ebstein et al. (1976) reported that patients on chronic Li treatment had a markedly inhibited plasma cyclic AMP rise after epinephrine injection, compared with controls. The patients' Li levels were non-toxic and well within the therapeutic range. Ball et al. (1972) had previously shown that the plasma cyclic AMP rise after epinephrine injection is due to stimulation of a peripheral β-adrenergic adenylate cyclase. At once it was clear that Li could inhibit adenylate cyclase in vivo at therapeutic concentrations. Moreover, glucagon-induced rises in plasma cyclic AMP in humans were then shown not to be inhibited in patients receiving chronic Li treatment (Ebstein et al. 1977). Thus Li inhibition of adenylate cyclase could be specific to some receptor-linked adenylate cyclases. Presumably such specificity could occur because the adenylate cyclase does differ in minor ways from tissue to tissue. Only minor differences in Li sensitivity of adenylate cyclase would be required to achieve specificity, as Li is a drug with a very narrow therapeutic index. For instance, a "therapeutic target" adenylate cyclase might be most sensitive to Li inhibition, which should occur in such a tissue below 2.0 mM Li. Other adenylate cyclases would need to be only slightly less sensitive to Li to be unaffected during clinical treatment which is carried out carefully to remain below blood Li levels of 2.0 mM.

A prime candidate for the target "adenylate cyclase" of Li treatment is the NE-sensitive adenylate cyclase, which appears to be inhibited at lower concentrations than the dopamine-sensitive adenylate cyclase (Reches et al. 1978), the parathyroid hormone-sensitive adenylate cyclase (Spiegel et al. 1976), or the glucagon-sensitive adenylate cyclase (Ebstein et al. 1977). Some adenylate cyclases such as the TSH-sensitive adenylate cyclase (Wolff et al. 1970) and the ADH-sensitive cyclase (Geisler et al. 1972), are inhibited by Li, perhaps to a lesser degree than the NE-sensitive adenylate cyclase, but enough to relate to Li side effects on the kidney and thyroid (Ebstein and Belmaker 1977 & 1979). Some adenylate cyclases have not yet been studied for Li effects, such as the serotonin-sensitive adenylate cyclase (Von Hungen et al. 1975). Preliminary work on the adenosine-sensitive adenylate cyclase suggests that it is sensitive to Li inhibition at 1.0 mM Li (Ebstein et al. 1978).

Recent experimental work suggests that adenylate cyclase assays in cell-free homogenates are much less sensitive to Li inhibition than tissue slice assays containing whole cells (Wang et al. 1974). This explains the need for such high Li concentrations in the earliest pioneering work on Li and adenylate cyclase. A recent study (Ebstein et al. 1980) of Li effects on NE-sensitive adenylate cyclase in rat cortical tissue slices found clear Li inhibition in vitro by 2.0 mM (Table 1). The isoproterenol-induced cyclic AMP accumulation in

<table>
<thead>
<tr>
<th>Drug</th>
<th>No drug (basal)</th>
<th>50 μM NE</th>
<th>Li 1 mM</th>
<th>50 μM NE + Li 1 mM</th>
<th>Li 2 mM</th>
<th>50 μM NE + Li 2 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3.0</td>
<td>1.4</td>
<td>3.5</td>
<td>0.7</td>
<td>2.0*</td>
<td></td>
</tr>
<tr>
<td>(100%)</td>
<td>(600% rise)</td>
<td>(280% of basal)</td>
<td>(13% inhibition of rise)</td>
<td>(140% of basal)</td>
<td>(47% inhibition of rise)</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05 50 μM NE + Li vs 50 μM NE
the same study was significantly inhibited by 1.0 mM Li. Chronic treatment of
rats with Li leading to plasma levels of 1.73 mM and ex vivo examination of NE-
stimulated cyclic AMP accumulation showed a significant inhibition (Ebstein et
al. 1980). Plasma levels of 1.73 mM are higher than the levels usual in psychia-
tric clinical practice today. Critics of the adenylate cyclase theory of Li
action have suggested that these levels, low as they are, still represent the
beginning of Li toxicity.

However, human cortex contains a NE-sensitive adenylate cyclase system
that is several fold more active than that of the rat. That is, maximal rises
of cyclic AMP are 12-40 fold over basal in human cortex (Fumaqalli et al. 1971;
Shimizu et al. 1971) compared with 2-20 fold increases in rats (Daly 1977).
Such a more active system could well be more sensitive to Li inhibition. We
recently were able to examine Li effects on NE-sensitive adenylate cyclase in
human brain tissue obtained from the edges of surgically removed brain tumors.
The brain tumors were sent to pathology where the healthy tissue was separated
from tumor and kept on ice until arrival in the laboratory. Slices were cut
with a McIlwain tissue chopper set at 0.5 mm and preincubated for at least 10 min
in Krebs Ringer bicarbonate buffer containing 1.28 mM CaCl₂, gassed with 95% O₂;
5% CO₂. At the end of this period the slices were collected by filtration on a
Buchner funnel and distributed among vials containing Li and/or noradrenaline as
indicated in the tables for a further 20 min incubation. The contents of the
vials were then centrifuged, the medium decanted and the slices homogenized in
1 ml 95% ethanol. 50 µl aliquots of the supernatant were assayed for cyclic AMP
by a protein binding method based on that of Brown et al. (1971), using a kit
supplied by the Radiochemical Centre, Amersham, U.K.

Addition of Li alone to the slices resulted in a rise in cyclic AMP levels
at 0.5 mM, although this did not reach statistical significance, followed by a
decline to baseline levels (Table 2). This effect parallels that found with rat
brain slices (Ebstein et al. 1980), although in that case the stimulatory effect
was maintained at 1 mM. The effects of Li on noradrenaline stimulation are shown
in Table 3. NE alone produced a 10-fold stimulation of cyclic AMP levels in 4
of the experiments shown and in two of these cases (patients 33 F and 8 M) Li at
0.5 mM inhibited the rise, although at higher concentrations (2 mM or 5 mM) a return
to the levels obtained with NE alone was seen. In two other cases NE only
produced a 2-fold stimulation, and in these cases inhibition was only obtained
at 2 mM and 5 mM Li. The results when pooled gave significant inhibition at 1, 2
and 5 mM Li. There was no correlation between the degree of stimulation by NE
and the degree of inhibition of the rise by Li. In tissues consisting only of
tumor (results not shown), NE did not stimulate cyclic AMP accumulation and in
tumor tissue there was no effect of Li on cyclic AMP levels in either the
presence or absence of NE.

<table>
<thead>
<tr>
<th>mM Li</th>
<th>Cyclic AMP, pmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.6 ± 9.7 (7)</td>
</tr>
<tr>
<td>0.5</td>
<td>64.4 ± 18.1 (5)</td>
</tr>
<tr>
<td>1</td>
<td>49.5 ± 12.6 (4)</td>
</tr>
<tr>
<td>2</td>
<td>45.3 ± 14.3 (5)</td>
</tr>
<tr>
<td>5</td>
<td>38.7 ± 10.6 (5)</td>
</tr>
</tbody>
</table>

Results are mean ± SEM of the number of observations in parentheses.
Table 3. Effect of Li on noradrenaline-stimulated cyclic AMP levels in incubated slices of human brain

<table>
<thead>
<tr>
<th>Patients (age, sex, brain area, tumor type)</th>
<th>33 F</th>
<th>69 F</th>
<th>40 M</th>
<th>8 M</th>
<th>31 M</th>
<th>28 F</th>
<th>68 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 F Left frontal</td>
<td>33 F Right temporal</td>
<td>33 F Frontal</td>
<td>33 F Frontal</td>
<td>33 M Frontal</td>
<td>33 M Left frontal</td>
<td>33 M Right fronto-parietal</td>
<td></td>
</tr>
<tr>
<td>Cystic astrocytoma</td>
<td>Glioblastoma</td>
<td>Glioblastoma</td>
<td>Burkitt's lymphoma</td>
<td>Recurrent oligodendro-glioma</td>
<td>Astrocytoma</td>
<td>Glioblastoma</td>
<td>Mean±SEM of all values</td>
</tr>
<tr>
<td>CAMP, pmol/mg protein</td>
<td>76.3</td>
<td>58.2</td>
<td>44.0</td>
<td>10.5</td>
<td>47.6</td>
<td>315.0</td>
<td>195.3</td>
</tr>
<tr>
<td>50µM NA</td>
<td>570.0</td>
<td>564.7</td>
<td>161.3</td>
<td>100.0</td>
<td>538.5</td>
<td>577.8</td>
<td>384.0</td>
</tr>
<tr>
<td>0.5mM Li+NA</td>
<td>320.8</td>
<td>675.7</td>
<td>110.3</td>
<td>32.8</td>
<td>555.1</td>
<td>608.0</td>
<td>323.0</td>
</tr>
<tr>
<td>1mM Li+NA</td>
<td>277.1</td>
<td>480.0</td>
<td>124.0</td>
<td>30.0</td>
<td>379.1</td>
<td>590.0</td>
<td>321.8</td>
</tr>
<tr>
<td>2mM Li+NA</td>
<td>302.0</td>
<td>501.3</td>
<td>-</td>
<td>91.4</td>
<td>546.1</td>
<td>351.7</td>
<td>175.4</td>
</tr>
<tr>
<td>5mM Li+NA</td>
<td>539.6</td>
<td>327.3</td>
<td>127.1</td>
<td>25.0</td>
<td>341.8</td>
<td>131.8</td>
<td>195.0</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.025 significance relative to values with NA alone by paired t test.

The degree of stimulation with NE in these experiments is similar to that obtained by Kodema et al. (1973) who reported a 15-fold rise in cyclic AMP values in cortical grey matter samples of human brain assayed after 40 min pre-incubation with 14C-adenine.

The present results suggest that small species differences exist in the sensitivity of NE-sensitive adenylate cyclase to Li inhibition. Since Li is a drug with such a narrow therapeutic index, such species differences are crucial in interpreting whether effects of Li are therapeutic or toxic. In humans, inhibition by Li of NE-sensitive adenylate cyclase occurs at therapeutic concentrations. Rat brain appears to be somewhat less sensitive to Li.

REFERENCES


