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The Differential Effect of Lithium on Noradrenaline- and Dopamine-Sensitive Accumulation of Cyclic AMP in Guinea Pig Brain

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Abstract. Lithium (Li) in its narrow therapeutic concentration range was found to inhibit only the noradrenaline- and not the dopamine-sensitive accumulation of cyclic AMP in guinea pig brain. The results suggest a pharmacological distinction between the antischizophrenic drugs that inhibit dopamine-sensitive cyclic AMP accumulation and Li, an antimanic agent that inhibits specifically only the noradrenaline-sensitive cyclic AMP accumulation.

Key words: Lithium – Noradrenaline- and dopamine-sensitive cyclic AMP accumulation – Differential inhibition – Guinea pig brain

In spite of the wide clinical use of lithium (Li) in psychiatry, the biochemical mechanisms of Li action in mania remain unknown (Schildkraut, 1973). One postulated primary site of Li action is as an inhibitor of noradrenergic adenylate cyclase (Ebstein et al., 1976; Forn, 1975). Our laboratory recently demonstrated an inhibitory effect of Li in vivo on the peripheral adrenaline-stimulated adenylate cyclase in man at therapeutic doses (Ebstein et al., 1976). If Li similarly inhibited brain noradrenergic adenylate cyclase, the functional excess of noradrenaline (NA) hypothesized to exist in mania (Schildkraut, 1965) might thus be controlled.

Li is a drug with a very narrow therapeutic index (Kerry, 1975). While this makes clinical management of manic-depressive patients more difficult and toxicity relatively frequent (Vacaflor, 1975), it may provide a strategy for identifying the biochemical mechanisms of Li's efficacy. Only those biochemical effects that appear within Li's narrow therapeutic dose range may legitimately be related to clinical efficacy. Another unique aspect of Li pharmacology important in identifying the biochemical substrate of Li action is the clinical specificity of Li in mania and this drug's lack of effectiveness in schizophrenic symptoms (Prien, 1975; Kline and Simpson, 1975). If the site of Li action in mania is a brain NA-stimulated adenylate cyclase, then it might be predicted that only a narrow concentration range of Li corresponding to the clinical dose would be effective and specific in inhibiting this enzyme. Furthermore, at this concentration Li would not be expected to inhibit the dopamine (DA-)sensitive adenylate cyclase, an enzyme that has been found to be inhibited only by drugs that possess antischizophrenic properties (Clement-Cormier et al., 1974; Miller et al., 1974).

Palmer et al. (1972) showed that Li at 1.0 mM concentration significantly inhibited the NA-induced rise in cyclic AMP in rat hypothalamic slices. Geisler and Klysner (1977) reported Li inhibition of DA-sensitive adenylate cyclase in rat brain homogenates, but at 10 mM (clinically toxic) concentrations. Therefore the aim of the present experiment was to compare the effect of Li at different concentrations on the rise in cyclic AMP induced by each of the two neurotransmitters. To accomplish this comparison a crude vesicular synaptosome preparation was chosen, since NA-stimulated rises in cyclic AMP are difficult to demonstrate in pure cell-tree homogenates and because DA-stimulated rises in cyclic AMP are difficult to demonstrate in tissue slices.

Materials and Methods

A crude vesicular synaptosome preparation was prepared from guinea pig cortex and caudate nucleus by a modification of the method first described by Chasin et al. (1974). The tissue was homogenized in a glass teflon homogenizer in 10 volumes Krebs Ringer phosphate (KRP) medium (including glucose). The homogenate was gassed with 95% O_2 – 5% CO_2 for 20s and incubated in the presence of ^3H adenine (5µCi/100mg wet weight tissue;

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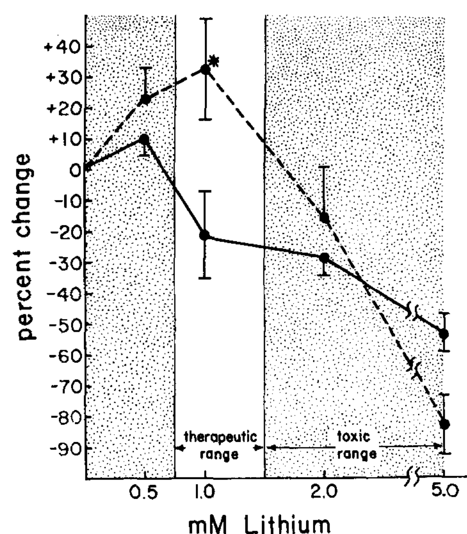

Fig. 1. The effect of Li on NA- and DA-sensitive cyclic AMP accumulation in guinea pig brain. DA-sensitive (caudate nucleus) activity (●----●); NA-sensitive (cortex) activity (——). * $P < 0.01$ (paired t-test)

Amersham 5Ci/mmol). After 40 min at 37° C in a shaking-water bath, the homogenate was centrifuged at 900 × g for 15 min and washed twice with an equal volume of KRP. After the second wash the pellet was resuspended in the original volume of KRP and divided into a number of separate vials and incubated after gassing for an additional 15 min at 37°C in the presence and absence of DA, NA, and various drugs. The reaction was stopped with 100 µl 1 N HCL. Cyclic AMP was purified by Dowex 50 chromatography as described by Krishna et al. (1968) and as modified by Chan and Lin (1974). Samples were counted in a Packard Tri-Carb scintillation counter.

Basal cyclic AMP accumulation was calculated as the amount of radioactive cyclic AMP formed from radioactive precursor in the absence of neurotransmitter and Li. The basal enzyme activity of the caudate nucleus preparation was 111 and of the cortex preparation 139 pmol cyclic AMP/h at 37° C/g wet weight tissue. Li (up to 5.0 mM) had no effect on basal activity from either cortex or caudate nucleus. Addition of neurotransmitter caused a rise in the radioactive cyclic AMP formation, from which basal activity was subtracted to yield the specific neurotransmitter-induced rise in cyclic AMP formation. On each experimental day the neurotransmitter-induced rise in cyclic AMP formation was determined in quadruplicate in the absence of Li, and then in duplicate at each of four Li concentrations. DA and NA stimulations were done on the same day with brain homogenates aliquoted from the same pool of 3 guinea pig brains. The percentage of Li inhibition was calculated by comparing the rise in cyclic AMP formation with neurotransmitter alone to the rises with neurotransmitter in the presence of various Li concentrations.

Results

The average stimulation above basal activity in the presence of 100 µM NA was 66% ± 3.2% (± SEM) in the cortex homogenate. Similar results were reported by Chasin et al. (1974). The average stimulation above basal activity in the presence of 1 mM DA was 28% ± 2.1% (± SEM) in the caudate nucleus homogenate. The higher concentration of DA in this preparation was required for maximal stimulation as described by Krueger et al. (1975). This DA-stimulated accumulation of cyclic AMP was reduced by 62% in the presence of 13 µM haloperidol and can therefore be ascribed to specific stimulation of the DA receptor.

Figure 1 shows the effect of different Li concentrations on the NA-sensitive accumulation of cyclic AMP obtained from guinea pig cortex and on the DA-sensitive accumulation of cyclic AMP from guinea pig caudate nucleus. Therapeutic concentrations of Li inhibit only the NA-sensitive accumulation of cyclic AMP. At 1.0 mM Li the difference between the NA-induced accumulation of cyclic AMP and the DA-induced accumulation of cyclic AMP is 54%, $P < 0.01$. Each point in Figure 1 is the average of 6 separate experiments for the caudate nucleus and 6 experiments for the cortex. In this series of experiments, limited in number by the aim of performing DA and NA stimulations under identical assay conditions, significant inhibition by Li of the NA-stimulated accumulation of cyclic AMP occurs at 2.0 mM and above ($P < 0.05$, Student's t-test) and significant inhibition by Li of the DA-stimulated accumulation of cyclic AMP occurs at 5.0 mM ($P < 0.05$, Student's t-test). However, the 22% inhibition by Li at 1.0 mM agrees with the data of Palmer et al. (1972), who showed significant inhibition by Li of the NA-stimulated accumulation of cyclic AMP at this Li concentration.

Discussion

These findings confirm (Palmer et al., 1972; Walker, 1974; Forn and Valdecasas, 1971) that therapeutic concentrations of Li inhibit the NA-induced accumulation of cyclic AMP and extend these previous reports to show that at low concentrations Li does *not* inhibit the DA-induced increase in cyclic AMP. This differential effect of Li may be relevant to Li's clinical profile. Since Li has no neuroleptic properties, its failure to inhibit the DA-sensitive accumulation of cyclic AMP agrees with the fact that in contrast to Li all known neuroleptic drugs inhibit the DA-sensitive enzyme (Clement-Cormier et al., 1974; Miller et al., 1974). The relative *increase* of DA-sensitive cyclic AMP accumulation in the presence of low concentrations of Li, although not statistically significant, may relate to reported exacerbations by this drug in some schizophrenic patients (Shopsin et al., 1971; Johnson et al., 1968). At clinically toxic concentrations (above 2 mM), Li loses its specificity and inhibits the DA-sensitive cyclic AMP accumulation as well as the NA-sensitive one. This finding offers a possible biochemical explanation for the Parkinsonian symptoms that have been reported in Li toxicity (Vacaflor, 1975; Shopsin et al.,

1970; Kirk et al., 1972), since Parkinsonian symptoms are thought to be related to reduction in DA neurotransmission.

Walker (1974) reported a specific inhibitory effect of a single Li concentration (0.5 mM) on a NA-sensitive adenylate cyclase from several brain regions and a lack of Li effect on the DA-sensitive enzyme obtained from caudate nucleus. However, Walker's results are difficult to evaluate since the NA-induced accumulation of cyclic AMP was only 14% above basal activity, and she used a frozen and thawed enzyme homogenate. In contrast to the homogenate used by Walker, only crude synaptosome or tissue slice preparations have consistently been shown to exhibit properties of a true adrenergic receptor (Chasin et al., 1974; Robinson and Sulser, 1976). Walker also reported an inhibitory effect of Li on the basal activity of the NA-stimulated enzyme, thus further complicating an evaluation of her small Li effect on the NA-stimulated enzyme activity. We find no inhibition by Li (up to 5.0 mM) of basal adenylate cyclase activity (results not shown).

An advantage of the crude synaptosome preparation used in the present experiment over tissue slices is the elimination of transsynaptic factors as possible mediators of the Li effect. The present experiments used a compromise preparation designed to achieve a DA and NA response in the same system. Only in this way can the effect of Li on the two cyclases be compared. Small differences in Li effects at different concentrations can be crucial in understanding Li's effects, but if different preparations are used the concentration-dependent differences may be merely artifacts. The inhibitory effect reported here by Li of cyclic AMP accumulation is most likely a direct effect of Li on adenylate cyclase activity. Unpublished observations in our laboratory have demonstrated that in the presence of a phosphodiesterase inhibitor Li still inhibits cyclic AMP accumulation. These results therefore rule out any effect of Li on cyclic AMP accumulation via activation of the degradative enzyme, phosphodiesterase. Furthermore, Li has been shown to directly inhibit adenylate cyclase activity from several tissues (Wolfe et al., 1970; Burke, 1970; Murphy et al., 1973; Wang et al., 1974).

The present strategy for investigating Li's mode of action is comparable to that employed by Snyder et al. (1974) in identifying a single common property of neuroleptic drugs. Since Li is unique in its class as a drug, we have used its narrow therapeutic range to exclude one biochemical action (DA-sensitive cyclic AMP accumulation blocking) as irrelevant and to include another biochemical action (NA-sensitive cyclic AMP accumulation blocking) as a candidate for a biochemical mechanism of therapeutic action. The difficulties of extrapolating from in vitro results to in vivo therapeutic mechanisms and from guinea pig brain to human brain are well known, however. More evidence is needed on the concentration of Li attained at specific cellular and subcellular sites in human brain during Li therapy in order for the strategy of comparing therapeutic and toxic effects to be fully exploited. Cross-species differences may be especially important in the interpretation of such effects as those reported here, which depend on narrow concentration gradients. Further development of techniques for measuring DA-sensitive adenylate cyclase in true tissue slices or NA-sensitive adenylate cyclase in true homogenates will allow easier investigation of Li's effects. In addition, the effect of therapeutic concentrations of Li on serotonin-sensitive adenylate cyclase and on acetylcholine-sensitive guanylate cyclase must be examined to test further the specificity of Li's effect on the NA-sensitive adenylate cyclase.

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