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Lithium inhibition of the adenosine-induced increase of adenylate cyclase activity

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Adenosine has been shown to stimulate accumulation of cyclic (c) AMP in brain tissue (Sattin & Rall, 1967; Rall & Sattin, 1970; Huang, Shimizu & Daly, 1971; Schultz & Daly, 1973). Although the exact mechanism of adenosine action is unknown this molecule most likely interacts directly with adenylate cyclase (Daly, 1976). In brain tissue slices adenosine appears to potentiate the effects of some neurohormones on formation of cAMP (Sattin & Rall, 1967; Rall & Sattin, 1970; Huang & others, 1971, Schultz & Daly, 1973; Daly, 1976). The precise function of adenosine in the cns is still unclear although the available evidence would suggest a general modulatory role in regulating neural transmission in several different brain areas (Daly, 1976).

Lithium, an effective drug in the treatment of mania (Schou & Thomsen, 1975), is known to inhibit a number of adenylate cyclases both within the cns (Dousa & Hechter, 1970; Forn & Valdecasas, 1971) and outside it (Wolff, Berens & Jones, 1970; Geisler, Wraae & Olesen, 1972; Ebstein, Belmaker & others, 1976). However, its inhibitory effect on this enzyme is not ubiquitous since some hormone-specific adenylate cyclases are not inhibited (Ebstein, Kara & Belmaker, 1977; Olesen, Jensen & Thomsen, 1974). The increasing importance attributed to adenosine in central neurotransmission prompted us to examine the effect of lithium on the adenosine-stimulated accumulation of cAMP in a crude synaptosome preparation from guinea-pig cortex and caudate nucleus. There has been no previous report of a specific inhibitor of the adenosine-induced stimulation of cAMP accumulation.

A crude vesicular synaptosome preparation was prepared by a modification of the method of Chasin, Mamrek & Samaniego (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 5% CO_2 in oxygen for 20 s and incubated in the presence of [^{3}H]adenine (5μCi/100 mg wet weight tissue; Amersham, 5Ci m mol^{-1}). After 40 min at 37° 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 separate vials and incubated after gassing for an additional 15 min at 37° in the simultaneous presence and absence of adenosine (10μM), noradrenaline (100μM) or lithium (1, 2 and 5mM). The reaction was stopped with 100 μl 1N HCl. cAMP was purified by Dowex 50 chromatography and precipitation with $ZnSO_4$ and Na_2CO_3 as described by Krishna, Weiss & Brodie, (1968). Samples were counted in a Packard Tri-Carb scintillation counter.

Basal formation of cAMP was calculated as the amount of radioactive cAMP formed from radioactive precursor in the absence of adenosine, noradenaline and lithium. Lithium (up to 5·0mM) had no effect on basal activity from either cortex or caudate nucleus. Addition of adenosine or noradrenaline caused a rise in the radioactive cAMP formation, from which basal activity was subtracted to yield the specific stimulus-induced rise in cAMP formation. On each experimental day the rise in cAMP formation was determined in quadruplicate in the absence of lithium, and then in duplicate at each of four lithium concentrations. The percentage inhibition caused by lithium was calculated by comparing the rise in cAMP formation with adenosine or noradrenaline alone to the rises in the presence of various lithium concentrations. The average stimulation due to adenosine in the cortex was 79 ± 10% and in the caudate nucleus was 46 ± 9%.

Fig. 1 shows the effect of different concentrations of lithium on the adenosine-induced accumulation of cAMP in guinea pig-cortex and caudate nucleus. Starting at 1mM, it inhibited the adenosine-stimulated

* Correspondence.

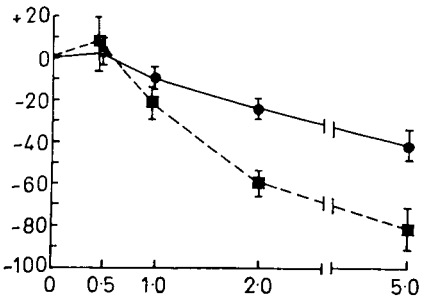

FIG. 1. Effect of lithium on adenosine-induced accumulation of cAMP in guinea-pig cortex (●—●) and caudate nucleus (■- - ■). Each point on the curve represents the average of 8 determinations. The concentration of adenosine is 10 μM. Ordinate: % change. Abscissa: Lithium (mM).

increase in cAMP concentration in both caudate nucleus (−22% $P<0.05$ paired t-test) and cortex (−12% $P<0.05$, paired t-test). At 2·0 and 5·0mM the caudate nucleus is significantly more sensitive to lithium than the cortex ($P<0.05$, Student's t-test).

In separate experiments the combined effect of adenosine and noradrenaline was examined in guinea-pig cortex. Noradrenaline alone elevated cAMP concentrations by 38 ± 6% (s.e.m., n = 12 experiments) and adenosine alone elevated them by 44 ± 6% (s.e.m., n = 12); the combination stimulated cAMP synthesis by 75 ± 16% (s.e.m., n = 12).

The inhibitory effect of therapeutic and near therapeutic concentrations of lithium on the adenosine-stimulated accumulation of cAMP provides an additional possible biochemical mechanism for the clinical efficacy of lithium. In therapeutic concentrations it has been previously reported to inhibit the noradrenaline induced rise in cAMP accumulation (Forn & Valecasas, 1971; Palmer, Robison & others, 1972; Walker, 1974; Reches, Belmaker & Ebstein, in preparation) whereas inhibition of the dopamine-induced rise in cAMP formation activity was achieved only at clinically toxic doses (Reches & others). Until the role of adenosine in neural transmission becomes more defined it is difficult to evaluate the full significance of the inhibition by lithium of adenosine-stimulated increases in cAMP concentration. If, however, as has been suggested (Daly, 1976) adenosine plays a modulating role in neural transmission by interacting with neurotransmitter-specific adenylate cyclases, then the inhibitory effect of lithium reported here may be an integral part of its action in mania.

In tissue slices adenosine potentiates the action of some neurohormones in a greater than additive fashion (Sattin & Rall, 1967; Rall & Sattin, 1970; Huang & others, 1971; Schultz & Daly, 1973; Daly, 1976). However, in the crude synaptosome preparation used in the present study the effect of adenosine and noradrenaline are strictly additive. These results suggest that separate receptors exist for adenosine and noradrenaline. Synergistic effects between adenosine and noradrenaline reported in tissue slices (Sattin & Rall, 1967; Rall & Sattin, 1970; Huang & others, 1971; Schultz & Daly, 1973; Daly, 1976) may therefore be due to trans-synaptically mediated events which are eliminated in the crude synaptosome preparation used by us.

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