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Chronic lithium prevents reserpine-induced supersensitivity of adenylate cyclase

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Pert et al (1978) reported that chronic oral 0·2% lithium (Li) can block the development of increases in dopamine receptor number in the rat caudate nucleus induced by chronic haloperidol. Li plasma concentrations were 0·8–1·0 mM. These authors suggested that Li may act therapeutically by stabilizing receptor number changes. Treiser & Kellar (1979) found that chronic 0·2% oral Li, yielding plasma concentrations of 0·8–1·1 mM, was able to prevent the development of reserpine-induced increases in rat cortex β-adrenoceptor number. Chronic Li alone was found by both Rosenblatt et al (1979) and Treiser & Kellar (1979) to cause a slight decrease in β-adrenoceptor number. Since noradrenaline-stimulated adenylate cyclase activity and radioligand measured β-adrenoceptor number are closely parallel in many systems (Lefkowitz & Williams 1978), we decided to investigate the effect of chronic Li on reserpine-induced increases in rat cortical noradrenaline-sensitive adenylate cyclase activity (Baudry et al 1976).

Male albino rats (Sabra strain, 200–250 g) obtained from the Hebrew University were used. Rat food containing 0·15% LiCl was prepared by grinding regular rat pellets to a fine powder and thoroughly mixing with LiCl. The Li-treated rats were maintained on this diet for 3–5 weeks and then received a series of 4 days of i.p. injections of reserpine or 0·9% NaCl (saline) while continuing to receive Li orally. Reserpine dose was 5 mg kg^{-1} on the first day and 2·5 mg kg^{-1} on the following 3 days. Animals were decapitated 24 h after the last reserpine dose and the brains rapidly removed and placed on a precooled cutting block at 4–8°C. Carotid blood at death was taken for Li determination, which averaged 0·4 mM. Cortical 1 mm cubes were prepared using a McIllwain Tissue Chopper. The tissue was incubated in Krebs Ringer bicarbonate solution containing glucose as described by Kakiuchi & Rall (1968) with continuous gassing (95% O_2 and 5% CO_2). After 20 min preincubation the Ringer solution was changed and the tissue incubated for an additional 10 min. At the end of the second incubation period noradrenaline was added for an additional 12 min. The reaction was stopped by addition of 95% ethanol. The tissue was homogenized in ethanol and an aliquot evaporated down and the cyclic (c)AMP assayed using a kit supplied by the Radiochemical Centre Amersham, U.K. Protein was determined by the procedure of Lowry et al (1951).

The results shown in Table 1 demonstrate that chronic Li pre-treatment can prevent reserpine-induced increases in noradrenaline-induced cAMP accumulation. This is the first demonstration that Li prevents receptor supersensitivity as measured by adenylate cyclase activity. The results parallel those of Treiser &

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Table 1. The effect of chronic Li pretreatment on the reserpine-induced increase in cAMP accumulation. cAMP values in p mol mg^{-1} protein represent the mean of 5 experiments on separate days, each experiment used four rat brains in each of the four groups.

	Control	Reserpine	Lithium	Reserpine + Li
Basal	91 ± 36*	108 ± 20	72 ± 16	66 ± 19
10 μM NA	154 ± 27	452 ± 109	181 ± 30	378 ± 65
50 μM NA	207 ± 14	615 ± 97[a]	250 ± 23	310 ± 73[b]

* s.e.m.
(a) $P < 0.01$ (one-tail) Student's $t = 3.6$, reserpine vs control
(b) $P < 0.03$ (one-tail) Student's $t = 2.23$, reserpine plus Li vs reserpine

Kellar (1979) who used radioligand measurement of β-adrenoceptor number. The discrepancy between the slight decrease in β-adrenoceptor number reported with chronic Li alone (Treiser & Kellar 1979) and the lack of effect of chronic Li alone on noradrenaline-sensitive adenylate cyclase (Table 1) may be due to an α-component of the noradrenaline-sensitive adenylate cyclase activity, as α-receptor number tends to increase with chronic Li treatment (Rosenblatt et al 1979).

It is clear from Table 1 that the concentrations of Li attained in the present paradigm cannot be shown to directly inhibit noradrenaline-sensitive adenylate cyclase ex vivo. Li has previously been shown to inhibit rat cortical noradrenaline-sensitive adenylate cyclase activity in vitro at 1·0–2·0 mM concentrations (Forn & Valdecasas 1971; Reches et al 1978). Chronic Li administration to rats yielding mean plasma Li concentrations of 1·7 mM caused ex vivo inhibition of rat cortical noradrenaline-sensitive adenylate cyclase activity. However, no supersensitivity or change in adenylate cyclase activity was observed 2 or 4 days after cessation of chronic Li treatment (Ebstein et al 1979). It was suggested that ability to block noradrenaline-sensitive adenylate cyclase without inducing compensatory supersensitivity may be a unique property of Li, relating to its therapeutic mechanism of action, and differentiating it from β-adrenoceptor blocking drugs like propranolol that induce compensatory receptor sensitivity changes (Wolfe et al 1978).

The effect of Li to directly inhibit noradrenaline-sensitive adenylate cyclase seemed to require close to 2·0 mM concentrations (Ebstein et al 1980), whereas Li prevention of β-adrenoceptor number changes occurs at lower concentrations (Treiser & Kellar 1979). In the present study we have shown that low Li concentrations, incapable of directly inhibiting noradrenaline-sensitive adenylate cyclase, are capable of preventing the reserpine-induced increase in noradrenaline-sensitive adenylate cyclase activity. These results suggest that the ability of Li to block the reserpine-induced increase in adenylate cyclase activity is not dependent on the previously demonstrated effect of Li (at 1·7 mM plasma concentrations) to directly inhibit noradrenaline-sensitive adenylate cyclase. However, the removal of cortical tissue after chronic Li treatment and incubation in Li-free medium during the pre-incubation and noradrenaline-stimulation leads to significant washout of Li from the brain tissue. Direct effects of Li that require its continued presence may well be underestimated by these methods. Similarly, in vitro estimation of Li effects on cAMP accumulation in brain slices is limited by the slow diffusion of Li into tissues, and thus these methods may also underestimate the effects of low Li concentrations. It is only a preliminary conclusion, therefore, that Li action to prevent receptor supersensitivity occurs at clinically 'prophylactic' concentrations (0·4–1·1 mM) whereas direct inhibition of noradrenaline-sensitive adenylate cyclase occurs only at 'acute treatment' Li concentrations (1·0–2·0 mM).

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