INHIBITION OF THYROTROPIN-STIMULATED ADENYL CYCLASE ACTIVITY OF BEEF THYROID MEMBRANES BY LOW CONCENTRATION OF LITHIUM ION

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Summary

Lithium inhibits the TSH-induced stimulation of adenyl cyclase activity in beef thyroid membranes without affecting basal activity. Half-inhibition occurs at 4-8 mM Li^+ when the Mg^{++} concentration is 2.5 mM and at greater Li^+ concentrations as the Mg^{++} concentration is raised. A relation of the inhibition to the acute antithyroid effects of Li^+ is suggested.

We have shown recently (1) that lithium administration to rats acutely blocks several steps in thyroidal iodine metabolism. This includes inhibition of 1) the ability to accumulate iodide, 2) the organification of iodine measured as the radioiodine uptake, and 3) the release of iodine from the gland. It is known that these parameters are under the control of the thyrotropic hormone (TSH). Since a number of responses to TSH are apparently mediated by the adenyl cyclase system (2), it seemed possible that Li^+ exerted some of its inhibitory effects on iodine metabolism via this enzyme system. This view was strengthened by the report (3) that large concentrations of Li^+ produced partial inhibition of the ACTH-induced stimulation of the adenyl cyclase of fat cell ghosts.

MATERIALS AND METHODS

All experiments were carried out on beef thyroid membranes that were stored in liquid nitrogen. Beef thyroids were homogenized in 0.25M sucrose containing 3.0 mM Tris HCl pH 7.4, 1.0 mM dithiothreitol, and 1.0 mM ethylene glycol-bis (β-amino ethyl ether)N,N'-tetracetic acid (EGTA) as the Mg^{++} salt.

After removal of debris at 100xg, the upper portion of a twice washed 11,000xg pellet was purified on a discontinuous sucrose gradient (30, 40 and 45% sucrose) in a SW 25.2 Spinco rotor at 2°C. The middle interface was collected, diluted with water or 0.25M sucrose, sedimented, and stored frozen in the 0.25M sucrose medium. Details of this preparation will be described elsewhere.

Adenyl cyclase was assayed at 37°C for 10 min, according to the method of Krishna, et al. (4), as modified by Birnbaumer et al. (3). The total incubation volume was 60 µl containing 2.7 mM ATP-α-^{32}P, (obtained from the International Chemical and Nuclear Corp.) 10 mM theophylline, 0.1% crystalline bovine serum albumin, 25 mM Tris HCl, pH 7.40, 10 mM creatine phosphate, 20 µg of rabbit muscle creatine kinase (Sigma), and bovine serum albumin to concentrations of 0.1%. Fluoride was present at 10 mM where indicated. The $MgCl_2$ concentration was varied as indicated in the text. Lithium and sodium were added as the chlorides; bovine TSH (4 units/mg) was added in 0.1% albumin at a concentration of 200 mU/ml. ATPase was determined as previously described (5).

RESULTS AND DISCUSSION

As shown in Fig. 1, low concentrations of Li^+ markedly reduced the stimulation of thyroid membrane adenyl cyclase activity produced by TSH. In four experiments and with three membrane preparations the concentration yielding a 50% reduction in the TSH effect varied from 4-8 mM. This was true only when the Mg^{++} concentration was 2.5 mM. When this was increased to 5 mM Mg^{++} the 50% inhibition point occurred at 30 mM Li^+ (not shown). This suggests that Li^+ competes with Mg^{++} in this system, and plots of the reciprocal of the rate of TSH-stimulated adenyl cyclase activity against Li^+ concentration at various Mg^{++} levels are consistent with competitive kinetics.

That this Li^+ effect was not merely one of ionic strength is shown by the curve depicting inhibition of the TSH-stimulated adenyl cyclase activity by Na^+ ion (Fig. 1). Even at 50 mM Na^+ (plus the ~6 mM present in the

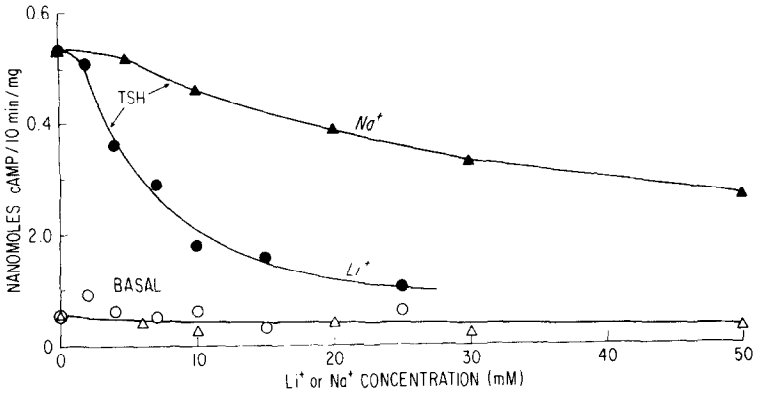

Fig. 1 - The inhibitory effect of Li^+ and Na^+ ions on beef thyroid adenyl cyclase. The Mg^{++} concentration is 2.5 mM. 0-0 and ●-● stand for Li^+ experiments; Δ-Δ and ▲-▲ stand for Na^+ experiments. 0-0 and Δ-Δ stand for basal activity and ●-● and ▲-▲ for cyclase activity stimulated with 200 mu/ml of bovine TSH.

normal incubation medium), inhibition was only approximately 50%. Potassium ion had an inconstant effect, increasing basal activity in three membrane preparations an average of 27% and TSH-stimulated activity an average of 79% at 30 mM K^+, while having no effect in a fourth, highly active preparation.

Since it has been shown that there are two Mg^{++} requirements in adenyl cyclase of fat cells, one for the formation of Mg-ATP the other on the enzyme (3), and assuming that a similar two-site requirement existed for the thyroidal adenyl cyclase, it seemed important to know which of these two sites was the locus of the Li^+ effect. We, therefore, measured the effect of Li^+ on the ouabain-sensitive ATPase present in these same membrane preparations. As shown in Table I, 50 mM Li^+ was without effect. This suggests that Li^+ acts on the Mg^{++} bound to the cyclase rather than that bound to ATP.

In view of the possible interaction of lithium with divalent cations or Ca^{++} and Mg^{++} - dependent processes (6,7,8), we studied in greater detail the role of Mg^{++} on the inhibition by Li^+ of TSH stimulation of thyroid membrane adenyl cyclase. It is clear from Fig. 2 that the Mg^{++} concentration has a profound effect on the ability of Li^+ to block the TSH-induced stim-

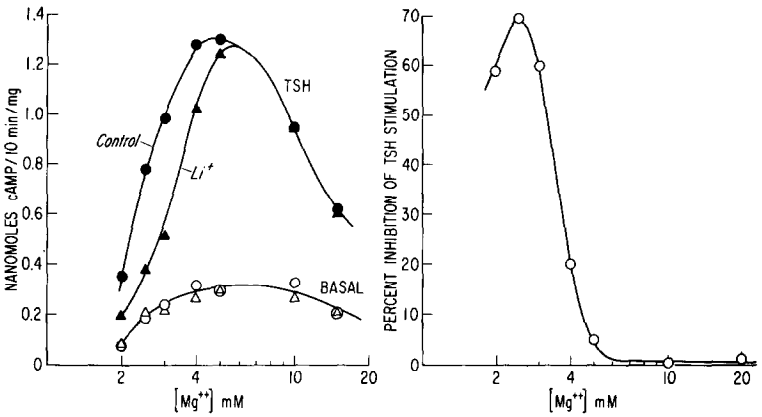

Fig. 2 - The effect of Mg^{++} concentration on the Li^{+}-inhibition of TSH-stimulated adenyl cyclase activity · Li^{+} concentration is 10 mM, TSH concentration 200 mu/ml.
Left panel: 0-0 and ●-● stand for controls and Δ-Δ and ▲-▲ stand for Li^{+} experiments · 0-0 and Δ-Δ stand for basal activity and ●-● and ▲-▲ for TSH-stimulated adenyl cyclase.
Right panel: Percent inhibition is expressed as

$$1 - \frac{\text{TSH - basal activity (with } Li^{+})}{\text{TSH - basal activity (without } Li^{+})}$$

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ulation of adenyl cyclase activity. The control and Li^{+} curves become superimposable at Mg^{++} concentrations greater than 5 mM. When plotted as TSH "effect," i.e., TSH-stimulated activity minus basal activity, it can be seen that 10 mM Li^{+} had a maximal inhibitory effect at 2.5 mM Mg^{++}.

Fluoride stimulation of adenyl cyclase activity was reduced at much higher Li^{+} concentrations. In four membrane preparations the concentration for 50% inhibition exceeded 50 mM Li^{+}.

The cation potencies may be written in the order $K^{+}<Na^{+}<Li^{+}$; this is also the well-known order of potencies affecting macromolecular conformations (9,10). Mg^{++} is the next member in this increasing order of potencies. However, the denaturing effects occur at much larger concentrations than required in the present study. It is of interest that for dipeptidyl arylamidase II, Li^{+} is inhibitory (20% at 9 mM) in the absence of a requirement for a divalent metal ion. The order of potencies for the alkali cations is, however, the reverse from that shown above (11). We have, at present,

no explanation for the interaction between Li^+ and Mg^{++}. While the crystal radii of these two cations are quite similar [Li^+ = 0.60 Å, Mg^{++} = 0.65 Å (Pauling)], it is uncertain to what extent these are applicable rather than the very different hydrated radii [Li^+ = 2.30 Å, Mg^{++} = 3.44 Å (Moelwyn-Hughes)]. It is known, however, that the reciprocal of the crystal radius can be one of the many parameters determining the stability or formation constants of coordination complexes for elements within the same period (12). Thus factors involving the interaction of Li^+ with the Mg^{++} site on the enzyme may include the crystal radius as a determinant.

As has been found by others (3) basal adenyl cyclase activity is increased by raising the concentration of Mg^{++} in the medium. Unlike the fat cell, the thyroidal basal activity plateaus, or may even fall, at high Mg^{++} concentrations (Fig. 2). Another difference from the fat cell is that the hormone stimulated activity also falls with large Mg^{++} levels (Fig. 2).

The intrathyroidal Li^+ concentrations that have been achieved in acute experiments in rats are in the range of 5-10 mM (Berens, unpublished obser-

TABLE I

Lithium and Beef Thyroid ATPase Activity

Li^+ concentration (mM)	Mg^{++} Activity (Pi(μmoles/mg/h)	Mg^{++}-Na^+-K^+ Activity* (Pi(μmoles/mg/h)
0	34.6	21.2
5	33.8	22.6
20	34.2	22.2
50	34.6	25.4

The medium contained: 5.0 mM ATP, 5.0 mM Mg^{++}, 0.1M Tris HCl pH 7.4, 25 mM Na^+, 4.6 mM K^+, 1.0 mM ouabain where indicated, and 35 μg of membrane protein added to start the reaction. All values are averages of duplicates.

*Ouabain sensitive ATPase.

vations). Assuming that this Li^+ is uniformly distributed in the gland, such concentrations fall within the range of adenyl cyclase sensitivity and it is therefore possible that inhibition of this enzyme system accounts for the acute inhibitory effects of Li^+ on iodine metabolism. These findings do not, however, rule out Li^+ effects at other loci in the thyroid gland.

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