Influence of Lithium on Cell Function in Two Different Cell Systems

(lithium / FRTL-5 / CHO / cAMP / TSH)

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Abstract. Lithium is widely used in the treatment and prophylaxis of bipolar psychiatric disorders. It accumulates in the thyroid gland and can cause goitre or thyroid dysfunction. The mechanisms of various effects of the lithium ion on thyroid cells have not been completely clarified. The aim of our work was to establish whether lithium, in the presence or absence of TSH, stimulates the synthesis of cAMP; as model systems we used a strain of rat thyroid follicular cells FRTL-5 and a line of Chinese hamster ovary fibroblasts with the human TSH receptor (CHO-R). Lithium at concentrations of 0.35 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM without TSH and at selected concentrations with TSH stimulation significantly increased cAMP synthesis in FRTL-5 and in CHO-R cells when compared with controls without lithium. These results are different from the published data, which have been unable to confirm the influence of lithium on cAMP synthesis or have even reported the inhibition of cAMP synthesis. However, in most published investigations only lithium in combination with TSH was tested. In conclusion, lithium was found to stimulate cAMP synthesis in FRTL-5 cells and in CHO-R cells.

As lithium carbonate, lithium is widely used in the treatment and prophylaxis of bipolar affective disorders (Schou, 1989). Therapeutic concentrations of lithium should be between 0.5 mM and 1.2 mM (Kallner and Petterson, 1995). Lithium accumulates in the thyroid gland; its intrathyroidal concentration can be 2.5–5 times higher than its serum concentration (Salata and Klein, 1987; Braverman and Utiger, 1996). During lithium therapy, higher incidences of goitre (Perrild et al., 1990; Boccheta et al., 1991; Boccheta et al., 1996), hypothyroidism (Leroy et al., 1988; Clower, 1989) and, in rare cases, hyperthyroidism (Barclay et al., 1994) have been observed. *In vivo* lithium inhibits processes such as iodine uptake, the coupling of iodothyrosines, the release of thyroxine and triiodothyronine (Davies and Franklyn, 1991; Braverman and Utiger, 1996).

Signal pathways that are stimulated by the lithium ion have not been completely identified. Since a number of thyroid cell functions are mediated by the cAMP pathway (Ciullo et al., 2001; Richards, 2001; Dremier et al., 2002), the influence of lithium on cAMP synthesis has been studied in various cell systems *in vitro*. Lithium at a concentration of 5 mM inhibited the TSH-stimulated but not the basal synthesis of cAMP in canine thyroid slices (Tseng et al., 1989). In porcine thyroid cells, lithium suppressed cAMP synthesis induced by TSH stimulation (Tsuchiya et al., 1990). In FRTL-5 cells, Urabe et al. (1991) could not confirm the inhibition of TSH-stimulated cAMP synthesis; they presumed the activation of other signal systems (protein kinase C system).

The aim of our study was to test whether lithium inhibits, stimulates or has no effect on cAMP synthesis. The effects of lithium on cAMP synthesis were tested in the presence and absence of TSH stimulation, using a strain of rat thyroid follicular cells FRTL-5 and a line of Chinese hamster ovary fibroblasts with TSH receptor (CHO-R).

Material and Methods

Cell culture

Experiments were performed using an FRTL-5 (Fischer rat thyroid cells in low serum) cell line (Ambesi-Impiombato et al., 1980) and a CHO-R cell line (Chinese hamster ovary fibroblasts with the human TSH receptor). FRTL-5 cells maintain most of the differentiated functions of normal thyroid cells, but are unable to organify iodide. CHO-R cells have a transfected human TSH receptor on their surface and can synthesise cAMP under stimulation with TSH.

FRTL-5 cells were grown in the Coon modified Ham F-12 medium (Sigma Chemical Co., Deisenhofen, Germany) supplemented with 5% calf serum (Gibco BRL, Paisley, UK) and a six-hormone mixture consist-

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Abbreviations: cAMP – cyclic adenosine 3',5'- monophosphate, CHO-R – Chinese hamster ovary fibroblasts with the human TSH receptor, FRTL-5 – Fischer rat thyroid cells in low serum, TSH – thyrotropin.

ing of insulin (10 µg/mL), transferrin (5 µg/mL), hydrocortisone (0.36 ng/mL), somatostatin (10 ng/mL), glycyl-L-histidyl-L-lysine acetate (2 ng/mL) and thyrotropin (1 mU/mL), all purchased from Sigma Chemical Co.

CHO-R cells were cultured in the RPMI-1640 medium, supplemented with glutamin (1 mM) and 10% foetal calf serum, all purchased from Gibco BRL.

Both cell lines were grown in a Heraeus-CO_2-autozero incubator (Heraeus Instruments, Hanau, Germany) in an atmosphere of 5% carbon dioxide and 95% air at 37˚C, 100% humidity.

Cyclic AMP assay

The amount of 9.6 x 10^4 FRTL-5 cells were seeded in 24-well plates. They grew for three days in the six-hormone medium and in the five-hormone medium (without TSH) for a further seven days. The wells were washed twice with Hanks' balanced salt solution (HBSS) and 10 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid). Lithium carbonate, dissolved in hypotonic HBSS without NaCl + 10 mM HEPES (Kohn and Valente, 1989), was added in the following concentrations: 0 mM (controls), 0.35 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM with TSH and without it, all in quadruplicate. In each well, 0.5 mM IBMX (3-isobutyl-1-methyl-xanthine), an inhibitor of phosphodiesterase, which breaks down cAMP, was added.

The amount of 4.6 x 10^4 CHO-R cells were seeded in 24-well plates. They grew for two days in the RPMI 1640 medium. After two days, lithium carbonate dissolved in HBSS without NaCl + 10 mM HEPES was added in the following concentrations: 0 mM (controls), 0.35 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM with TSH and without it, all in quadruplicate. Additionally, TSH in concentrations of 0 mU/mL, 0.01 mU/mL, 0.1 mU/mL and 1 mU/mL was tested. In each well, 0.5 mM IBMX was added.

Plates with both cell lines were incubated for 2 h at 37˚C in a humidified atmosphere of 5% carbon dioxide and 95% air. The supernatant was aspirated; cAMP was determined in the supernatant using an Immunotech radioimmunoassay kit (Immunotech International, Marseille Cedex, France). The amount of cAMP was expressed as the number of picomoles of cyclic AMP per well.

Statistics

The results of experiments are the averages of quadruplicate determinations ± SD and have been statistically analysed using an analysis of variance and t-test.

Results

The influence of lithium without TSH stimulation on cyclic AMP production in FRTL-5 cells is shown in Fig. 1. Lithium at concentrations of 0.35 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM significantly stimulated cAMP production when compared with controls without lithium. With TSH stimulation (Fig. 2), lithium significantly stimulated cAMP synthesis at concentrations of 0.35 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM. In FRTL-5 cells we did not observe any inhibition of cAMP synthesis under the influence of lithium, in the presence or absence of TSH stimulation.

Lithium in concentrations of 1 mM, 1.4 mM, 1.7 mM and 2 mM without TSH stimulation significantly increased cAMP synthesis in CHO-R cells when compared with controls without lithium, as shown in Fig. 3. In the presence of TSH stimulation (Fig. 4), lithium increased cAMP synthesis at concentrations of 1 mM, 1.7 mM and 2 mM. Additionally, TSH alone at concentrations of 0.01 mU/mL, 0.1 mU/mL and 1 mU/mL significantly stimulated cAMP synthesis when compared with controls without TSH (Fig. 5). In CHO-R cells we did not observe any inhibition of cAMP synthesis under the influence of lithium, with or without TSH stimulation.

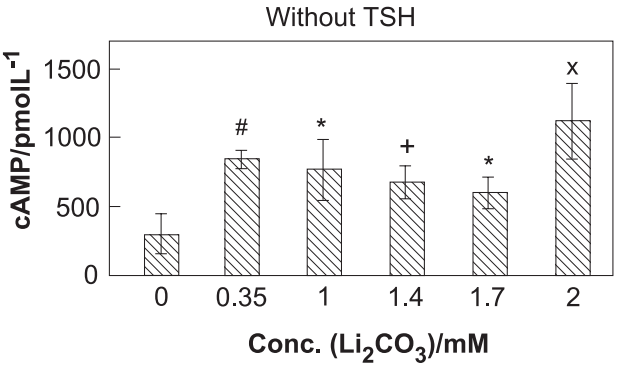

Fig. 1. Influence of lithium on cAMP production in FRTL-5 cells. Bars represent average values of quadruplicate determinations ± SD. Significant differences of results compared with controls without lithium are depicted (* = P < 0.05, + = P < 0.01, **x** = P < 0.005, # = P < 0.001).

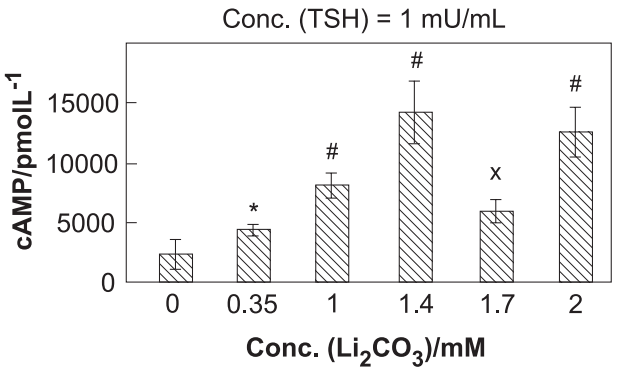

Fig. 2. Influence of lithium + TSH on cAMP production in FRTL-5 cells. Bars represent average values of quadruplicate determinations ± SD. Significant differences of results compared with controls without lithium are depicted (* = P < 0.05, + = P < 0.01, **x** = P < 0.005, # = P < 0.001).

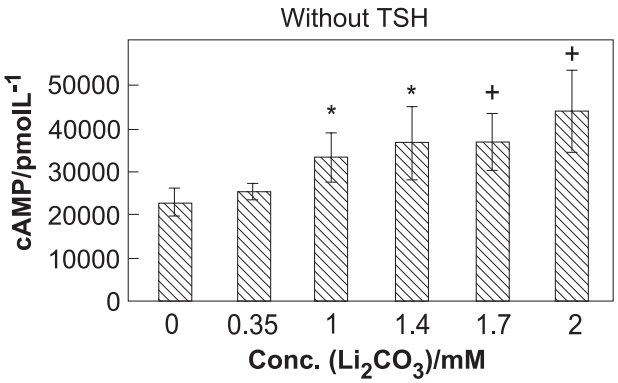

Fig. 3. Influence of lithium on cAMP production in CHO cells. Bars represent average values of quadruplicate determinations ± SD. Significant differences of results compared with controls without lithium are depicted (* = P < 0.05, + = P < 0.01, **x** = P < 0.005, # = P < 0.001).

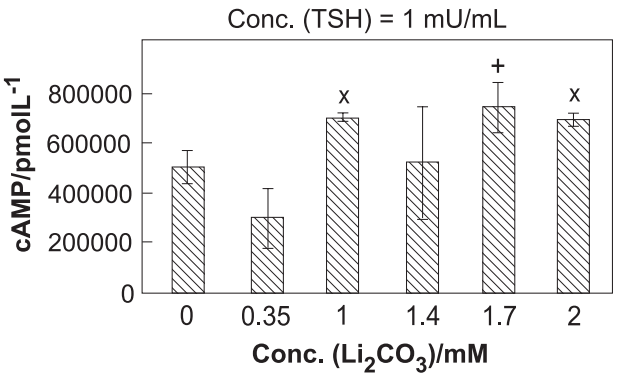

Fig. 4. Influence of lithium + TSH on cAMP production in CHO cells. Bars represent average values of quadruplicate determinations ± SD. Significant differences of results compared with controls without lithium are depicted (* = P < 0.05, + = P < 0.01, **x** = P < 0.005, # = P < 0.001).

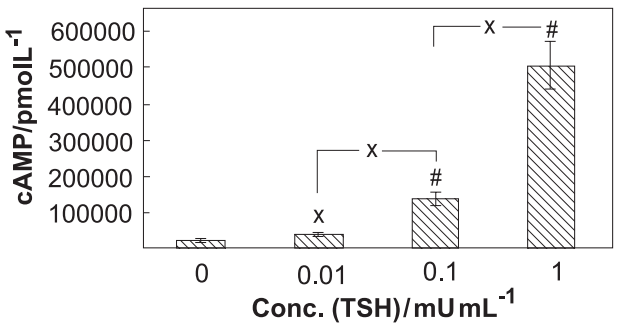

Fig. 5. Influence of TSH on cAMP production in CHO cells. Bars represent average values of quadruplicate determinations ± SD. Significant differences of results compared with controls without TSH are depicted (* = P < 0.05).

Discussion

In our study, the stimulative effect of lithium on cAMP synthesis in FRTL-5 cells and in CHO-R cells in the presence and absence of TSH stimulation at selected concentrations between 0.35 mM and 2 mM is shown. We did not observe any inhibition of cAMP synthesis under the influence of lithium.

Our results are different from the observations of Tsuchiya et al. (1990), who reported that, in porcine thyroid cells, lithium inhibited cAMP synthesis in the presence of TSH. However, they did not test the influence of lithium on its own. Mori et al. (1989) observed a decrease in cAMP content in the thyroid at a concentration of 10 mM of lithium on perfused mouse thyroids in the presence of TSH. There, the cAMP content was measured only in the presence of TSH and at concentrations above the therapeutic range. In canine thyroid slices, lithium at concentrations between 5 and 100 mM did not stimulate cAMP production, while cAMP synthesis was inhibited at a concentration of 5 mM of lithium in the presence of TSH (Tseng et al., 1989). Recently, Tasevski et al. (2000) observed a slight inhibition of cAMP synthesis at a concentration of 10 mM of lithium in FRTL-5 cells; otherwise, no effect of lithium on cAMP production in the presence or absence of TSH was observed. Discrepancies in cAMP concentrations in different studies could be due to different lithium concentrations, different methods for measuring cAMP, and different cell systems. In our work, we used the well-established method for extracellular cAMP determination (Kohn and Valente, 1989) in a hypotonic buffer with a recommended incubation time (two hours).

Our results are partly compatible with the results of Urabe et al. (1991), who observed that lithium in FRTL-5 cells and in isolated porcine thyroid follicles did not significantly inhibit TSH-induced cAMP synthesis, while at a concentration of 2 mM of lithium a slight stimulation of cAMP synthesis was observed. Urabe et al. (1991) also reported that lithium without TSH did not stimulate cAMP synthesis. However, their cell systems were under the influence of lithium for 48 h, while in our experiment the duration of incubation was two hours. Hence, the differences in results could, at least partly, be explained by different incubation times. With a longer incubation time and in the presence of TSH, activation of G-protein-coupled receptor kinase (GRK), which phosphorylates the TSH receptor, can occur. GRK then attracts inhibitory proteins (arrestins) to the receptor and blocks its ability to interact with G proteins (Nagayama et al., 1996). As a consequence, cAMP concentrations decrease. Additionally, TSH stimulates activation of the cAMP-specific phosphodiesterase, which breaks down cAMP and decreases cAMP concentrations (Oki et al., 2000; Ito et al., 2001).

Based on the discrepant data on the effects of lithium on cAMP synthesis and on the differences between our results and the results of other authors, we decided to test the effects of lithium on cAMP synthesis in CHO-R cells as well. As mentioned before, the CHO-R cells have a transfected functional human receptor for TSH

on their surface and can activate G proteins and synthesize cAMP (Perret et al., 1990). They are widely used for biological activity measurements of different substances and antibodies (Perret et al., 1990; Van Sande et al., 1990; Costagliola et al., 1992; Michelangeli et al., 1994). Our experiments performed on CHO-R cells confirmed the results that we obtained on FRTL-5 cells. By contrast, Van Sande et al. (1990) observed that lithium at a concentration of 10 mM (only this concentration was tested) had no influence on TSH-stimulated cAMP synthesis in CHO-R cells. However, they did not test the influence of lithium on its own.

The results of our experiments with cAMP synthesis indicate that lithium could stimulate cAMP synthesis and that lithium does not inhibit TSH-stimulated cAMP synthesis. One possible mechanism could be the interference of lithium – as a monovalent cation – with the TSH receptor, probably by changing its tertiary structure (Singer and Rotenberg, 1973). The changed TSH receptor could activate the adenyl cyclase through the G_s proteins (Michelangeli et al., 1994). A second mechanism of the lithium effect could be the stimulation of the more distal site, for example the adenyl cyclase molecule itself. Otherwise, the lithium ion is supposed to stimulate the thyroid cell through the protein kinase C system (Boehm et al., 1980) and, as recently shown by Tasevski et al. (2000), through *de novo* cholesterol synthesis and G-protein prenylation, an important transduction pathway in FRTL-5 cells.

In conclusion, we have found the stimulative effect of lithium on cAMP synthesis in FRTL-5 cells and in CHO-R cells in the presence and absence of TSH stimulation. No inhibition of cAMP synthesis under the influence of lithium was observed.

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