Influence of Lithium on Growth and Viability of Thyroid Follicular Cells

(lithium / FRTL-5 / ^{3}H-thymidine incorporation / ^{51}Cr release)

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Abstract. Lithium accumulates in the thyroid gland and can cause goiter or thyroid dysfunction. The aims of our work were: 1) to verify whether lithium stimulates proliferation of thyroid cells; as methods, the ^{3}H-thymidine incorporation assay and the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were used; as a model system the FRTL-5 (Fischer rat thyroid cells in low serum) cell line was selected, 2) to test whether lithium can have a cytotoxic effect on FRTL-5 cells, using the cytotoxicity assay with ^{51}Cr release and the trypan blue exclusion method. Without TSH stimulation, lithium at 0.35–2 mM concentrations significantly increased the ^{3}H-thymidine incorporation. A similar effect was observed in the case of the MTT assay: without TSH stimulation, lithium at 0.4–2 mM concentrations showed a significant stimulation of proliferation. Surprisingly, under TSH stimulation, lithium at the 2 mM concentration significantly inhibited proliferation of FRTL-5 cells. With the cytotoxicity assay, lithium was found to increase ^{51}Cr release at 1.4–2 mM concentrations. Additionaly, the percentage of viable FRTL-5 cells at 0.35–2 mM concentrations of lithium was lower than in the controls without lithium. In conclusion, lithium was found to stimulate proliferation of FRTL-5 cells in conditions without TSH and, surprisingly, lithium in higher concentrations diminished proliferation of FRTL-5 cells under TSH stimulation. A cytotoxic effect of higher lithium concentrations was observed.

Lithium as lithium carbonate has been widely used in treatment and prophylaxis of recidivant bipolar affective disorders in therapeutic concentrations between 0.5 mM and 1.2 mM (Rosenthal and Goodwin, 1982; Schou, 1989; Kallner and Petterson, 1995). Intrathyroid concentrations of lithium can be 2.5–5 times higher than its serum concentrations (Salata and Klein, 1987). Lithium therapy has been associated with higher incidence of goiter (Schou et al., 1968; Lazarus and Bennie, 1972; Perrild et al., 1990; Boccheta et al., 1996), hypothyroidism (Lindstedt et al., 1977; Leroy et al., 1988; Yassa et al., 1988; Clower, 1989), and, rarely, hyperthyroidism (Rosser, 1976; Barclay et al., 1994). *In vivo* lithium inhibited iodine uptake, coupling of iodothyrosines, and release of thyroxine and triiodothyronine (Bhattacharyya and Wolff, 1976; Davies and Franklyn, 1991). *In vitro* lithium increased ^{3}H-thymidine uptake in porcine primary cultures and in FRTL-5 cells (Urabe et al., 1991). Additionally, some cases of lithium-associated thyroiditis have been reported. A cytotoxic effect of lithium has been assumed (Kontozoglou and Mambo, 1983). Presumably, the cytotoxic effect of lithium has not yet been tested *in vitro*.

The first aim of our work was to verify whether lithium had a mitogenic effect. For this purpose we used the ^{3}H-thymidine incorporation assay and the MTT assay. The second aim was to test whether lithium could have a cytotoxic effect on FRTL-5 cells. We used the cytotoxicity assay with ^{51}Cr release and the trypan blue exclusion assay for cell viability estimation.

Material and Methods

Cell culture

The experiments were performed using the FRTL-5 cell line (Ambesi-Impiombato et al., 1980). FRTL-5 cells maintain most of the differentiated functions of normal thyroid cells but are unable to organify iodide.

FRTL-5 cells were grown in a 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 consisting 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.

Cells were grown in a Heraeus-CO_2-auto-zero incubator (Heraues Instruments, Hanau, Germany) in an atmosphere of 5% carbon dioxide and 95% air at 37°C, 100% of humidity.

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^{3}H-thymidine incorporation: growth assay

The quantity of 6.7 x 10^4 FRTL-5 cells were seeded in 24-well plates. They grew for 3 days in the six-hormone medium and, for further 7 days, in the five-hormone medium (medium without TSH). The final volume of the culture medium was 500 µl. The wells were then washed once with the five-hormone medium. Lithium carbonate (Fluka Chemie, Buchs, Switzerland), dissolved in the five-hormone medium, was added in the following concentrations: 0 mM (controls without lithium), 0.35 mM, 0.7 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM. The amount of 18.5 kBq of ^{3}H-thymidine (Amersham, Little Chalfont, UK) was added to each well. The plates were incubated for 72 h at 37°C in the incubator. After 72 h, the wells were washed three times with a cold phosphate-buffered saline solution. Then, 1 ml of 5% trichloroacetic acid was added. After 10 min at 4°C, the supernatant was aspirated and 500 µl of diphenylamine solution were added (Valente et al., 1983). The plates were incubated at room temperature for 24 h. From each well, 100 µl of solution were taken for the ^{3}H-thymidine content measurements in a liquid scintillation counter.

MTT assay

The quantity of 1.5 x 10^4 FRTL-5 cells were seeded in 96-well plates, maintained in the six-hormone medium for 3 days, and in the five-hormone medium for 7 days. The final volume of the culture medium was 100 µl. The wells were then washed once with the five-hormone medium. Cells were stimulated with lithium carbonate in 0 mM (controls), 0.4 mM, 0.8 mM, 1 mM, 1.6 mM and 2 mM concentrations without TSH and with 1 mU/ml of TSH for 3 days. After 3 days, the medium was replaced by the same medium, and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Sigma Chemical Co.) solution (25 mg/ml PBS) was added to each well (Šentjurc et al., 1998). Plates were incubated for 3 hours at 37°C. Formazan crystals were dissolved in 100 µl of dimethyl sulphoxide (Sigma Chemical Co.). The plates were shaken and the absorbance of the solution was measured at 570 nm using an Anthos spectrophotometer (Anthos Labtec Instruments, Salzburg, Austria).

^{51}Cr release from FRTL-5 cells: cytotoxicity assay

The quantity of 7.5 x 10^4 FRTL-5 cells were seeded in 24-well plates. They grew for 3 days in the six-hormone medium, and for 7 days in the five-hormone medium. The final volume of the culture medium was 500 µl. Then the wells were washed twice with HBSS (Hanks' balanced salt solution). Into each well, HBSS with 5% calf serum and 185 kBq of ^{51}Cr in the form of $Na_2{}^{51}CrO_4$ (Amersham) were added. Plates were incubated for 60 min at 37°C, and for further 30 min at 4°C. Cells were then washed twice with the HBSS. Lithium carbonate, dissolved in the five-hormone medium, was added in the following concentrations: 0 mM (controls without lithium), 0.35 mM, 0.7 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM without TSH and with 1 mU/ml of TSH. Maximal cell lysis was achieved by incubating cells with the detergent NP 40 (20% solution). The plates were incubated for 24 h at 37°C in the incubator. Aliquots of supernatant were measured in a gamma-counter. The percentage of specific lysis was calculated according to the formula: {[cpm (lithium + medium) – cpm (medium alone)]/[cpm (NP 40) – cpm (medium alone)]} x 100 (Chiovato et al., 1994).

Trypan blue exclusion method

Suspension of FRTL-5 cells in HBSS with different lithium concentrations (0 mM, 0.35 mM, 0.7 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM) was mixed with a 0.4% solution of trypan blue (Sigma Chemical Co.). Five to fifteen minutes later, coloured (non-viable) and dye-excluding (viable) cells were counted in the Bürker-Türks' chamber. The results were expressed as a percentage of viable cells according to the formula: [number of viable cells (non-coloured)/number of all cells] x 100 (Chiovato et al., 1994).

Statistics

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

Results

The influence of lithium on ^{3}H-thymidine incorporation into FRTL-5 cells in the absence of TSH stimulation is shown in Figure 1. Lithium at 0.7 mM, 1 mM, 1.4 mM, 1.7 mM and 2 mM concentrations significantly stimulated ^{3}H-thymidine incorporation when compared with the controls without lithium.

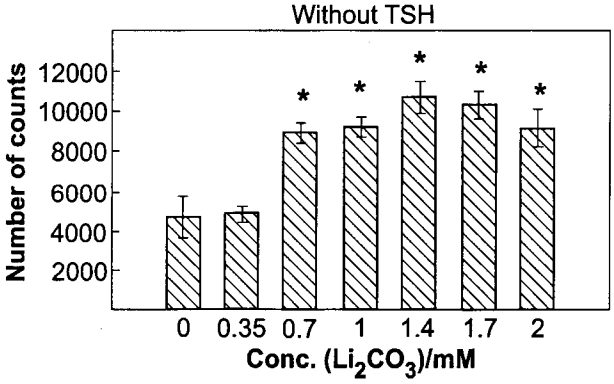

Fig. 1. Influence of lithium on ^{3}H-thymidine incorporation into DNA of FRTL-5 cells. Bars represent average values of quadruplicate determinations ± SD. Significant differences of results compared with controls without lithium are depicted by asterisks (* = $P < 0.05$).

Similarly, in the case of the MTT assay, lithium at 0.4 mM, 0.8 mM, 1.0 mM, 1.6 mM and 2 mM concentrations and in the absence of TSH stimulation significantly increased the absorbance at 570 nm when compared with the controls without lithium (Fig. 2). However, under stimulation by TSH in the 1 mU/ml concentration, lithium at 0.4 mM, 0.8 mM, 1 mM, and 1.6 mM concentrations did not change the absorbance at 570 nm when compared with the controls without lithium. Surprisingly, lithium at the 2 mM concentration significantly diminished the absorbance at 570 nm when compared with the controls without lithium (Fig. 3).

In the absence of THS, lithium at 1.4 mM, 1.7 mM and 2 mM concentrations significantly increased ^{51}Cr release from the FRTL-5 cells in the cytotoxicity assay (Fig. 4). These results are confirmed by the data shown in Table 1. The higher was the lithium concentration, the lower was the percentage of viable cells, as assessed with the semiquantitative trypan blue exclusion method (second column). Additionally, a higher percentage of specific lysis was observed at higher lithium concentrations (third column). Although lithium with 1 mU/ml of TSH did not influence ^{51}Cr release from FRTL-5 cells (Fig. 5), again, a higher percentage of specific lysis was observed at higher lithium concentrations (Table 1, fourth column).

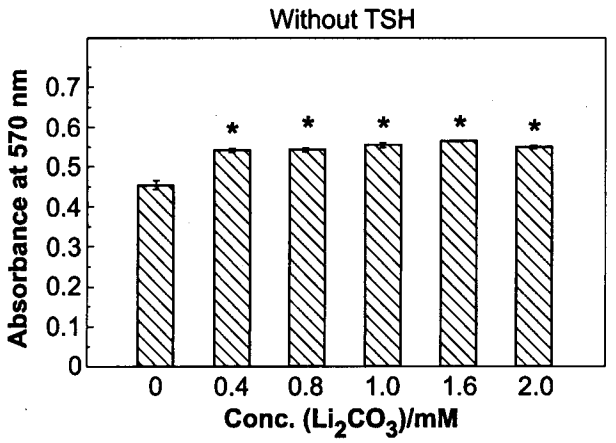

Fig. 2. Influence of lithium on proliferation of FRTL-5 cells (MTT assay). Bars represent average values of quadruplicate determinations ± SD. Asterisks represent statistically significant ($P < 0.05$) results when compared with controls without lithium.

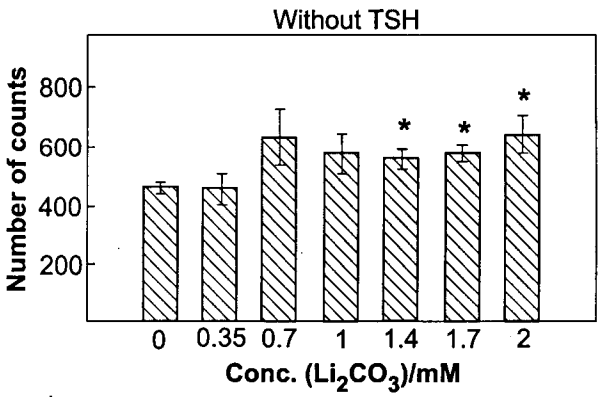

Fig. 4. Influence of lithium on ^{51}Cr release from FRTL-5 cells (cytotoxicity assay). Bars represent average values of quadruplicate determinations ± SD. Asterisks represent statistically significant ($P < 0.05$) results when compared with controls without lithium.

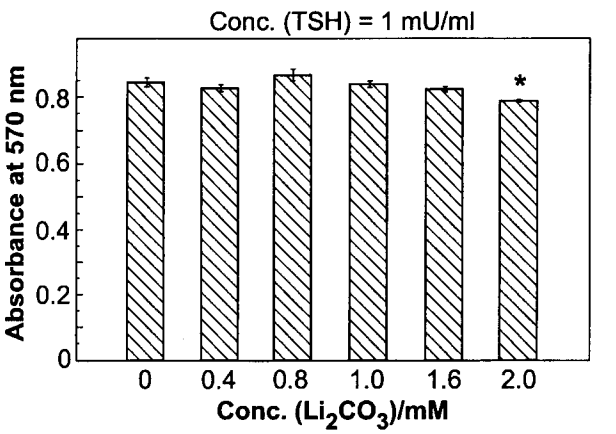

Fig. 3. Influence of lithium + TSH on proliferation of FRTL-5 cells (MTT assay). Bars represent average values of quadruplicate determinations ± SD. Asterisks represent statistically significant ($P < 0.05$) results when compared with controls without lithium.

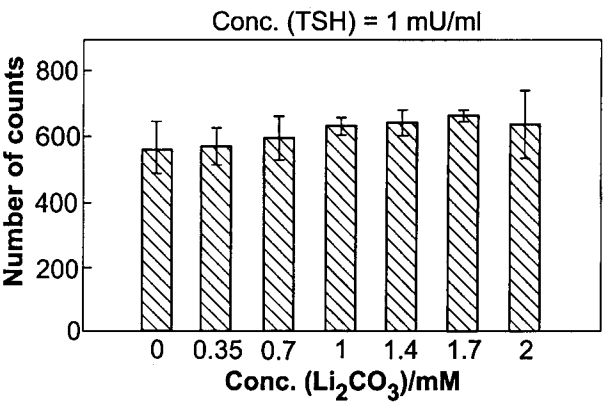

Fig. 5. Influence of lithium + TSH on ^{51}Cr release from FRTL-5 cells (cytotoxicity assay). Bars represent average values of quadruplicate determinations ± SD.

Table I. Viability (second column) and specific lysis without TSH (third column) and with TSH (fourth column) of FRTL-5 cells with respect to concentration of lithium as Li_2CO_3 (first column).

Conc. (Li_2CO_3)/mM	% of viable cells	% of specific lysis – without TSH	% of specific lysis – with TSH
0	99.0	0	0
0.35	90.2	0.5	0.7
0.7	86.4	17.7	3.4
1	85.1	11.9	7.5
1.4	87.2	10.2	8.5
1.7	86.3	11.7	10.8
2	84.1	15.5	8.1

Discussion

In the present study, we have confirmed the stimulative effect of selected lithium concentrations on proliferation of FRTL-5 cells, and, surprisingly, we have noticed an inhibitory effect of higher lithium concentrations on FRTL-5 cells proliferation under the stimulation by TSH. Additionally, a cytotoxic effect of higher lithium concentrations has been observed.

The results obtained by the ^{3}H-thymidine incorporation assay and by the MTT assay in the absence of TSH stimulation confirm the *in vivo* observations that lithium stimulates proliferation of thyroid cells and, therefore, leads to enlargement of the thyroid gland (Perrild et al., 1984; Boccheta et al., 1991). These results are compatible with those obtained with porcine thyroid follicles (Tsuchiya et al., 1990), where the 0.5 mM concentration of lithium without TSH was found stimulative. Additionally, our results agree with a study of Urabe et al. (1991), where lithium without TSH stimulated proliferation of FRTL-5 cells. Tasevski et al. (2000) observed the same effect at concentrations equal or higher than 1 mM. Lithium has been shown to have an effect on thyroid cells through a number of cell-signalling pathways (Manji et al., 1995), but probably not through the stimulation of adenylate cyclase and the synthesis of cAMP (Wolff et al., 1970; Mori et al., 1989; Tsuchiya et al., 1990; Van Sande et al., 1990). The lithium ion could have influence on the thyroid cells partly through the protein kinase C system (Urabe et al., 1991) and, as recently shown by Tasevski et al. (2000), through *de novo* cholesterol synthesis and G-protein prenylation.

We have observed an inhibitory effect of a higher lithium concentration (2 mM) on FRTL-5 cells proliferation under stimulation by TSH. The results are partly in contradiction with the findings of Urabe et al. (1991), who found a significant stimulation of ^{3}H-thymidine incorporation in FRTL-5 cells in the presence of 37 μU/ml of TSH and at the 10 mM concentration of lithium. Our results are in agreement with the observation in porcine thyroid cells (Urabe et al., 1991), where thyroid cell growth in the presence of both 1 mU/ml of TSH and lithium was found to be suppressed when compared with lithium alone. Presumably, the reduction of proliferation could be partly due to the inhibitory effect of higher lithium concentrations on cAMP synthesis, stimulated by TSH (Mori et al., 1989; Tsuchiya et al., 1990), and consequently, on cell proliferation, and partly due to the toxic effect of higher lithium concentrations (Kontozoglou and Mambo, 1983). Another possibility in the MTT assay is a changed mitochondrial activity rather than the decreased number of FRTL-5 cells. In the absence of TSH, the proliferative effect of lithium probably prevails over its cytotoxic effect. Only under the stimulation by TSH, which in this case controls the cell proliferation, the cytotoxic effect of lithium might become evident.

Indeed, the assumption of a cytotoxic effect of lithium has been confirmed by our results with the cytotoxicity assay without TSH, by the percentage of specific lysis and by the results obtained with the trypan blue exclusion method. Our results are in agreement with literature data, where several cases of lithium-associated thyroiditis have been reported. McLaren and Toft (1981) observed atypical changes in thyroid cell morphology, pleomorphism of the cells, disrupted architecture of the gland from a hyperthyroid patient treated with lithium and antithyroid drugs. Mizukami et al. (1995) published data on histopathological alterations of the thyroid gland with extensive follicular cell disruption. Lithium might directly damage thyroid follicular cells.

In conclusion, lithium was found to stimulate proliferation of FRTL-5 cells in conditions without TSH stimulation. Surprisingly, under the stimulation by TSH, lithium diminished proliferation of FRTL-5 cells. A cytotoxic effect of higher lithium concentrations in the absence of TSH was observed.

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