Thyroid and pituitary thyroxine-5'-deiodinase activity and thyrotrophin secretion in lithium-treated rats

T G P Frankenfeld, V M Corrêa da Costa, C C A Nascimento-Saba¹,
T M Ortiga-Carvalho, R M M Santos, P C Lisboa¹, D P Carvalho
and D Rosenthal

Laboratório de Fisiologia Endócrina, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Cidade Universitária, 21949-900-Rio de Janeiro, Brasil

¹Depto Ciências Fisiológicas, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro 20550-030-Rio de Janeiro, Brasil

(Correspondence should be addressed to D Rosenthal; Email: doris@biof.ufrj.br)

(Requests for offprints should be addressed to T G P Frankenfeld)

Abstract

Some authors have reported increased serum thyrotrophin (TSH) in animals chronically treated with lithium, suggesting that lithium might decrease pituitary thyroxine (T_4)-5'-deiodinase activity. On the other hand, the effect of lithium treatment on thyroidal T_4 -5'-deiodinase activity is also unknown. The present study was undertaken to evaluate the effects of lithium treatment on pituitary and thyroid T_4 -5'-deiodinase activity. Serum and pituitary TSH levels and thyroidal and pituitary T_4 -5'-deiodinase activities were determined in 3-month-old isogenic male Dutch-Miranda rats treated with lithium for 8 weeks.

Chronic lithium treatment produced a slight increase in pituitary TSH content, but no change in serum TSH, and a significant increase in the thyroidal T_4 -5'-deiodinase activity. However, the pituitary T_4 -5'-deiodinase activity was unaffected by lithium administration. As far as we know, the present data show for the first time that chronic lithium treatment can increase the thyroxine to triiodothyronine conversion in the murine thyroid gland, be it directly or indirectly.

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Introduction

Lithium administration may alter thyroid function directly or indirectly, but the mechanisms involved are still unclear. Some authors have suggested that an important effect of lithium could involve a decrease of the peripheral metabolism of thyroid hormones in humans (Carlson *et al.* 1973) and rats (Voss *et al.* 1977). A direct inhibitory effect of lithium on the murine pituitary type II thyroxine-5'-deiodinase was also referred to by St Germain (1987). The conversion of thyroxine (T_4) to 3,3',5'-triiodothyronine (T_3) in the pituitary has been shown to be of primordial importance for the negative feedback of thyroid hormones on thyrotrophin (TSH) secretion (Silva *et al.* 1978). Several studies have shown that the rat pituitary tissue contains both the propylthiouracil (PTU)-sensitive type I iodothyronine-deiodinase activity (DI-I), similar to that found in liver and kidney, and the PTU-insensitive type II iodothyronine-deiodinase activity (DI-II), which seems to account for a high percentage of the total T_4 to T_3 conversion in the pituitary gland (Van Doorn *et al.* 1983, Visser *et al.* 1983).

Some authors have previously found increased TSH secretion in humans during lithium treatment (Lauridsen *et al.* 1974, Maarbejerg *et al.* 1987, Deodhar *et al.* 1999). We have previously shown that thyroid hormone serum levels, thyroperoxidase activity and the 24-h thyroid radioiodide uptake were unchanged after chronic lithium treatment in rats (Frankenfeld *et al.* 1992), but a possible transitory effect of lithium on TSH secretion or a direct effect on pituitary thyroxine-5'-deiodinase (T_4 -5'-DI) activity have not yet been evaluated. In rats, the thyroid gland is a major source of circulating T_3 (Chanoine *et al.* 1993), in part through intraglandular T_4 -deiodination by type I DI, that might also be affected by chronic lithium use.

In order to better evaluate the effects of chronic lithium treatment in rats, in this study we evaluated the thyroid and pituitary thyroxine 5'-deiodinase activities as well as the pituitary TSH content and serum TSH, since it is known that both the thyroid and the pituitary glands concentrate lithium (Berens *et al.* 1970, Nelson *et al.* 1976, Frankenfeld *et al.* 1992).

Materials and Methods

Animals and hormone measurements

The study protocol was approved by the Institutional Use of Animals in Research Committee (CAUAP, IBCCF), and the procedures used are in compliance with the International Guiding Principles for Biomedical Research Involving Animals, CIOMS/Switzerland, and the guiding principles for Care and Use of Animals from the American Physiological Society.

Isogenic male Dutch-Miranda rats, 3 months old, maintained in a temperature-controlled room (22–25 °C) with a ratio of 12 h light:12 h darkness, were randomly divided into experimental and control groups of three animals each. During the following 8 weeks the experimental groups received 12.5 mM LiCl in the drinking water, while the paired control groups received tap water. Pelleted commercial animal chow (Purina) was offered *ad libitum*. We used two experimental protocols: (A) blood samples were collected from the jugular vein every week, for serial determinations of serum T_4 , T_3 and TSH, and (B) at the end of the experimental period blood was collected from the jugular vein, the animals were killed under ether, and the thyroid and pituitary glands were rapidly removed. Serum was stored at –20 °C. For the pituitary gland TSH determination, individual glands were homogenised in 500 µl ice-cold phosphosaline buffer, pH 7.6, containing 1% bovine serum albumin; the homogenates were stored at –20 °C until TSH determination. Serum and pituitary TSH were determined by a specific RIA using a kit for rat TSH determination supplied by the National Hormone and Peptide Program/NIDDK (Bethesda, MD, USA), and was expressed in terms of the preparation provided (RP-1 in the serial experiments (A) and RP-2 for pituitary TSH and serum TSH in the 8-week lithium-treated rats (B)). Serum T_4 and T_3 were measured by RIA (Mallinckrodt, St Louis, MO, USA). For the deiodinase assays, the thyroid and pituitary glands were rapidly excised, weighed, pooled (six glands per pool) and stored at –70 °C for a maximum period of 48 h.

T_4 -5'-deiodinase activity assay

Minor modifications of previously described methods were used for determination of thyroid and pituitary T_4 -5'-deiodinase activities (Costa & Rosenthal 1996, Costa *et al.* 2001). The pooled thyroid glands were homogenised in 50 mM Tris-HCl buffer, pH 7.4, containing 250 mM sucrose and 5 mM dithiothreitol (DTT). The pooled pituitaries were homogenised in 50 mM Tris-HCl buffer, pH 7.6, containing 250 mM sucrose and 10 mM DTT. The homogenates were centrifuged at 12 000 g (4 °C, 20 min). The supernatant was centrifuged twice at 100 000 g (4 °C, 60 min), and the washed pellet was taken up in homogenising buffer without sucrose. The micro-

somal fractions were diluted to a protein concentration of 20–50 µg protein/100 µl for the thyroid and to 50–80 µg protein/100 µl for the pituitary gland. Propylthiouracil (PTU, 1 mM) was added to one of the pituitary microsomal fraction aliquots to inhibit DI-I activity. Protein concentration was determined by the method of Bradford (1976).

The deiodinase assay mixture contained 100 µl microsomal fraction and 2 µM T_4 in a final volume of 125 µl, and was incubated at 37 °C, for 20 min in the thyroid DI assays, and for 60 min in the pituitary DI assays. The reaction was stopped by the addition of 250 µl ice-cold 95% ethanol. The tri-iodothyronine formed was determined in the alcoholic extracts by a specific RIA, as previously described (Costa & Rosenthal 1996). The T_4 -5'-deiodinase activity was expressed as picomoles T_3 formed per milligram protein (pmol T_3 /mg protein). The pituitary type I DI activity was estimated from the difference between total and type II DI activities in the presence of PTU.

Statistical analysis

The deiodinase activity results are expressed as means $\pm$ S.E.M. Serum TSH values were analysed after logarithmic transformation. Analysis of variance, randomised block experimental design, complemented by multiple comparison tests when adequate, were used for the data statistical evaluation (SuperANOVA program, Abacus Concept, Berkeley, CA, USA).

Results

As previously reported (Frankenfeld *et al.* 1992) no signs indicative of toxic effects were seen during the 8-week period of lithium administration.

Pituitary TSH content is increased although not significantly in the lithium-treated animals, but no change in serum TSH was detected during the experimental period (protocol A; Table 1) or at the end of lithium treatment (protocol B; Table 2). No significant differences were found in the serial measurements of serum T_4 and T_3 in the lithium-treated rats (Table 1).

Thyroid T_4 -5'-deiodinase activity was significantly increased in the lithium-treated rats ($P < 0.005$). Only a slight and not significant increase in type I deiodinase activity was detected in the pituitary, while the type II deiodinase activity was unaffected (Fig. 1).

Discussion

Goiter and hypothyroidism have been reported to occur occasionally during chronic lithium treatment (Schou *et al.* 1968, Leppaluoto *et al.* 1973, Kusalic & Engelsmann

Table 1 Serial measurements of serum TSH, T₄ and T₃ in chronically lithium-treated rats (protocol A). TSH concentrations are expressed in terms of the RP-1 preparation provided by the National Hormone and Peptide Program/NIDDK. Results were analysed after logarithmic transformation and are shown as means and, within parentheses, limits defined by mean $\pm$ S.E.M. T₄ and T₃ results are expressed as means $\pm$ S.E.M. The values are means of at least five different animals.

	TSH (ng/ml)	T ₄ (μ g/dl)	T ₃ (ng/dl)
Basal	2.72 (2.65–2.81)	3.23 $\pm$ 0.14	107.60 $\pm$ 10.23
1 week	3.05 (2.88–3.23)	2.80 $\pm$ 0.33	118.30 $\pm$ 8.23
2 weeks	2.81 (2.63–3.02)	3.54 $\pm$ 0.12	107.00 $\pm$ 7.03
3 weeks	2.90 (2.80–3.00)	2.63 $\pm$ 0.29	100.70 $\pm$ 6.77
4 weeks	3.32 (3.15–3.51)	3.37 $\pm$ 0.33	98.14 $\pm$ 11.05
5 weeks	3.16 (3.00–3.34)	3.04 $\pm$ 0.38	100.10 $\pm$ 2.98
6 weeks	3.10 (2.96–3.52)	4.18 $\pm$ 0.10	68.40 $\pm$ 6.07
8 weeks	3.20 (3.09–3.33)	3.83 $\pm$ 0.12	110.70 $\pm$ 6.92

1999). Several mechanisms have been proposed to explain lithium effects on thyroid function (Lazarus 1998, Sigrid *et al.* 2000). It has been suggested that, in humans, lithium might have a direct inhibitory action on thyroid hormone secretion, which might result in a compensatory increase in serum TSH (Sedvall *et al.* 1969, Spaulding *et al.* 1972). Various studies have reported sub-clinical hypothyroidism associated with increased serum TSH and/or TSH response to exogenous thyrotrophin-releasing hormone in patients treated with lithium (Lazarus 1998, Kliner *et al.* 1999).

In previous studies we found no significant changes in 24-h thyroid radioiodide uptake, thyroid radiolabelled iodoamino acids content, thyroid peroxidase activity or serum T₃ and T₄ in chronically lithium-treated rats

Table 2 Serum and pituitary TSH in control and chronically lithium-treated rats at the end of the treatment period (protocol B). TSH concentrations are expressed in terms of the RP-2 preparation provided by the National Hormone and Peptide Program/NIDDK. Results were analysed after logarithmic transformation and are shown as means and, within parentheses, limits defined by mean $\pm$ S.E.M.

	n	Control	Lithium
Serum TSH (ng/ml)	10	0.82 (0.74–0.90)	0.88 (0.80–0.96)
Pituitary TSH (μ g/g)	8	7.93 (7.18–8.77)	10.67 (9.75–11.67)

Figure 1 Thyroid and pituitary T₄-5'-deiodinase activities at the end of the treatment period in control and chronically lithium-treated rats. **P* < 0.05 compared with controls.

(Frankenfeld *et al.* 1992). These observations suggested that chronic lithium effects on murine thyroid glands were either irrelevant or minimised by increased thyroid stimulation by TSH. Child *et al.* (1976) reported a transient decrease in serum T₃ or T₄ in lithium-treated rats during the second and third weeks of treatment. Although serum TSH was not determined in their study, the fact that goiter appeared during the third and fourth weeks of treatment, and that serum thyroid hormones returned to normal levels during the fourth week, are consistent with an increment of TSH effects on the thyroid gland. In the present study, we found no expressive changes in serum TSH levels during the whole period of lithium treatment.

The thyroid and the pituitary glands concentrate lithium (Berens *et al.* 1970, Nelson *et al.* 1976, Frankenfeld *et al.* 1992). A direct *in vitro* inhibitory effect of lithium on renal DI-I activity has been reported by Mannisto (1974), but St Germain (1987) found no acute change in kidney DI activity *in vivo* after lithium injection.

A significant inhibitory effect of lithium on the type II deiodinase activity has been found in cultured pituitary and neural tissues by St Germain (1987), and a decreased type II deiodinase activity was also found in several brain regions of rats treated with lithium for 14 days (Baumgartner *et al.* 1997). Nevertheless, we found no significant differences in type I or type II pituitary T₄-5'-deiodinase activities in chronically lithium-treated rats. It is interesting to note that acute toxic doses of lithium also failed to produce detectable effects on serum TSH or on the hypothalamic or pituitary D-II activity (Eravci *et al.* 2000). Thus, different doses and/or periods of treatment may be responsible for the apparent discrepancy between the various studies.

A significantly enhanced thyroidal T₄-5'-deiodinase activity in long-term lithium-treated rats was found in the present study. The increase of *in vivo* thyroidal DI-I activity was also found after only 2 weeks of lithium treatment, using an alternative methodology (results not shown, Frankenfeld *et al.* 2001). As far as we know, these are the first reports of *in vivo* lithium effects on thyroid

gland T_4 -5'-deiodinase activity. The increased thyroidal DI activity suggests an enhanced TSH effect on the thyroid, even if the serum TSH is not increased, since TSH is the major regulator of T_4 deiodination in the thyroid gland (Erickson *et al.* 1982). Nevertheless, a direct lithium effect on the thyroidal deiodinases cannot be discarded. These hypotheses must be further tested. Further studies on the chronic effects of lithium on the thyroid-pituitary axis are still necessary until we completely understand how this cation can change thyroid gland function and regulation.

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