CHANGES IN CYCLIC NUCLEOTIDES OF RAT THYROID BY CHRONIC ADMINISTRATION OF LATS AND TSH

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ABSTRACT

The effects of LATS and TSH on the cyclic nucleotide content and enzymatic activity in rat thyroid was observed during the continuous administration of LATS or TSH for 6 days. Serum T4 and T3 levels were increased significantly compared with the saline controls. The cyclic nucleotide (cAMP and cGMP) levels and enzyme activities per wet weight of tissue were determined.

The thyroid weight in both the LATS and TSH groups increased approximately two-fold, but cAMP and cGMP content per wet weight did not significantly change. Neither cyclic nucleotide showed any significant change in plasma.

The cAMP-PDE activity in the thyroid significantly increased in both the LATS and TSH groups, but the cGMP-PDE activity was unchanged. Neither was cyclic nucleotide-PDE activity changed in the plasma.

The ATPase activity in the thyroid increased markedly in both the LATS and TSH groups, while 5'-nucleotidase activity did not change.

These data suggest that LATS and TSH appear to have a stimulatory effect on the metabolism of cAMP, but do not affect the metabolism of cGMP.

Although the pathological significance of LATS-immunoglobulin found in the serum of patients with hyperthyroidism is not clear, it is generally considered that LATS may have thyroid stimulating effect as a consequence of its reaction
with an antigen on the mammalian thyroid cell (Dorrington & Munro 1966; McKenzie 1967; Burke 1971).

Evidence has been accumulated that the thyroid stimulating activity of LATS is surprisingly similar to that of TSH with respect to iodine metabolism, glucose metabolism, phosphate metabolism, RNA and protein synthesis and endocytosis (Dorrington & Munro 1966; McKenzie 1967; Burke 1971).

The mode of action of LATS is believed to involve the activation of adenyl cyclase leading to an enhanced cyclic AMP production in the thyroid gland (Kaneko et al. 1970; Levey & Pastan 1970; Zakarija & McKenzie 1973).

In this study, the effects of chronic administration of LATS and TSH on cyclic AMP and cyclic GMP metabolism on rat thyroid have been investigated.

**MATERIALS AND METHODS**

Male Wistar rats, weighing 80–90 g were studied. Each group of 5 rats was injected with test material ip daily for 6 days. Rats were fed a regular diet obtained from Oriental Comp. (1.06 mg/100 ml iodine content), and allowed tap water ad libitum.

The LATS-I₄G fraction was purified from LATS-positive serum by affinity chromatography on protein A-bound Sepharose (Ochi et al. 1976). Four mg of the LATS-I₄G fraction produced 800 % response at 24 h according to a modified McKenzie bioassay (Ochi & DeGroot 1969). LATS-I₄G (40 or 120 mg), TSH (Armour, Thytopar) (1 or 3 U) or I₄G (human Cohn Fr II, Calbiochem) (40 or 120 mg) were injected once daily. Saline was injected ip as a control.

Rats were sacrificed by ether anaesthesia 24 h after the last injection. Organs (thyroid, pituitary, adrenal and testis) and blood were obtained promptly. Blood was collected in EDTA to a final concentration of 5–10 mM. Plasma was separated immediately by centrifugation at 4°C. Each organ was immediately frozen in acetone containing dry ice. Glands were weighed in a cold room and homogenized in a small glass homogenizer containing 0.02 mM Tris-HCl, pH 7.6.

The determination of cAMP and cGMP was performed by Yamasa's radioimmunoassay (RIA) kit (Tokyo) according to the method of Steiner et al. (1969). Homogenized tissue was brought to 1 ml and to 6% TCA as a final concentration, and centrifuged at 4000 r.p.m. for 15 min at 4°C. Five ml of water saturated ether was added to this supernatant fraction. The mixture was shaken vigorously, and then the ether fraction was removed. This procedure was repeated 3 times. Finally, the residue fraction was dissolved in 1 ml of water after drying. One tenth ml was used for succinylation. Plasma was succinylated directly. Each sample was assayed as specified in the protocol of the RIA kit.

Phosphodiesterase (PDE) activity for cAMP and cGMP was measured by a modification of the method of Russell et al. (1973). PDE activity was assayed with a reaction mixture of 0.5 ml containing 20 mM Tris-HCl, pH 7.5, 2 mM MgCl₂, 0.4 µM [3H]cAMP or [3H]cGMP, and enzyme sample. The samples were then placed in boiling water for 3 min, cooled, and then incubated for an additional 20 min after the addition of 50 µg snake venom. Half a ml of 0.1 mM adenosine or guanosine was added, after which 1.0 ml of Amberlite (G-400 resin, 1:2 slurry in H₂O) was added, and a 0.5 ml aliquot of the supernatant solution was counted after centrifugation.

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ATPase was assayed according to a modification of the method of Wolff (Wolff & Halmi 1963; Wolff & Jones 1971). Reaction mixture containing 3 mM ATP, 5 mM MgCl₂ in 0.2 ml of Tris-HCl buffer, pH 7.4, was incubated for 30 min at 37°C. Inorganic phosphate was analysed by the Fiske-SubbaRow method (Wolff & Halmi 1963; Wolff & Jones 1971). 5'-Nucleotidase was assayed with 5'-AMP as substrate by the method of Wattiaux-DeConinck & Wattiaux (1969).

Plasma thyroxine (T₄) and triiodothyronine (T₃) concentrations were determined by the radioimmunoassay kits of the Dainabott Lab (Tokyo).

RESULTS

1) Effect of LATS treatment on plasma T₄ and T₃ levels

Both the LATS group and the TSH group showed increased T₄ and T₃ levels in the plasma in comparison with the IgG group and the saline control. The T₄ levels were 6.0 and 6.7 µg/100 ml after treatment with 40 mg and with 120 mg of LATS-IgG, respectively. The TSH group (3 U) showed a comparably increased T₄ level. T₃ increased significantly in both the LATS and TSH groups compared to the saline control group (Fig. 1).

2) Effect of LATS treatment on thyroid and pituitary weight

The thyroid weight increase after prolonged treatment with LATS or TSH was compared with that of the saline control group (Fig. 2). The LATS group showed a significant increase in thyroid size. The wet weight increased about 1.4 and 1.8 times, respectively, after treatment with 40 mg and with 120 mg of LATS-IgG.

The TSH group also demonstrated thyroid enlargement, specifically 1.3- and 1.85-fold after treatment with 1 U and 3 U, respectively. However, no thyroid enlargement was observed after treatment with the IgG.

Fig. 1.
Effect of LATS treatment on T₄ and T₃ levels in plasma.
All values are expressed as mean ± sd.
3) **Effect of LATS treatment on cyclic nucleotides in the thyroid and plasma**

The cAMP content per wet weight of thyroid increased slightly but not significantly in the LATS group. There was no significant elevation of the cAMP content in the TSH group. The cGMP content of the thyroid did not change in either group. Both cAMP and cGMP levels in the plasma were significantly unaffected (Fig. 3).

The thyroidal cAMP per mg wet weight in the LATS (120 mg), TSH (3 U) and IgG (120 mg) group was 375 ± 40 (fmol), 325 ± 37, and 317 ± 42 (mean ± sd), respectively. The thyroidal cGMP per mg wet weight in the LATS,
TSH and IgG group was 25 ± 7 (fmol), 28 ± 8, and 27 ± 8, mean ± sd), respectively. Thus, the ratio of cAMP/cGMP was 15.0. This ratio was 11.7 in the TSH group and 11.6 in the IgG group.

4) Effect of LATS treatment on the activity of PDE, ATPase and 5'-nucleotidase

The cAMP-PDE activity per wet weight of thyroid was increased 1.5-fold and 1.7-fold by treatment with 40 mg and 120 mg of LATS-IgG, respectively. The TSH group also showed 1.3-fold and 1.6-fold increases in thyroid cAMP-PDE activity after treatment with 1 U and 3 U, respectively. On the contrary, thyroid cGMP-PDE activity did not significantly change in either group. The plasma levels of cAMP-PDE and cGMP-PDE activity were essentially unchanged in both groups (Fig. 4).

Although ATPase activity in the thyroid was markedly increased by either LATS or TSH treatment, 5'-nucleotidase did not change. Both enzyme activities were unaffected in the plasma (Fig. 5).

**DISCUSSION**

Recently, much research has been focused on cAMP and its related compounds. The *in vitro* stimulation of adenyl cyclase by TSH or LATS in the mouse thyroid has been previously reported (Kendall-Taylor 1972). Slight elevation
Fig. 5.

Effects of LATS and TSH treatment on enzyme activities in thyroid and plasma. Thyroid ATPase or 5'-nucleotidase activity was expressed per mg wet weight. Plasma enzyme activity was expressed per ml. IgG (120 mg) used as the control. All values are expressed as mean ± sd.

of cAMP levels in mouse thyroid by a single injection of LATS or TSH in vivo has been reported (Zakarija & McKenzie 1973). Increased cAMP levels in rat thyroid as a consequence of the protracted TSH stimulation seen during goitrogen administration have also been reported (Zakarija et al. 1973; Berthier & Lemarchand-Béraud 1976; Nakamura et al. 1976). This finding has also been confirmed by the present authors.

We have previously reported the thyroid stimulating effect on iodine uptake, RNA and protein synthesis, and G-6-PDH activity, by LATS in mouse thyroid (Ochi & DeGroot 1968, 1969, 1970, Ochi et al. 1973).

In the present experiments, the effect of LATS was studied in the rat, a species in which tissue quantity is not limiting. Plasma T₄ and T₃ levels were increased significantly by both LATS and TSH. The cAMP level per wet weight of tissue in rat thyroid was compared after prolonged LATS or TSH stimulation, although the cAMP level per mg RNA or DNA may be a more appropriate expression. The cAMP level did not significantly change with LATS or TSH treatment. Thus, we conclude that the effect of TSH and LATS on thyroid cAMP levels is minimal, even under prolonged stimulation.

It has been reported that chronic administration of a low iodine diet and propylthiouracil induced an increase in the activity of cAMP-PDE (Bastomsky
et al. 1971). In our study, thyroid cAMP-PDE activity was significantly increased by prolonged treatment with LATS and TSH.

It is generally accepted that the turnover of cAMP is quite rapid if compared to the synthetic capacity of adenylyl cyclase. For example, in rat brain the ratio of enzyme activity (cAMP-PDE/adenyl cyclase) has been reported to be as high as 500 (DeRobertis et al. 1967). Thus it seems plausible that the degradation of newly produced cAMP may occur immediately even if cAMP synthesis has already been augmented. The relative levels of cAMP and its hydrolyzing enzyme (cAMP-PDE) may in part be due to the differences in their labilities. However, cAMP levels within a compartment of the cell may be more important than the total cAMP level present in the gland.

The increase in thyroid cAMP-PDE activity by prolonged LATS or TSH stimulation suggests that an acceleration of cAMP turnover is quite possible. Negligible effects of TSH on thyroid cAMP content by the increase in PDE activity has already been suggested (Bastomsky et al. 1971; Zakarija et al. 1973). Rat thyroid cAMP content (treated with TSH or goitrogen in vivo) increased significantly, when the thyroid was incubated with theophylline in vitro. From the above findings Zakarija & McKenzie (1973, 1975) suggested the physiological significance of PDE for cAMP.

There are several available data for the role of cGMP in the thyroid function. Yamashita & Field (1972) reported no increase of guanylyl cyclase by TSH stimulation. On the other hand, the stimulation of thyroidal protein synthesis in vivo and in vitro by cGMP has been reported (Pisarev et al. 1971; Varrone et al. 1973). Champion et al. (1974) also reported that acetylcholine which has been shown to increase cGMP levels in the thyroid, inhibits TSH-induced increases in thyroidal cAMP.

In our experiment, the non-responsiveness of thyroid cGMP and the cGMP-PDE levels to LATS or TSH administration suggests no remarkable effect of either stimulator on the cGMP system.

Mg\(^{2+}\)-ATPase and 5'-nucleotidase have been found in the plasma membranes of various tissues. The activity of both enzymes has been found to be responsive to prolonged TSH stimulation in rat thyroid (Bastomsky et al. 1971; Matsuzaki 1976). Bastomsky et al. (1971) showed that the activity of these enzymes was increased by treatment with either a low iodine diet or with propylthiouracil, for 1 month. Matsuzaki (1976), however, showed that Mg\(^{2+}\)-ATPase activity increased but that of 5'-nucleotidase decreased after treatment with methylthiouracil for 15 days. In the present experiment, thyroid Mg\(^{2+}\)-ATPase was increased by both LATS or TSH treatment, but 5'-nucleotidase was not significantly affected.

These data indicate that the metabolism of cAMP system in the thyroid is stimulated by LATS or TSH. Moreover, the data suggest that LATS acts in much the same way as TSH in the stimulation of cAMP metabolism.
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