We wish to thank the consultant obstetricians at the West Middlesex Hospital for allowing us to study patients under their care, Dr. J. S. Stewart for helpful discussions, and Miss S. K. M. Lovell, Sisters M. E. A. Hall, C. Lewis, and N. Nolan and the nursing staff of the obstetric unit for kind help with these patients. We are also indebted to Mr. A. J. T. Bowden for invaluable technical help and Mrs. P. Lipscombe for programming assistance.

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Effects of Long-acting Thyroid Stimulator (LATS) and LATS Protector on Human Thyroid Adenyl Cyclase Activity

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British Medical Journal, 1973, **3**, 72-75

Summary

The long-acting thyroid stimulator (LATS) has been thought to be responsible for the hyperthyroidism of Graves's disease. It is detected by its effect on the mouse thyroid gland but cannot be found in all patients with hyperthyroidism. In an attempt to clarify the problem of LATS-negative hyperthyroidism, serum was obtained from untreated patients and its effect in vitro on human thyroid tissue examined, using the activation of adenyl cyclase as a measure of stimulation. Human thyroid adenyl cyclase was activated by both thyroid-stimulating hormone (TSH) and LATS. Thyroid tissue obtained from patients with Graves's disease was relatively less responsive to LATS than was non-toxic thyroid tissue. Of the 24 samples studied five contained LATS and all of these activated adenyl cyclase. The presence of LATS protector in LATS-negative hyperthyroid patients was confirmed but LATS-negative sera had no effect on human thyroid adenyl cyclase activity.

Introduction

The long-acting thyroid stimulator (LATS) is an immunoglobulin (Ig) G found in the serum of some but not all patients with Graves's disease (Adams and Purves, 1956). It has been shown experimentally to stimulate the thyroid in several species, having actions remarkably similar to those of thyroid-stimulating hormone (TSH); further, transplacental passage of LATS appears to be responsible for the development of neonatal thyrotoxicosis. For these reasons (Kriss, 1970; Munro, 1971) LATS was thought to be the cause of the hyperthyroidism in Graves's disease, and the apparent failure to find it in all patients has been attributed to inadequate sensitivity of the available bioassay methods.

It now seems doubtful whether LATS can be incriminated as the sole cause of hyperthyroidism (McKenzie, 1972; Solomon and Chopra, 1972; Volpé et al., 1972) and recently other hypotheses have been put forward to explain the problem of the LATS-negative hyperthyroid patient. The first of these postulates that, since LATS is a human IgG, there may in some cases be lack of cross reactivity with the mouse thyroid, the mouse being the species usually used for the detection of LATS. The second is based on the finding by Adams and Kennedy (1967) of LATS protector in the serum of LATS-negative hyperthyroid patients: this is an Ig, distinct from LATS, which protects LATS from neutralization by human thyroid protein. It does not bind to, or stimulate, the mouse thyroid and it has therefore been suggested that it may be a specifically human thyroid stimulator (Adams and Kennedy, 1971).

In this study an attempt has been made to explore these possibilities using human thyroid tissue. It is well established that the actions of both TSH and LATS on thyroid hormone secretion are mediated by cyclic AMP, and adenyl cyclase in the mouse thyroid was previously shown to be activated by LATS (Kendall-Taylor, 1972). This assay method for adenyl cyclase was therefore adapted for use with human thyroid tissue.

Patients and Methods

Blood Samples.—Serum was obtained from 24 patients all of whom had at the time unequivocal hyperthyroidism as judged by clinical examination, protein bound iodine estimation, and radioiodine uptake. None was receiving any drugs; though some of the patients had recurrent hyperthyroidism, none had received any treatment during the preceding year. Serum was also obtained from normal volunteers for use in control observations. Serum containing a high titre of LATS was obtained from a patient with treated hyperthyroidism and was used as a standard in all experiments. Samples were assayed for: (1) activation of adenyl cyclase in human thyroid tissue; (2) LATS activity; and (3) LATS protector. Immunoglobulin was prepared from serum by precipitation with 1.6 mol/l. ammonium sulphate and then dialysed and lyophilized. The effect of a two-fold concentrate of this material on human thyroid adenyl cyclase activity was examined and normal Ig was included in

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each experiment for comparison. For the assay of LATS or LATS protector unconcentrated serum, or an equivalent amount of Ig, was used.

Human Thyroid Tissue was obtained at surgery and used immediately. Patients with non-toxic goitres received no preoperative medication. Those with hyperthyroidism were prepared for surgery by treatment with antithyroid drugs; when euthyroid thyroxine was administered for two weeks pre-operatively, which is the preferred regimen in this centre. Part of the specimen was used for the adenyl cyclase assay. The remainder was homogenized, centrifuged at 105,000 *g* for 1½ hours, and the supernatant dialysed and lyophilized. This soluble human thyroid extract (HTE) was subsequently used for the detection of LATS protector.

Assay of Adenyl Cyclase Activity.—The method, based on that of Humes *et al.* (1969), has been described in detail for measurement of adenyl cyclase activity in mouse thyroid glands (Kendall-Taylor, 1972). Tissue is preincubated with ³H-adenine to form intracellular ³H-adenosine triphosphate and the cyclase activity measured by the amount of cyclic ³H-AMP formed during a second incubation period. Thyroid tissue was chopped into small pieces of uniform size (about 2 mg). These were placed together in a flask containing 5 ml Krebs-Ringer bicarbonate buffer plus glucose and albumin (pH 7.4) with 100 µCi ³H-adenine, and incubated with shaking at 37°C for 1½ hours. The buffer was discarded and the tissue washed repeatedly to remove excess label. Several slices, accurately weighed, were then incubated for one hour at 37°C in 0.5 ml buffer containing 10⁻³ mol/l. theophylline together with the Ig sample. The reaction was terminated and cyclic ³H-AMP extracted and counted as previously described. Two series of experiments were performed: (a) to assess the response of human thyroid adenyl cyclase to purified human TSH (kindly given by Dr. A. Stockell-Hartree) and to the LATS-Ig standard; and (b) to test whether the samples from the 24 hyperthyroid patients would increase cyclase activity.

Assay of LATS.—Thyroid glands from iodine-deficient mice were prelabelled *in vivo* by the injection of ¹³¹I. After five hours the mice were killed and the thyroid glands removed intact and incubated for 16 hours with serum samples. The release of ¹³¹I and ¹³¹I-labelled thyroid hormones into the incubation medium was measured and expressed as a percentage of the total counts in the medium plus gland. Where whole serum was used this was preheated at 56°C for one hour and additions were made of KClO₄ (to prevent re-uptake of ¹³¹I), glucose (to adjust serum osmolality), and penicillin and streptomycin. The method has been described in detail elsewhere (Ensor *et al.*, 1971).

Assay of LATS Protector.—LATS protector was detected by a method similar to that described by Adams and Kennedy (1971) except that the activity of the samples was measured by the *in-vitro* assay referred to above. Preliminary experiments determined the amount of HTE required to reduce, but not completely abolish, the stimulation of the mouse thyroid by LATS. The HTE was preincubated for one hour at 37°C with 1 ml heat-treated LATS-negative or normal serum (or an equivalent amount of Ig) plus 50 µl standard LATS serum, and the mixture subsequently assayed.

Results

Effect of TSH and LATS on Adenyl Cyclase Activity.—Both TSH and LATS activated adenyl cyclase in human thyroid tissue. As the supply of LATS was limited the sensitivity of the method was assessed for TSH and found to be about 0.3 mU/ml, a linear response being obtained over the range 0.1 mU/ml to 6 mU/ml (fig. 1); with the laboratory LATS standard preparation 1 mg/ml was readily detectable. The sensitivity was similar whether tissue was derived from patients with non-toxic goitre or with hyperthyroidism, but the response to maximal doses of TSH and LATS was less in the "toxic" glands. Moreover, whereas the TSH and LATS at these doses induced equivalent

FIG. 1—Effect of human TSH on adenyl cyclase activity in thyroid tissue from patients with Graves's disease. After preincubation with ³H-adenine, thyroid tissue was incubated for 20 min in presence of 10⁻³ mol/l. theophylline and TSH; formation of cyclic ³H-AMP measured cyclase activity. Results are mean of two experiments ± S.E. of mean.

FIG. 2—Effect of TSH or LATS on human thyroid adenyl cyclase activity showing difference in response between non-toxic goitres (mean of 3) and glands from patients with Graves's disease (mean of 3).

responses in non-toxic glands, the response of "toxic" tissue to LATS was significantly less than to TSH (fig. 2).

Effect of Ig from Hyperthyroid Patients on Cyclase Activity.—Ig preparations (four replicates of each sample) were assayed at two-fold, and in some experiments five-fold, concentration using both non-toxic and toxic glands. Only five out of 24 activated adenyl cyclase and these were all subsequently found to contain LATS. The remaining 19 samples, in none of which was LATS detected, were without any stimulatory action on human thyroid adenyl cyclase in individual experiments, and the mean of all experiments showed no significant difference between LATS-negative and normal Ig (see table).

LATS Protector.—Samples from 12 of these LATS-negative hyperthyroid patients and from normal subjects were assayed for LATS protector. This was deemed to be present when assay of LATS and HTE in the presence of hyperthyroid serum resulted in a significantly greater response than to the LATS-HTE complex itself. To clarify this, results of a representative experiment are shown in fig. 3. This also shows differences in serum concentrations of LATS protector in individual hyperthyroid patients: the presence of unconcentrated sample 2 or of normal serum had no effect on the assay response to LATS-HTE—that is, LATS protector not found. With sample 4 the

FIG. 3—LATS protector effect. LATS serum 50 μ l + HTE (12 mg/ml) was preincubated at 37°C for 1 hr in presence of 1 ml hyperthyroid LATS-negative serum (samples 2, 3, and 4). Mixture was assayed by its effect on release of ¹³¹I in vitro from prelabelled mouse thyroid glands.

binding of LATS to HTE is partly prevented, whereas with sample 3 it is completely abolished, the response then being similar to that obtained with LATS alone. The response to each sample alone was the same as in control preparations—that is, LATS could not be found. This LATS protector effect was not seen with the five normal sera examined, but was found in nine of the 12 LATS-negative hyperthyroid samples. Immunoglobulin from these nine sera containing LATS protector did not activate adenyl cyclase (see table).

Effect of Immunoglobulin from Hyperthyroid Patients on Adenyl Cyclase Activity in Human Thyroid Tissue

	No. of Subjects	Mean Cyclic ³ H-AMP (c.p.m./20 mg)	S.E. of Mean	Range
Normal	8	392	50	180-825
Hyperthyroid LATS-negative	19	395	61	147-880
Hyperthyroid LATS-positive	5	3,946	1,306	1,011-7,685
Hyperthyroid with LATS protector	9	495	90	147-880

Discussion

It has been clearly shown that LATS stimulates human thyroid adenyl cyclase activity. Though this was not unexpected it has not been shown before and supports the view that in patients in whom it is present LATS is at least partly responsible for the thyroid overactivity.

There are few reports of the experimental effects of LATS on human thyroid. One of the chief problems is the difficulty of obtaining suitable tissue and post mortem normal thyroid glands lose their responsiveness within a few hours of death (personal observation). Glands from patients with Graves's disease were stimulated by both TSH and LATS, though they were less responsive than non-toxic goitres. Though other factors may be involved, the most likely explanation would seem to be a persistent effect of the antithyroid drugs given previously and discontinued 10 days or so preoperatively. Of particular interest was the finding that the "toxic" glands were relatively less responsive to LATS than to TSH. None of the patients from whom these glands were obtained had detectable LATS in their serum but it is possible that cell-bound LATS may be present in the glands, limiting their response. Perhaps

more likely, LATS protector may bind to the cell membrane and partly interfere with the stimulation by LATS. Though to date neither LATS nor LATS protector has been found in these glands, the theory gains some support from the finding of immunoglobulins deposited in the thyroid follicular basement membrane in Graves's disease (Doniach, 1967; Werner *et al.*, 1972).

Adams and Kennedy (1967) found LATS protector in the serum of LATS-negative hyperthyroid patients, and this study confirms the presence of a factor in such patients that interferes with the binding of LATS to thyroid protein. Unfortunately insufficient of the original samples remained to determine whether it could be found in Ig concentrates of all these LATS-negative patients. Using the adenyl cyclase assay it has not been possible to confirm the hypothesis that LATS protector is a thyroid stimulator, distinct from LATS, specific for the human thyroid. It may be objected that the method was not sensitive enough to detect a small degree of stimulation, but this seems improbable as Ig concentrates were used and LATS protector could be found in unconcentrated serum by the mouse thyroid assay. Furthermore, the adenyl cyclase assay clearly differentiated those patients with LATS from those without and in no case did a sample containing LATS fail to activate adenyl cyclase. A further possibility is that if LATS protector is a thyroid stimulator it may not be mediated by cyclic AMP, and, though this would be surprising, it remains to be investigated.

Present day opinion regards as oversimplified the view that LATS is the sole cause of the hyperthyroidism in Graves's disease. Alternative hypotheses have been put forward but none completely fits the facts. For example, Solomon and Chopra (1972) suggest that the primary lesion in Graves's disease may be an abnormality in the plasma membrane along the basal surface of the follicular cell, which results in continuous activation of adenyl cyclase. However, it has been shown here that adenyl cyclase in "toxic" glands is activated by TSH and LATS and moreover the basal level of activity is not appreciably different from non-toxic glands. Another possibility, suggested by McKenzie (1972), to explain the problem of the apparently LATS-negative patient, is a difference in responsiveness to LATS between mouse and man; he concluded that failure to find LATS by mouse bioassay does not indicate its absence. Again the present study tends to repudiate this, since there is remarkably good correlation in response to LATS between mouse and human thyroid. The role of LATS protector in the pathogenesis of Graves's disease is far from clear. Though it does not appear to activate adenyl cyclase it may result in thyroid stimulation by some other mechanism, and at present this would seem to be one of the more fruitful possible lines of investigation.

I wish to thank Mrs. J. Moulson for her excellent technical help, Dr. S. Dirmikis who prepared the human thyroid extract, Professor D. S. Munro for his helpful criticism of the manuscript, and Mr. C. H. Talbot who performed the thyroidectomies.

ADDENDUM.—Since this paper was accepted for publication a report has appeared (Shishiba *et al.*, 1973) in which LATS protector was found to stimulate the formation of intracellular colloid droplets in human thyroid tissue.

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Pharmacological Studies with Lincomycin in Late Pregnancy

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British Medical Journal, 1973, 3, 75-78

Summary

The placental transmission of lincomycin was studied in 60 patients in late pregnancy. A peak maternal blood level of 12.5 µg/ml was recorded 45 minutes after injection, and detectable levels were still present up to 42 hours after a single injection. A peak cord blood level of 2.7 µg/ml was recorded 55 minutes after injection; cord blood levels were about a quarter of the maternal blood levels, and in most cases no levels were detectable 24 hours after a single injection. The passage of lincomycin into and out of the liquor was slower and more variable, but some hours after injection the liquor levels were always higher than the maternal or cord blood levels, and detectable levels were still present in the liquor 52 hours after a single injection. Repeated injections did not lead to any significant accumulation of lincomycin. The only side effect was a possible case of neuromuscular block in a mother delivered by caesarean section. No infant was adversely affected.

Introduction

Improved aseptic techniques and the active management of labour, as advocated by O'Driscoll *et al.* (1969, 1970), have helped almost to eliminate the problem of intrauterine infection during labour. Occasional cases still occur, especially after premature rupture of the membranes. More commonly intrauterine infection occurs after septic abortion in early pregnancy. In the three years 1967-9, 88 maternal deaths occurred in England and Wales from sepsis, 12 after delivery, 62 after abortion, and 14 after surgical intervention in pregnancy (D.H.S.S., 1972).

The organisms usually isolated in cases of intrauterine infection are streptococci or bacteroides. Gram-negative organisms may also be commonly isolated but their pathological role is not clear, while infection with *Clostridium welchii* or staphylococcus pyogenes may also occur, but less frequently. *Bacteroides* species vary greatly in their sensitivity to antibiotics, and a combination of penicillin and chloromycetin has commonly been used to treat obstetric cases (Pearson and Anderson, 1967, 1970). Recently lincomycin has been shown to be very effective

in the treatment of bacteroides infection (Geddes *et al.*, 1967; Tracy *et al.*, 1972). In addition to its activity against bacteroides, lincomycin is also very active against Gram-positive organisms—notably, staphylococci, haemolytic streptococci, and pneumococci. It is not active against Gram-negative bacilli or gonococci.

Gray *et al.* (1964) found that lincomycin crossed the placental barrier in rats without causing side effects, and Medina *et al.* (1964) recorded levels of 1.5-6.9 µg/ml in cord blood and liquor two to four hours after an intramuscular injection of 600 mg. However, despite its potential value in the treatment of intrauterine infection, there have been no detailed studies of the placental transfer of lincomycin. The present investigation was undertaken in order to determine whether lincomycin crossed the placental barrier in concentrations sufficient to justify its use in the treatment of intrauterine infection with bacteroides or Gram-positive cocci. Lincomycin was studied in preference to clindamycin because it can be given intramuscularly and is therefore more suitable for use during labour.

Patients and Methods

Clinical Procedures.—Sixty patients who were admitted to hospital for caesarean section, admitted for induction of labour because of postmaturity, or admitted in early labour with intact membranes were studied. They were divided into three groups: (1) 32 patients were each given lincomycin 600 mg by intramuscular injection at selected times from 20 minutes to 12 hours before artificial rupture of the membranes (A.R.M.). Lincomycin 600 mg was continued at 12-hourly intervals until delivery, but because of the active management of labour only seven patients received two doses and none received more than two. (2) 22 patients were given one intramuscular dose of lincomycin 600 mg at selected times between 12 and 52 hours before A.R.M. They were not given lincomycin during labour. (3) Five patients received two, and one patient received four, 12-hourly doses of lincomycin 600 mg before A.R.M. was performed. None of these patients received lincomycin after A.R.M. Liquor was collected at the time of A.R.M. by rupturing the hind-waters with a Drew-Smythe catheter; the forewaters were also ruptured after collection of the sample. Maternal blood was collected at the time of A.R.M. and at delivery. Cord blood was collected at delivery. The administration of the drug was part of a research procedure, and full consent of the patients was given.

Laboratory Methods.—Antibiotic levels were determined by the cup plate method using *Sarcina lutea* as test organisms incorporated into Difco antibiotic medium 11. Standard solutions of antibiotic were prepared in a diluent appropriate to the material under assay. All specimens were stored at -20°C until assay, which was performed within 72 hours of collection.

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