The Long-acting Thyroid Stimulator

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It is sixteen years since Adams and Purves (1956) first reported the detection of the long-acting thyroid stimulator (LATS) in the serum of patients with thyrotoxicosis. This discovery provoked a controversy about the significance of LATS in the pathogenesis of Graves' disease, which still continues. Because LATS activity can be recovered with γG-immunoglobulin (McKenzie, 1962; Adams and Kennedy, 1962; Meek *et al.*, 1964; Kriss *et al.*, 1964) and absorbed by thyroid subcellular particles or extracts (Kriss *et al.*, 1964; Beall and Solomon, 1966; Dorrington *et al.*, 1966a) it has been suggested that LATS may be a member of the group of autoantibodies to various thyroid components which have been recognised in disturbances of human thyroid function (Doniach and Roitt, 1957) including thyrotoxicosis. It must be acknowledged that the hypothesis that LATS-γG is a thyroid autoantibody with thyroid stimulating properties has no precedent in the pathogenesis of other autoimmune diseases. For detailed discussion of the scientific background to this subject the reader may consult several recent reviews (Dorrington and Munro, 1966; McKenzie, 1968; Werner and Naumann, 1968; Munro, 1970).

Here it is hoped to provide a broad survey representing various points of view regarding LATS and to show why scepticism regarding its importance in the pathogenesis of thyrotoxicosis persists. Patients with solitary toxic adenoma of the thyroid, in whom LATS cannot be detected, are excluded from the discussion.

ASSAY OF LATS

Although the original observations about LATS were made in guinea-pigs (Adams and Purves, 1953), the most widely used assay method was described by McKenzie (1958), who used mice. There are several modifications of McKenzie's original assay but all measure thyroid stimulation by following, in serial blood samples, the discharge of radioiodine from the thyroids of suitably prepared mice. In preparing the mice, a period of dietary iodine depletion is followed by a single injection of radioiodine and treatment with exogenous thyroid hormone to suppress endogenous thyroid stimulating hormone (TSH) secretion. In the actual assay the withdrawal of a control

blood sample before the injection of standard or test solutions allows the radioactivity of subsequent blood samples to be expressed as a percentage of the corresponding control sample from each mouse. The times of subsequent blood sampling are selected to fit in with the peak of the shorter time course of action of both TSH (usually 2 to 3 hr) and the later effect of LATS (usually 9 to 24 hr). It is by their characteristic time courses of action that TSH and LATS are recognised.

Other methods for the detection of LATS *in vitro* have been described but none has better sensitivity than the McKenzie assay. A major difficulty (*see* below) is that the sensitivity of the McKenzie assay may not allow the detection of LATS in assays of some unconcentrated serum samples that can subsequently be shown to contain LATS after suitable concentration procedures have been used (Carneiro *et al.*, 1966a). Thus, there is clearly a need for better assay methods and until they are available it will always be possible to explain away the failure to detect LATS in individual patients by referring to the relative insensitivity of the McKenzie assay.

CLINICAL CORRELATIONS

All surveys of sera drawn from normal subjects and from patients with a variety of thyroid disorders have shown an important correlation between the detection of LATS and a history of past or present Graves' disease. The report by Wall *et al.* (1969) that LATS may be found in asymptomatic relatives of patients with Graves' disease is analogous to the detection of an increased incidence of thyroid autoantibodies in asymptomatic relatives of patients with other thyroid disturbances.

There are two clinical 'markers' that are frequently associated with a high serum LATS level. The first to be described is the mother with a history of Graves' disease, who has had a baby who suffered from the very rare neonatal thyrotoxicosis (Major and Munro, 1960; Rosenberg *et al.*, 1963; McKenzie, 1964; Sunshine *et al.*, 1965; Holmes *et al.*, 1965). The second 'clinical marker' is the much commoner localised or pretibial myxoedema (Kriss *et al.*, 1964; Pimstone *et al.*, 1964; Bonnyns *et al.*, 1968). In groups of patients with these 'markers' there are some in whom the levels are not outstanding but, in general, they are efficient indicators of very high LATS levels.

It must be stressed that other patients, without either of these associations, may have equally high LATS levels but they cannot be detected clinically.

Finally, the conclusion that most workers have reached is that there is no relationship between serum LATS and the severity of the associated eye involvement in Graves' disease (Major and Munro, 1962; McKenzie and McCullagh, 1968).

CHEMICAL NATURE OF LATS

It is generally agreed that LATS activity may be separated from serum as an immuno-electrophoretically pure protein which is chemically and immunologically a member of the γG class of immunoglobulins (Adams and Kennedy, 1962; McKenzie, 1962; Meek *et al.*, 1964; Kriss *et al.*, 1964). As occurs with known antibodies (Sela and Mozes, 1966), LATS-γG may be found in different γG fractions with a wide range of electrophoretic mobility (Smith *et al.*, 1969a). Proteolysis of γG molecules with LATS activity has shown that the thyroid stimulating groups are in the Fab or Fab′ fragments (Meek *et al.*, 1964; Kriss *et al.*, 1965; Dorrington *et al.*, 1965, 1966b). Separation of H and L chain from LATS-γG has located the thyroid stimulating activity in the Fd part of the H chain, and reaggregation revealed that the intact molecule was necessary for maximum activity (Smith *et al.*, 1969b).

These observations all confirm the close similarities between LATS and γG antibodies and its differences from TSH. It is on this basis that LATS is thought to be an antibody.

RELATIONSHIP BETWEEN LATS AND THYROID AUTOANTIBODIES

There has been a series of investigations into the possible relationship between serum LATS and the titres of the known thyroid autoantibodies. The results are conflicting but on balance have failed to reveal any association; the relative sensitivity of the methods of measurement varies widely and, if LATS is indeed a human thyroid autoantibody, its detection by the McKenzie bioassay method might be influenced by variations in cross-reactivity with mice (Major and Munro, 1962; Hoffman and Hetzel, 1966; Bonnyns and Vanhelst, 1969). However, a study by Volpé *et al.* (1969) reported that LATS persisted after total thyroid ablation with radioiodine, whereas the titres of thyroid autoantibodies declined markedly.

ABSORPTION OF LATS BY THYROID EXTRACTS

The concept of LATS as an antibody prompted the work on LATS absorption. Kriss *et al.* (1964) first reported that serum LATS activity was lost when incubated with whole thyroid homogenate. When similar studies were done with thyroid subcellular particles the microsomal fraction was usually found to be the most effective in absorbing LATS (Beall and Solomon, 1966; Dorrington *et al.*, 1966b). Other tissues were either inactive or much less effective (Benhamou-Glynn *et al.*, 1967).

Berumen *et al.* (1967) showed that the greater part of the LATS binding activity in whole thyroid homogenate can be recovered in a soluble form, thus opening the way to attempts at further purification.

Recently, Florsheim *et al.* (1970) reported that the injection of thyroid extracts or thyroxine into mice prepared for the McKenzie assay apparently inhibited the response to LATS. This must cast some doubt on many studies in which loss of LATS activity by absorption has been detected by comparison of the responses to LATS in serum and to the LATS serum-thyroid extract incubation mixture injected into mice prepared for the McKenzie assay. However, studies on the dissociation of LATS after absorption (Smith, 1970) and the demonstration of LATS absorption by an *in vitro* assay (Brown and Munro, 1967) both confirm that LATS binding does occur, at least in some circumstances.

If LATS is an autoantibody the purification of the binding activity might reveal the antigen and allow the development of an improved competitive binding type of assay. The difficulties of thyroidal protein fractionation are likely to hinder the attainment of both these objectives.

MODE OF ACTION OF LATS

Earlier research established that LATS had thyroid stimulating actions other than the ability to stimulate radioiodine release. It was shown that LATS increased thyroidal uptake of radioiodine (McKenzie, 1960; Major and Munro, 1962), caused histological activation of the thyroid (McKenzie, 1960), and increased the rate of cell division (Garry and Hall, 1970).

Similarly, biochemical studies have shown that LATS stimulates glucose oxidation (Scott *et al.*, 1966; Shishiba *et al.*, 1970) and increases both the uptake of ^{32}P into phospholipid and RNA synthesis (Field, 1968; Ochi and DeGroot, 1968).

Comparisons of the *in vitro* response of thyroid tissue incubated with TSH, LATS, cyclic adenosine monophosphate (cyclic-AMP) or its dibutyryl derivative, show that their effects are all very similar (Ensor and Munro, 1969; McKenzie, 1967; Kendall-Taylor and Munro, 1970). This suggests that both TSH and LATS, in common with many other hormones, act through the mediation of cyclic-AMP.

Further evidence in support of this view comes from the measurement after stimulation of thyroidal cyclic-AMP concentrations (Klainer *et al.*, 1962; Gilman and Rall, 1966; Levey *et al.*, 1969) and thyroidal adenyl cyclase activity (Kaneko *et al.*, 1970; Levey and Pastan, 1970; Kendall-Taylor, 1972). Both TSH and LATS have lipolytic activity (Kendall-Taylor and Munro, 1971).

The overall picture is one of striking similarity between the actions of TSH and LATS. In general, the differences between the time courses of action of TSH and LATS, which are seen in *in vivo* experiments, do not persist *in vitro*.

There is, however, one exception to this generalisation, namely, the effects of LATS on thyroidal adenyl cyclase activity, which are delayed when compared with TSH (Levey and Pastan, 1970; Kendall-Taylor, 1972).

CONCLUSION

Even from this brief and incomplete review of the evidence there can be no doubt that LATS is a true thyroid stimulator associated in some way with Graves' disease and chemically quite different from TSH.

Apart from chorionic thyroid stimulating hormone or molar thyroid stimulating activity, which are not relevant to this discussion, LATS and TSH are the only clearly recognised thyroid stimulating hormones. Serum TSH is low in untreated thyrotoxicosis yet measurements made while patients are on treatment indicate that the normal 'negative feed-back' mechanism is intact (Adams and Kennedy, 1965; Kriss *et al.*, 1967). Why then is LATS not universally accepted as the cause of the excessive thyroid activity of Graves' disease? The chief argument against LATS as the cause of thyroid over-activity in thyrotoxicosis is the failure to demonstrate LATS in some patients who undoubtedly have severe Graves' disease. The proportion of 'LATS negative' patients has varied widely in different laboratories (McKenzie, 1968), but may be as high as 90 per cent, and no one has claimed to detect LATS in all patients even after the application of concentration procedures.

Kriss (1968) has consistently argued, from the striking clinical association between the presence of high LATS levels and localised or pretibial myxoedema, that LATS must be an 'epi-phenomenon', a consequence rather than the cause of thyrotoxicosis. In a detailed study, Chopra *et al.* (1970) failed to find any correlation between serum LATS activity and a large number of thyroid function tests, including 'non-suppressibility' of thyroidal 20-minute radioiodine uptake. All LATS measurements were made against a standard and this report is in agreement with other similar studies (Pinchera *et al.*, 1965; Bonnyns *et al.*, 1968). These authors are reluctant to accept the view that failure to detect LATS may only reflect the relative insensitivity of the assay method or a variable degree of interaction between LATS-γG and the mouse thyroid. Chopra *et al.* (1970) offer the hypothesis that the primary abnormality in Graves' disease may be an intrinsic disorder of the thyroid gland which renders it autonomous. There is no direct evidence to support this concept and it has been observed that the infusion of plasma from patients with Graves' disease will stimulate normal human thyroid function (Arnaud *et al.*, 1965). There is also evidence that, in patients in whom LATS can be detected in their unconcentrated serum, there is a strong positive correlation between circulating LATS and radioiodine turnover

(Carneiro *et al.*, 1966b). While concentration procedures increased the proportion of 'LATS-positive' sera (Carneiro *et al.*, 1966a), no conclusion could be drawn regarding the 45 per cent of patients in whom the initial serum LATS assay was negative. This conflict of evidence might well arise as a result of the differences between laboratories in the methods of study applied to the problem. If an improved LATS assay becomes available this is certainly the first problem to which it will be applied.

An alternative explanation arises in the description by Adams and Kennedy (1967) of a gamma globulin found in patients with thyrotoxicosis which protects LATS from absorption by thyroid extracts. This gamma globulin, which is called LATS protector, has recently been detected by the same authors in every one of a series of twenty patients with thyrotoxicosis (Adams and Kennedy, 1971). They failed to absorb LATS protector with mouse thyroid homogenate yet found that it was bound by human thyroid extracts. It was concluded that LATS protector may be the thyroid stimulator in thyrotoxic patients without detectable LATS. This work awaits confirmation, and direct evidence that LATS protector stimulates the thyroid is lacking, but the paper by Adams and Kennedy (1971) may be regarded as tacit recognition by one of the original discoverers that mechanisms other than LATS must be found to explain thyrotoxicosis in all patients.

At this stage no definite answer can be given to the question 'Does LATS cause thyrotoxicosis?' The problem would certainly come nearer to solution if a more sensitive assay became available, and further research on this problem and into 'LATS protector' is urgently needed.

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