Effects of Long-Acting Thyroid Stimulator on Thyrotropin Stimulation of Adenyl Cyclase Activity in Thyroid Plasma Membranes

KAMEJIRO YAMASHITA and JAMES B. FIELD

From the Clinical Research Unit and the Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

ABSTRACT Both thyroid-stimulating hormone (TSH) and long-acting thyroid stimulator (LATS) stimulated adenyl cyclase activity in plasma membranes obtained from bovine thyroid glands. The stimulation induced by LATS was much less than that obtained with maximal amounts of TSH. LATS inhibited TSH stimulation of adenyl cyclase activity while an equivalent amount of normal human γ-globulin did not influence basal or TSH-stimulated activity. The inhibition by LATS appeared to be noncompetitive and was greatest when the plasma membranes were initially exposed to LATS for 30 min at 0°C before being incubated with TSH for 10 min at 37°C. Inhibition could still be demonstrated when the plasma membranes were incubated for 30 min at 0°C with TSH before the addition of LATS. Prolonging the period of incubation of plasma membranes with LATS from 30 to 60 min did not augment the stimulation of adenyl cyclase or increase the inhibition of the effect of TSH. Papain digests of LATS also increased adenyl cyclase activity of thyroid plasma membrane and inhibited the stimulation induced by TSH. The inhibitory effect of LATS was not completely specific for TSH and thyroid plasma membranes since glucagon stimulation of adenyl cyclase in hepatic plasma membranes was also inhibited, but to a lesser extent. In contrast to the results obtained with thyroid plasma membranes, LATS did not influence basal adenyl cyclase activity in hepatic plasma membranes. Furthermore equivalent amounts of normal human γ-globulin also decreased glucagon stimulation of adenyl cyclase activity in plasma membranes obtained from liver. The present data suggest that LATS stimulation of adenyl cyclase in thyroid plasma membranes might be due to a change in the membrane configuration rather than binding to a specific receptor site. Such modification of the membrane structure could interfere with the binding of TSH to specific receptors or to the subsequent stimulation of adenyl cyclase. However, the results do not exclude the possibility that some component in the preparation other than LATS might be responsible for the inhibition of the stimulation by TSH.

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INTRODUCTION

Recent evidence indicates that thyroid-stimulating hormone (TSH [1]) regulates most, if not all, of the thyroid gland functions through activation of the adenyl cyclase-cyclic AMP system (1–4). TSH action is initiated by the binding of the hormone to receptors on the plasma membrane followed by activation of adenyl cyclase and the formation of cyclic AMP. We have previously reported that the plasma membrane fraction prepared from bovine thyroid glands contains most of the adenyl cyclase activity and this preparation is more sensitive to TSH stimulation than whole homogenate of thyroid (5).

Long-acting thyroid stimulator (LATS) is found in the serum of many patients with Graves' disease and has been implicated in its etiology (6–8). Moreover, LATS reproduces most of the metabolic effects of TSH on thyroid tissue (9–16). Kaneko, Zor, and Field reported that LATS increased cyclic-AMP levels and adenine-^{3}H incorporation into ^{3}H labeled cyclic AMP in dog thyroid slices (17). Levey and Pastan also reported that LATS stimulated adenyl cyclase activity in bovine and canine thyroid homogenates (18). These results indicate that LATS, like TSH, produces its effects as a consequence of activation of the adenyl cyclase-cyclic AMP system.

The present studies were undertaken to examine the effects of TSH and LATS, either alone or in combination, on the adenyl cyclase activity in thyroid plasma

[1] *Abbreviations used in this paper:* Cyclic AMP, cyclic 3',5' adenosine monophosphate; LATS, long-acting thyroid stimulator; TSH, thyroid-stimulating hormone.

membranes. It was hoped that such experiments would provide additional information concerning the binding sites for TSH and LATS.

METHODS

Bovine thyroids, obtained from a local abattoir, were transported to the laboratory on ice. Plasma membranes were prepared by the method which we reported previously (5). Plasma membranes from rat livers were prepared by the same method. Plasma membranes, suspended in 1 mM $NaHCO_3$, were used for the assay on the day of preparation or were stored at −20°C and used within a week. Such storage was associated with some decrease in basal adenyl cyclase activity but no change in responsiveness to TSH. Adenyl cyclase activity in plasma membranes was measured during a 10 min incubation as previously described (2). ^3H labeled cyclic AMP was used to monitor recovery of ^{14}C labeled cyclic AMP. The interaction between LATS and TSH on plasma membrane adenyl cyclase activity was studied by the three following procedures. (*a*) Plasma membranes (20–50 μg protein in 50 μl of 0.04 M Tris-HCl buffer, pH 7.8, containing 3.5×10^{-3} M $MgCl_2$, 10^{-2} M theophylline, and 1 mg/ml albumin) and appropriate amounts of LATS (10 μl of solution) were initially incubated together in a small test tube for 30 min at 0°C and then appropriate amounts of TSH (in 10 μl of solution) and ATP-^{14}C (in 50 μl of the above Tris-HCl buffer which also contained an ATP generating system) were added to initiate the 10 min incubation for adenyl cyclase assay at 37°C. The TSH and LATS were dissolved in the Tris-HCl buffer. This same sequence was used to examine the effect of γ-globulin, normal plasma, and antithyroglobulin antibody on TSH stimulation of thyroid plasma membranes and the effect of LATS on glucagon stimulation of hepatic adenyl cyclase activity. (*b*) Both LATS (in 10 μl) and TSH (in 10 μl) were incubated together for 30 min at 0°C and then plasma membranes (in 50 μl) and ATP-^{14}C (in 50 μl) were added to initiate the 10 min incubation at 37°C. (*c*) Plasma membranes (in 50 μl) and TSH (in 10 μl) were initially incubated together for 30 min at 0°C and then LATS (in 10 μl) and ATP-^{14}C (in 50 μl) were added to initiate the final 10 min incubation at 37°C. In some experiments the incubation at 0°C was for 60 min. The results are expressed as counts of ATP-^{14}C converted to ^{14}C labeled cyclic AMP per minute per milligram of protein of plasma membranes. Each result is the average ±SEM of triplicate determinations or the average of duplicate determinations.

Bovine TSH (2 U/mg) was a generous gift of the Endocrinology Study Section, National Institutes of Health. Three different LATS preparations were tested and gave identical results when compared on an equivalent unit basis. They varied in concentration from 27 to 39 mg protein/ml

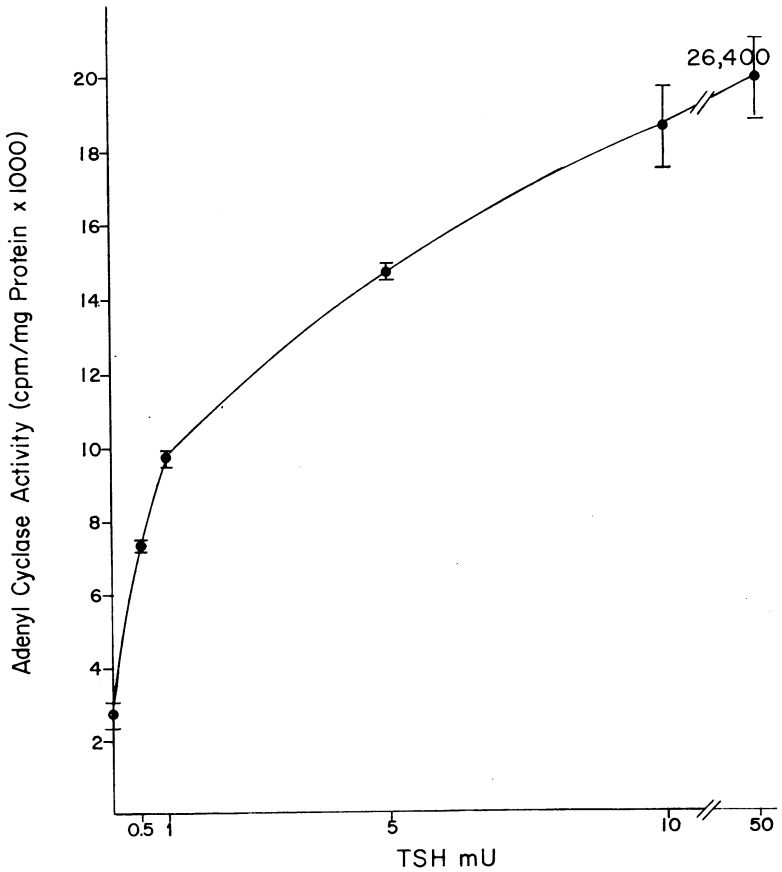

FIGURE 1 Effects of increasing amounts of TSH on thyroid plasma membrane adenyl cyclase activity.

and from 32.4 to 50 Kriss U/mg. The papain-digested LATS (7.4 mg/ml) was obtained from one of the LATS preparations. The LATS and papain-digested LATS were kindly supplied by Dr. Joseph Kriss, Stanford University School of Medicine, Palo Alto, Calif. Antithyroglobulin antibody preparation (8.3 g protein/100 ml) was kindly provided by Dr. Reed Larsen, Department of Medicine, University of Pittsburgh School of Medicine. 8-ATP-^{14}C (25 mCi/mmole) and ^{3}H labeled cyclic AMP (2.35 Ci/mmole) were obtained from Schwartz Bio-Research Inc., Orangeburg, N. Y.

RESULTS

As little as 0.5 mU TSH/0.12 ml markedly increased adenyl cyclase activity in plasma membrane fractions and larger doses of TSH caused further stimulation (Fig. 1). However, in whole homogenates prepared from the same thyroids, 0.5 mU TSH/0.12 ml had no effect. (Control = 217 cpm/mg protein, and 219 cpm/mg protein with TSH). Although 1 and 10 mU TSH significantly increased the activity in whole homogenates (259 cpm/mg protein), the effect was much less than that observed using plasma membrane fractions. LATS (0.05 U/0.12 ml) also significantly augmented adenyl cyclase activity in plasma membranes, and increasing effects were obtained with larger doses up to 0.25 U/0.12 ml (Fig. 2). However, no further increase was obtained using up to 2.7 U/0.12 ml even though the stimulation was considerably less than the maximum caused by

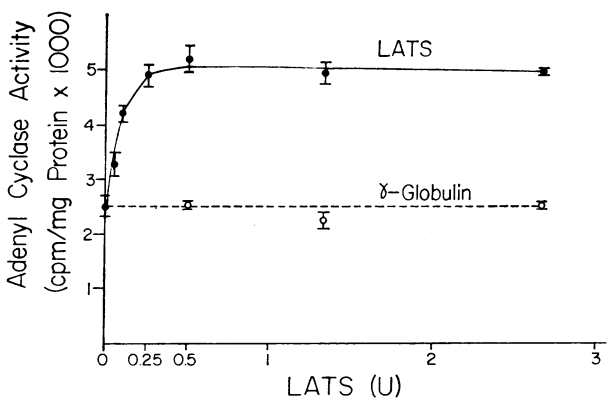

FIGURE 2 Effects of increasing amounts of LATS on thyroid plasma membrane adenyl cyclase activity. The amount of human γ-globulin was equivalent in weight to that of the LATS.

TSH. Amounts of γ-globulin equivalent in weight to that of LATS had no significant effect. In other experiments, increasing the LATS to 10 U/0.12 ml did not increase stimulation of adenyl cyclase.

The data in Fig. 3 demonstrate that incubation of plasma membranes with LATS for 30 min at 0°C inhibited the subsequent TSH stimulation of plasma membrane adenyl cyclase activity. A progressively greater inhibition resulted from larger amounts of LATS and

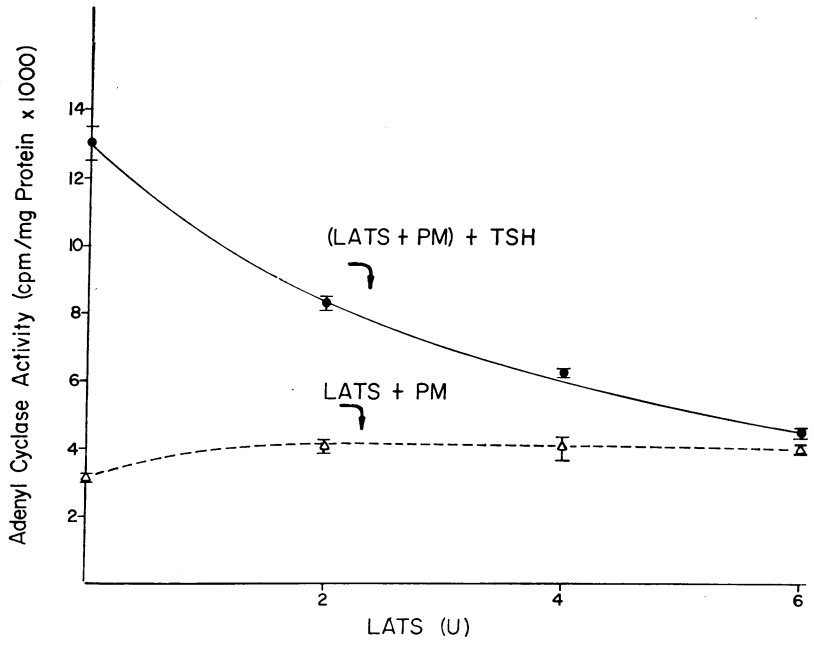

FIGURE 3 Inhibition of TSH stimulation of thyroid plasma membrane adenyl cyclase activity by varying amounts of LATS. Plasma membranes were incubated with the appropriate amount of LATS for 30 min at 0°C and then 10 mU TSH (upper curve) or Tris-HCl buffer (lower curve) was added and the mixture incubated for 10 min at 37°C.

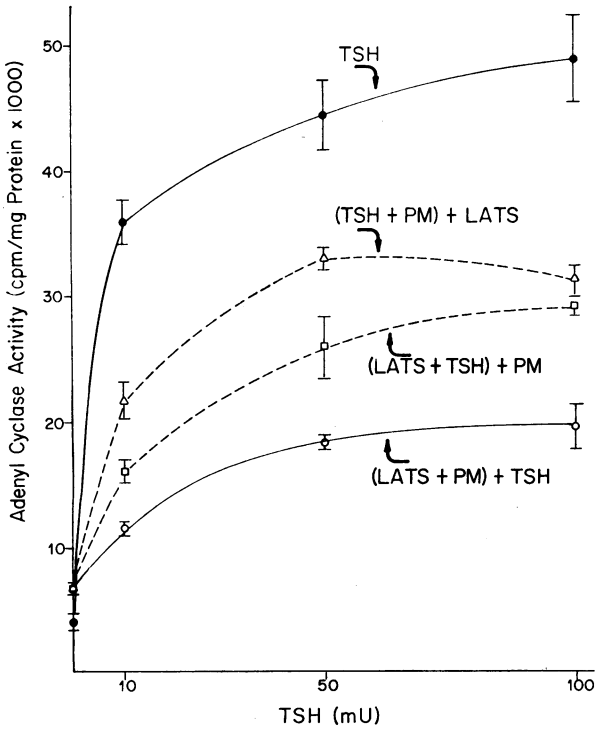

FIGURE 4 Effects of amounts and order of addition of LATS on the dose response curve of TSH stimulation of thyroid plasma membrane adenyl cyclase activity. Plasma membranes were incubated with TSH for 30 min at 0°C and then 10 μl Tris-HCl buffer added to initiate the 10 min assay for adenyl cyclase at 37°C (top curve). Plasma membranes (PM) were incubated with TSH for 30 min at 0°C and then 6 U LATS was added to start the 10 min assay for adenyl cyclase (second curve). TSH and LATS (6 U) were incubated for 30 min at 0°C and then plasma membranes (PM) were added to initiate the 10 min adenyl cyclase assay (third curve). Plasma membranes were incubated with LATS (6 U) for 30 min at 0°C and then TSH was added for the assay of adenyl cyclase (lowest curve).

6 U almost completely inhibited the effect of 10 mU TSH. The extent of the LATS inhibition of TSH depends upon the order in which LATS and TSH are added to the plasma membranes (Fig. 4). The top curve demonstrates the stimulation induced by increasing amounts of TSH. The next curve represents the results when differing amounts of TSH were added to plasma membranes for 30 min at 0°C and then LATS (6 U) was added to initiate a 10 min incubation at 37°C. LATS significantly reduced the stimulation of each dose of TSH. Even greater inhibition was produced by initially incubating the TSH and LATS together for 30 min at 0°C and then addition of plasma membranes for a further 10 min incubation at 37°C. The greatest reduction in TSH stimulation was obtained when LATS and plasma membranes were initially incubated together for 30 min at 0°C and then TSH added for the final 10 min incubation at 37°C. When these data are analyzed using a Lineweaver-Burke plot, the inhibition induced by LATS appears to be noncompetitive (Fig. 5).

The data in Fig. 6 evaluated the effect of different incubation times at 0°C with either TSH or LATS on adenyl cyclase activity. The top curve demonstrates that preincubation of TSH with plasma membranes for 30 or 60 min did not augment the stimulation of adenyl cyclase activity. However the LATS stimulation was increased somewhat after a 30 or 60 min incubation with the plasma membranes at 0°C. Although longer exposure of the plasma membranes (30 min and 60 min) to LATS seemed to increase the inhibition of TSH stimulation, the differences were not statistically significant.

Amounts of normal human γ-globulin equivalent to those of LATS did not change basal adenyl cyclase activity or the stimulation induced by TSH (Fig. 7). Basal adenyl cyclase activity was also not modified by normal rabbit plasma (containing 200 μg protein) or the same protein concentration of plasma containing antithyroglobulin antibodies (Fig. 8). However, the TSH stimulation was partially inhibited by both of these preparations indicating that the inhibiting effect of LATS is not completely specific. The explanation for the inhibition by normal rabbit plasma and antithyroglobulin antibody plasma is not apparent. The inhibition was still present after dialysis of the plasma and could not be accounted for by the albumin content of the plasma (unpublished observations). The specificity of the inhibition caused by LATS was also investigated utilizing glucagon stimulation of

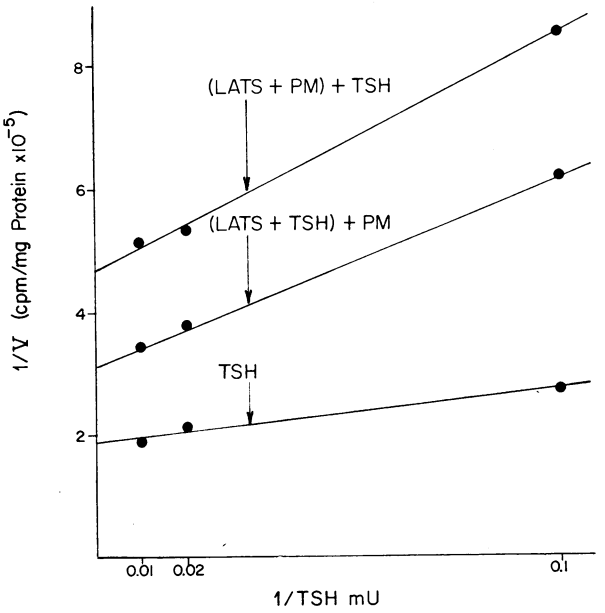

FIGURE 5 Lineweaver-Burke plot of some of the data in Fig. 4.

adenyl cyclase in plasma membranes prepared from rat liver. The data in Fig. 9 demonstrate that 10^{-9} M and 0.45×10^{-6} M glucagon stimulated adenyl cyclase activity in rat liver plasma membranes. LATS (6 U), by itself, had no significant effect on hepatic adenyl cyclase. However, when liver plasma membranes and LATS were incubated together for 30 min at 0°C and then glucagon added for a 10 min incubation at 37°C, there was some reduction of the stimulating effect of glucagon. Similar, but less, inhibition was obtained when an equivalent amount of normal human γ-globulin was substituted for the LATS. This amount of normal γ-globulin had no effect on basal adenyl cyclase activity. LATS inhibition of glucagon was much less than that with TSH.

Papain digests of LATS also augmented thyroid plasma membrane adenyl cyclase activity (Fig. 10). Even though the stimulation was much less than that induced by TSH, increasing the amount of papain digests of LATS did not induce any greater stimulation. These results are similar to those obtained with increasing concentrations of LATS. The papain digests of LATS also inhibited the TSH stimulation of adenyl cyclase. However, based on amounts that gave stimulation equivalent to LATS, the inhibition by papain digests of LATS was much less than that caused by LATS.

DISCUSSION

Previous studies (5) and the present data (Fig. 1) indicate that the adenyl cyclase activity in thyroid plasma membrane fractions was more sensitive to TSH stimulation than whole homogenate prepared from the same glands. The stimulation of adenyl cyclase induced by LATS (Fig. 2) is consistent with our previous report that LATS increased cyclic AMP levels and adenine-^{3}H incorporation into ^{3}H labeled cyclic AMP in dog thyroid slices (17). The greater sensitivity of the adenyl cyclase activity in the plasma membranes probably accounts for our previous failure to demonstrate LATS stimulation of the enzyme in whole thyroid homogenates. Wolff and Jones have also reported a bovine thyroid plasma membrane preparation containing adenyl cyclase activity which was stimulated by TSH (19). They, however, were unable to obtain stimulation using LATS. This discrepancy could be explained on the basis that their adenyl cyclase preparation was much less sensitive to TSH than ours. With our preparation 1 mU TSH increased adenyl cyclase activity fourfold (Fig. 1) while Wolff and Jones required 25–50 mU/ml to double the activity using their purified membrane fraction. Since the stimulation caused by LATS was almost always less than that induced by 1 mU TSH, it is not surprising that Wolff and Jones obtained negative results. The maximum stimulation caused by LATS, usually less than

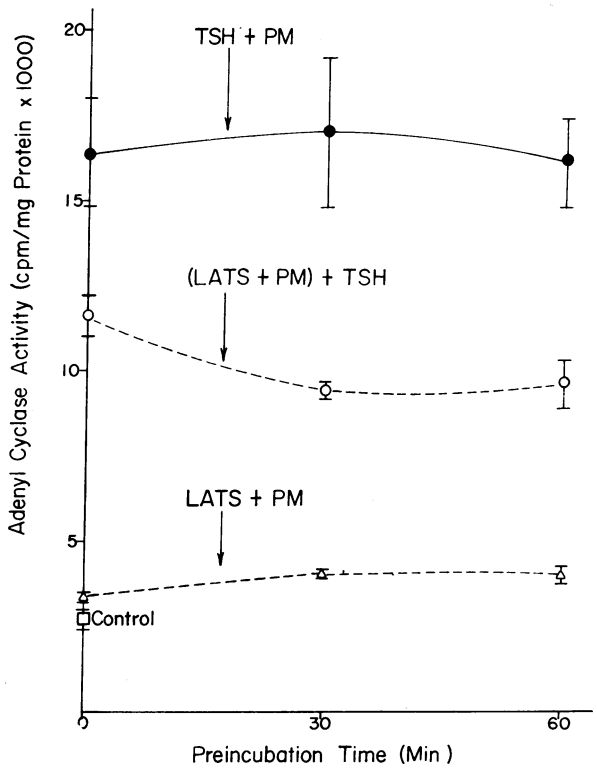

FIGURE 6 Effect of duration of preincubation with TSH (10 mU) or LATS (3 U) on thyroid plasma membrane adenyl cyclase activity. Plasma membranes (PM) were preincubated with TSH for 0, 30, or 60 min at 0°C before assay of adenyl cyclase activity for 10 min at 37°C (top curve). Plasma membranes were preincubated with LATS for 0, 30, or 60 minutes at 0°C and then TSH was added to initiate assay of adenyl cyclase activity (middle curve). Plasma membranes were preincubated with LATS for 0, 30, or 60 minutes at 0°C and then assayed for adenyl cyclase activity (lowest curve).

a doubling of adenyl cyclase activity, was attained with a dose of 0.25–0.5 U. The lack of effect of larger amounts of LATS does not reflect maximum stimulation of adenyl cyclase activity since much greater stimulation was obtained with increasing amounts of TSH.

LATS inhibition of TSH stimulation of adenyl cyclase was unexpected but would be consistent with the reports of McKenzie (20) and Burke (21) that administration of LATS to mice almost completely prevented the augmented ^{131}I release induced by TSH 2 hr later. The inhibiting action of LATS in these latter experiments could be accounted for on the basis of the present results. Assuming that the TSH stimulation of ^{131}I release reflects increased cyclic AMP, then the LATS inhibition of the increased plasma membrane adenyl cyclase induced by TSH would prevent the rise in cyclic AMP. An interaction between LATS and the plasma membrane is also indicated since LATS caused

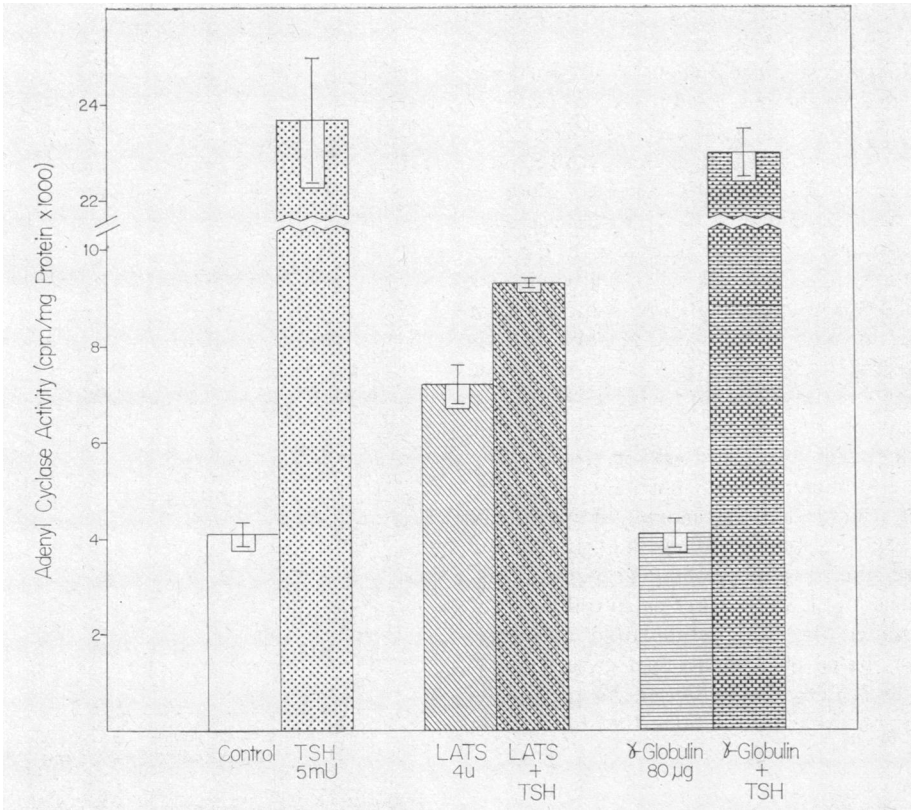

FIGURE 7 Effects of LATS and equivalent amounts of human γ-globulin on basal and TSH-stimulated adenyl cyclase activity in thyroid plasma membranes. Plasma membranes were incubated with LATS, γ-globulin, or Tris-HCl buffer for 30 min at 0°C and then either TSH or buffer added to initiate assay of adenyl cyclase.

some stimulation of adenyl cyclase activity. The greatest inhibition of the TSH effect was observed when the plasma membranes were initially exposed to LATS for 30 min at 0°C before the addition of TSH. Such a procedure would provide LATS a better opportunity to modify the membrane or occupy binding sites so that when TSH was added, it would be less able to combine with its receptors. The results of this experiment and those in which the order of addition of LATS, TSH, and plasma membranes were varied indicate that LATS can effectively interfere with TSH binding to the plasma membranes or its subsequent activation of adenyl cyclase.

Although the finding that increasing amounts of LATS produced greater and complete inhibition of TSH might suggest a competitive type of inhibition, examination of the data by Lineweaver-Burke plot demonstrates that the inhibition is probably noncompetitive. This conclusion casts doubt on the supposition that TSH and LATS are binding to the same receptors on the plasma membrane. In fact it is quite possible that LATS is not interacting with specific receptors at all but that it is causing a modification of the plasma membrane and/or TSH receptor sites. Such a modification itself might cause some, but limited, activation of adenyl cyclase activity and also prevent the interaction of TSH with its specific receptors or the subsequent stimulation of adenyl cyclase. The present data do not delineate between these possibilities. The combination of LATS and TSH producing maximum inhibition never reduced adenyl cyclase activity to the control level but only to that induced by LATS itself.

Since the LATS preparation does not represent a single, purified substance, the possibility must certainly be considered that the stimulating and inhibitory actions represent two separate substances in this impure preparation. This possibility cannot be excluded even though essentially identical results have been obtained using three different LATS preparations of varying degrees of purity. The stimulation of adenyl cyclase might represent the LATS in the preparation while the inhibition of TSH would be accounted for by another substance.

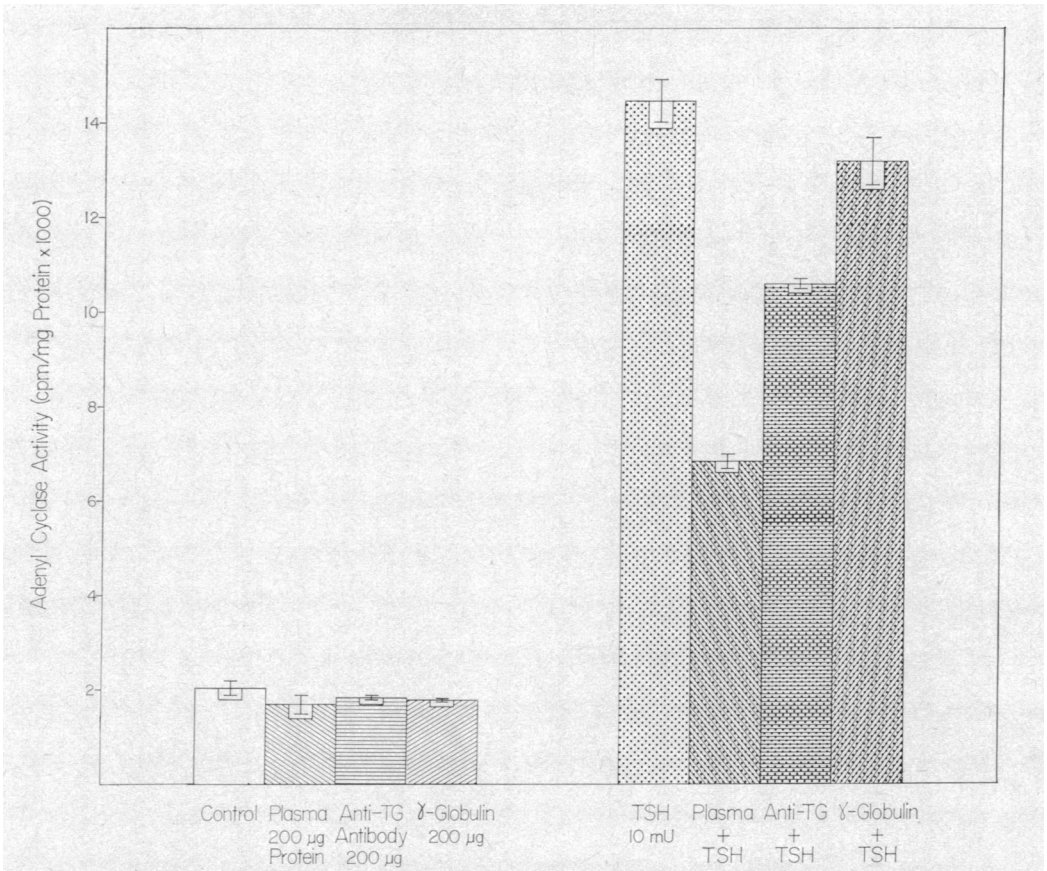

FIGURE 8 Effects of normal rabbit plasma, antithyroglobulin antibody plasma and human γ-globulin on basal and TSH-stimulated adenyl cyclase activity in thyroid plasma membranes. Plasma membranes were incubated with Tris-HCl buffer, normal rabbit plasma, rabbit plasma containing antithyroglobulin antibody, or human γ-globulin for 30 min at 0°C and then TSH (10 mU) or buffer was added to initiate the 10 min assay for adenyl cyclase.

The fact that increasing amounts of LATS did not mimic the action of greater doses of TSH in producing increasing effects cannot be interpreted as evidence for or against this point. If the inhibitory substance causes modifications in the plasma membrane, then increasing amounts of the LATS component would be incapable of augmenting adenyl cyclase just as greater amounts of TSH could not overcome the inhibition induced by the LATS. Recent studies have demonstrated the coexistence of antimicrosomal and antithyroglobulin antibodies in addition to LATS in the plasma of patients with Graves' disease (22–24). Our finding that antithyroglobulin antibodies also inhibit TSH stimulation of adenyl cyclase indicates that such antibodies might be responsible for the inhibition observed when LATS was tested. In addition, Mori, Fisher, and Kriss reported that LATS preparations contained an antimicrosomal antibody (anti-M) which appeared to be responsible for the inhibition of binding of ^{125}I labeled LATS-IgG to thyroid microsomes when unlabeled LATS was added (22). A similar conclusion was also suggested by the previous results of Pinchera et al. (23). Fagraeus, Jonsson, and ElKabir also felt that mixed hemadsorption reactions were probably due to the thyroid cell surface antibodies usually present in sera from patients with thyrotoxicosis rather than to the reactivity of LATS (25). The inhibition of TSH stimulation of plasma membrane adenyl cyclase by normal rabbit plasma was unexpected and unexplained. The inhibition was not removed by dialysis nor could it be attributed to albumin since addition of equivalent amounts of albumin to the incubation medium had no effect. It is not apparent whether the inhibition represents an interaction with the TSH itself or some component of the plasma membrane preparation.

Papain-digested LATS produced results which were similar to those using LATS. Basal adenyl cyclase activity was increased and the stimulatory effect of

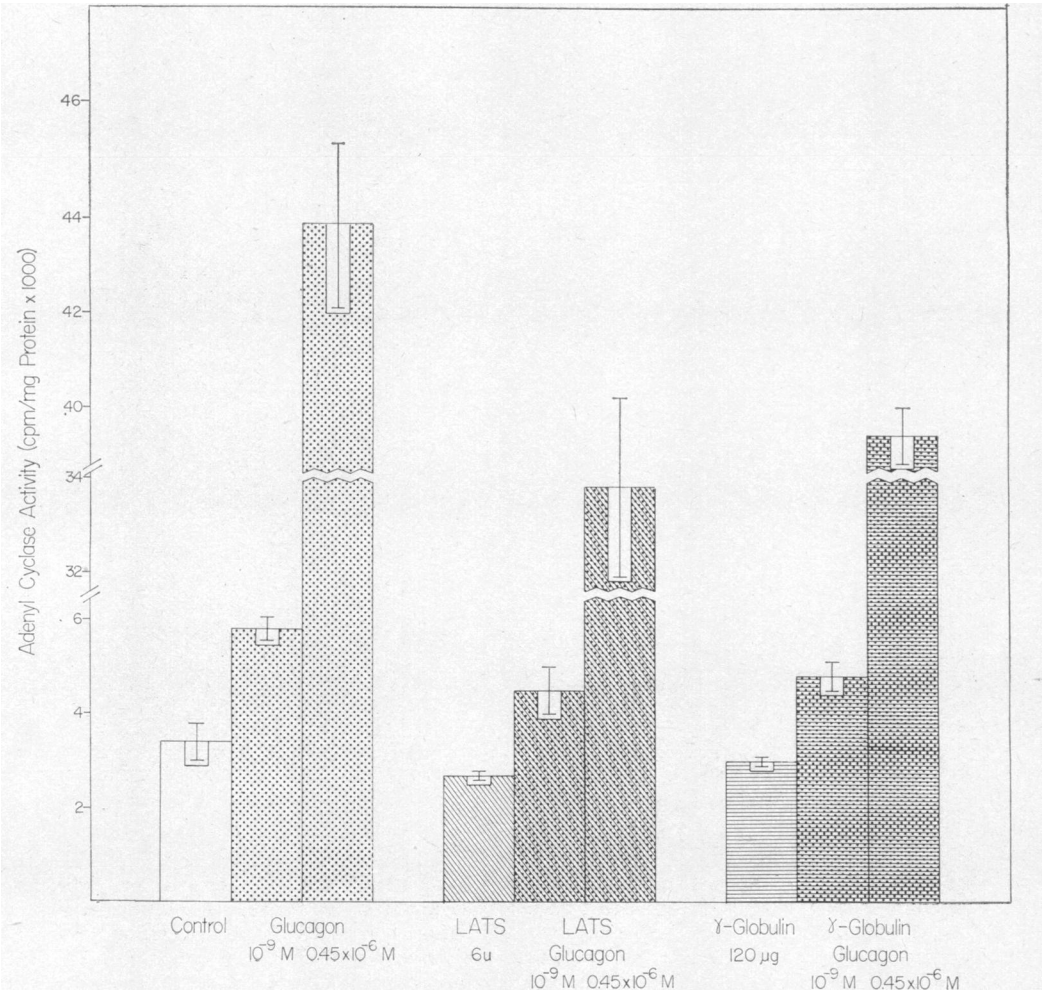

FIGURE 9 Effects of LATS and human γ-globulin on basal and glucagon-stimulated adenyl cyclase activity in rat liver plasma membranes. Plasma membranes were incubated with Tris-HCl buffer, LATS (6 U), or an equivalent amount of human γ-globulin for 30 min at 0°C and then glucagon (10^{-9} M and 0.45×10^{-6} M) or buffer was added to initiate the assay for adenyl cyclase.

TSH was inhibited. A dose of papain-digested LATS which caused stimulation equivalent to LATS was much less effective in inhibiting the action of TSH. This discrepancy might reflect the smaller molecular size of the papain digested LATS. That part of the LATS molecule necessary for activation of adenyl cyclase might be much smaller than that responsible for the inhibition of TSH action. The result could also be due to a greater effect of papain on the inhibitory component of the LATS preparation.

The effect of LATS on the stimulation of liver plasma membranes by glucagon provides further evidence for the concept that the LATS preparation might contain at least two biologically active components or that the effect of LATS involves some type of non-specific modification of the plasma membrane. In contrast to the results with thyroid plasma membranes, LATS did not modify the basal adenyl cyclase activity in liver plasma membranes. Thus, if LATS modifies the plasma membrane it does it differently in thyroid and liver. However, LATS inhibited the stimulation of adenyl cyclase induced by glucagon in hepatic plasma membranes. If the LATS preparation contains two biologically active components, the inhibitory one is active on both thyroid and liver plasma membranes. The inhibition of glucagon stimulation was not specific for LATS since similar results were obtained with equivalent amounts of normal γ-globulin. The effects of LATS on tissues other than the thyroid have not been extensively investigated. Field, Remer, Bloom, and

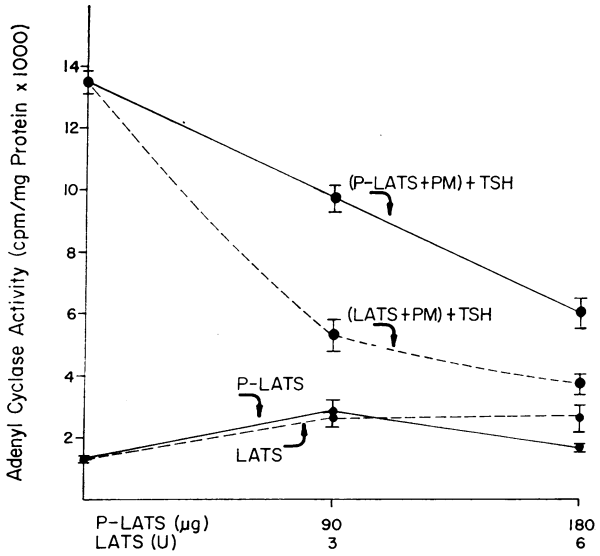

FIGURE 10 Comparison of effects of LATS and papain digests of LATS on TSH (10 mU) stimulation of thyroid plasma membrane adenyl cyclase activity. Plasma membranes (PM) were incubated with the appropriate amount of LATS or papain-digested LATS for 30 min at 0°C and then 10 mU TSH or Tris-HCl buffer was added for assay of adenyl cyclase activity at 37°C for 10 min.

Kriss reported that LATS sometimes increased glucose oxidation in dog liver and spleen slices, but the results were not as consistent as when thyroid slices were used (10). It has also been reported that LATS stimulates lipolysis in adipose tissue, an effect similar to that of TSH (26).

Unfortunately these data do not provide any more specific data relative to either the binding site for TSH or for LATS. Nor do they contribute to the controversy concerning the importance of LATS in the etiology of Graves' disease. They do indicate that LATS can stimulate adenyl cyclase in thyroid plasma membranes but such an effect might be due to a change in the membrane configuration unrelated to any specific binding sites. The inhibition by LATS or TSH effects could also represent a nonspecific effect or might reflect the presence of a contaminating substance in the LATS preparation.

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Dr. Yamashita is Research Associate, Clinical Research Unit, University of Pittsburgh School of Medicine.

Dr. Field is Director, Clinical Research Unit, and Professor of Medicine, University of Pittsburgh School of Medicine.

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