*EFFECTS OF THYROID HORMONES ON ADENYL CYCLASE IN ADIPOSE TISSUE AND ON FREE FATTY ACID MOBILIZATION**

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Some signs of hyperthyroidism, including enhanced responses to external stimulation and elevation of basal metabolic rate, are reminiscent of increased sympathetic activity. The possibility has been raised that the action of thyroid hormones is mediated through catecholamines.[1, 2] Furthermore, hypothyroidism is associated with depressed sympathetic responses to catecholamines.[3] The possibility that the thyroid hormones increase the rate of norepinephrine (NE) synthesis is not supported by a recent study from this laboratory showing that the turnover rate of cardiac NE is the same in normal and hyperthyroid mice.[4]

Published reports indicate that the exposure of hyperthyroid rats to cold or the injection of these animals with catecholamines elicits an exaggerated rise in plasma FFA.[5] In contrast, the responses to these stimuli are greatly diminished in thyroidectomized rats but are restored by treatment of the animals with thyroid hormones. Similarly, epinephrine elicits an exaggerated lipolytic response on incubation with epididymal fat pads from hyperthyroid rats, while the catecholamine elicits almost no response from fat pads of hypothyroid rats.[6–8] Again the lipolytic response is restored if the adipose tissue is taken from hypothyroid rats after treatment with thyroid hormone.

Since NE, epinephrine, isoproterenol, and corticotropin, added to adipose tissue *in vitro*, all stimulate lipolysis to almost the same maximum degree,[9, 10] it is generally assumed that the rate of this process is limited by the amount of lipase in adipose tissue. Thyroxine is usually thought to enhance the lipolytic effects of catecholamines by increasing the amount of catecholamine-sensitive lipase in adipose tissue.[6] Recent findings from this laboratory[11, 12] have demonstrated that the total lipase activity of adipose tissue is much higher than heretofore considered. This conclusion is based on experiments showing that NE in high concentrations induces only a fraction of the lipolytic response produced when phosphodiesterase is blocked by high concentrations of theophylline. From these results it was inferred that the maximal effects of theophylline are caused by a rise in cyclic 3′,5′-AMP to a level that completely activates lipase, while the effects of NE are limited by the level of cyclic 3′,5′-AMP produced when adenyl cyclase is completely activated.

The present study concerns the effects of theophylline and norepinephrine in adipose tissue from hypothyroid, hyperthyroid, and euthyroid rats. The results support the hypothesis that thyroxine enhances the action of catecholamines on the lipolytic system by increasing the amount of adenyl cyclase and not by increasing the total lipase activity.

Materials and Methods.—Experiments were carried out on male Sprague-Dawley rats. Animals were made hyperthyroid by injection of *l*-thyroxine (1 mg/kg s.c.) each day for

5 days and used for experiments 1 day after the last injection. Animals (Hormone Laboratories, Chicago) were made hypothyroid by surgical removal of the gland 6 weeks before the experiment. The rate of oxygen consumption was determined in a spirometer (model 160, Custom Engineering and Development Co., St. Louis, Mo).

Lipolysis in adipose tissue was determined by the rate of glycerol release from minces of epididymal fat pads.[13] Minced tissue weighing 50 mg was incubated for 1 hr, at 37°C, in 1 ml Krebs-Ringer phosphate buffer, pH 7.4, containing 5% albumin (fraction V, Nutritional Biochemical Corp., Cleveland, Ohio).

Protein in fat pad was estimated according to Sutherland *et al.*[14] Glycerol was assayed according to Lambert and Neish.[15] Phosphodiesterase in fat pads was determined as previously described.[12]

Adenyl cyclase in fat pads was assayed according to the method of Krishna *et al.*[16] from the rate of formation of H^3-cyclic AMP from H^3-ATP. In this method, fat pads were homogenized with 3 vol of a buffer containing Tris HCl, pH 7.3 (8×10^{-2} *M*), $MgSO_4$ (7×10^{-3} *M*), NaF (2×10^{-2} *M*), and theophylline (2×10^{-2} *M*), and a 0.4-ml aliquot was incubated at 30°C with 0.2 ml of H^3-ATP (6×10^{-3} *M*, 33 $\mu c/\mu$mole). The enzyme reaction was terminated by immersion in boiling water for 2–3 min after the addition of 0.1 ml of cyclic 3′,5′-AMP (5 mg/ml) as a carrier. The cyclic 3′,5′-AMP was separated from other radioactive material by chromatography on a Dowex 50-H^+ column and precipitation by zinc sulphate-barium hydroxide. The cyclic H^3-3′,5′-AMP was measured by scintillation spectrometry and the recovery determined from the UV absorption (260 mμ) of the unlabeled cyclic 3′,5′-AMP. The enzyme activity was measured from the linear rate of formation of H^3-cyclic 3′,5′-AMP over a 15-min period and was expressed as $\mu\mu$mole of cyclic 3′,5′-AMP/mg of protein/min. The identity of the H^3-cyclic 3′,5′-AMP isolated by the above procedure was authenticated by enzymatic conversion to 5′-AMP by cyclic 3′,5′-AMP phosphodiesterase, by chromatography in a number of ion-exchange and paper chromatographic systems, and by recrystallization to constant specific activity after addition of carrier cyclic 3′,5′-AMP.[16]

Results.—Effects of treatment with thyroxine: Rats were made hyperthyroid by the administration of thyroxine (1 mg/kg) for five days. At this time, the oxygen consumption was increased by about 40 per cent and the protein content of

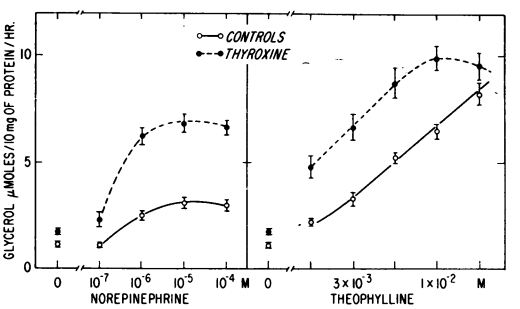


FIG. 1.—*Right,* lipolytic effects of theophylline in adipose tissue of control and hyperthyroid rats.

Left, lipolytic effects of NE in adipose tissue of normal and hyperthyroid rats. Rats were injected with *l*-thyroxine, 1 mg/kg s.c., every day for 5 days. Each point represents mean value of 6–8 experiments ± SE.

adipose tissue by about 15 per cent. Figure 1 (*right*) shows that adipose tissue from hyperthyroid rats was more sensitive to the lipolytic effects of theophylline than that from control animals. However, the maximum lipolytic response produced by high concentrations of theophylline was almost the same in the two groups. These results suggested that thyroxine treatment had not increased the quantity of adipose tissue lipase.

In addition, the maximum lipolytic response to NE of adipose tissue from hyperthyroid rats was two to three times that from control animals. Since NE

was added in amounts sufficient to activate adenyl cyclase completely, it was inferred that the amount of this enzyme had been increased by the action of thyroxine. Further evidence of this was seen from the lipolytic effects of NE and theophylline in various combinations. The NE-induced lipolysis in adipose tissue from normal and hyperthyroid rats was potentiated by the action of theophylline (Fig. 2). For example, at a concentration of 10^{-8} M, NE exerted little or no lipolytic effect on adipose tissue from normal or hyperthyroid rats. In the presence of 10^{-3} M theophylline, this concentration of NE elicited almost maximum lipolysis in adipose tissue of hyperthyroid rats and lipolysis to a lesser extent in adipose tissue from normal animals. Although adipose tissue from hyperthyroid rats was more sensitive to the drug combination, the maximum response was the

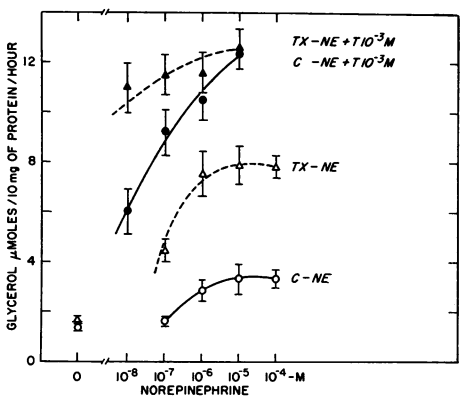


FIG. 2.—Lipolytic effects of NE and of combinations of NE and theophylline (T) in adipose tissue of normal and hyperthyroid rats. C, normal rats; TX, l-thyroxine–1 mg/kg s.c., every day for 5 days. Each point represents mean value of 6–8 experiments ± SE.

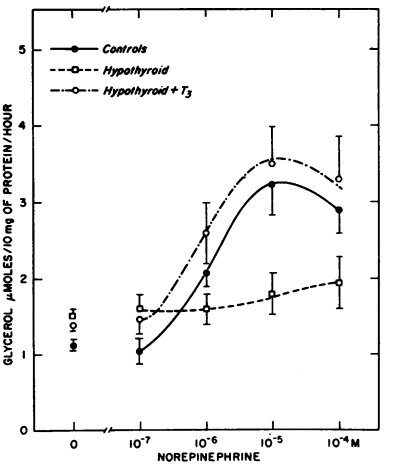


FIG. 3.—Lipolytic effects of NE on adipose tissue of control, thyroidectomized, and thyroidectomized rats treated with triiodothyronine (T_3). Rats were thyroidectomized 6 weeks previously. Triiodothyronine was given to thyroidectomized animals in a single dose of 250 μg/kg, 48 hr before the experiment. Each point represents mean value of 6 experiments ± SE.

same in both cases (Fig. 2). This is additional evidence that the amount of lipase was not changed by treatment with thyroxine.

Effects of thyroidectomy: The lipolytic responses to NE and theophylline were compared in adipose tissue from control and hypothyroid rats. The adipose tissue of thyroidectomized rats showed only a slight response to NE relative to the controls. However, within 48 hours after a single dose of triiodothyronine was given to the thyroidectomized animals, the responsiveness to NE was restored (Fig. 3).

The adipose tissue of thyroidectomized rats was found to be somewhat less

sensitive to low concentrations of theophylline. However, a high concentration of theophylline (10^{-2} *M*) elicited the same maximal rate of lipolysis in adipose tissue from hypothyroid, hyperthyroid, and normal animals. These results suggest that the amount of lipase was not changed by thyroidectomy.

Assay of adenyl cyclase: The most direct evidence that thyroid hormone had increased the amount of adenyl cyclase was shown from assays of the enzyme in adipose tissue from control, hyperthyroid, and thyroidectomized rats (Table 1).

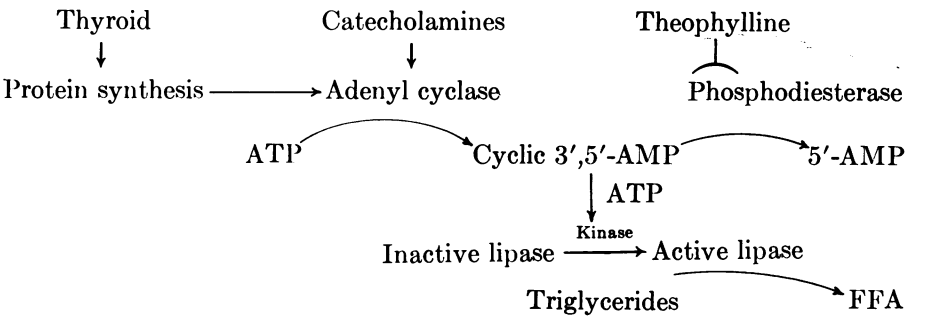
TABLE 1. *Effects of thyroxine on adenyl cyclase and phosphodiesterase activity and on lipolytic response to NE in epididymal fat pads.*

	Lipolytic activity (μmole glycerol/hr)	Adenyl cyclase activity (μμmole cyclic 3′,5′-AMP/min)	Phosphodiesterase activity (μμmole P_i/min)
Control	0.31 ± 0.05	90 ± 10	7910 ± 800
Hyperthyroid	0.68 ± 0.05	198 ± 20	7860 ± 1740
Hypothyroid	0.10 ± 0.05	50 ± 5	—

Rats received thyroxine, 1 mg/kg s.c., each day for 5 days and were killed on sixth day. The pads from left side were used for the assay of adenyl cyclase activity and the pads from right side were minced and incubated in NE (10^{-5} *M*) as described in *Methods*. Each value represents the mean value of 4 experiments ± SE. For each experiment the fat pads from 3 animals were pooled. Lipolytic activity was measured from release of glycerol, adenyl cyclase activity from formation of cyclic AMP, and phosphodiesterase activity from conversion of cyclic AMP via 5′-AMP to inorganic phosphate. Activities are expressed per milligram protein.

In hyperthyroid rats, the activity of adenyl cyclase and the lipolytic responses to NE had increased in almost the same proportion, whereas in thyroidectomized rats, the activity of adenyl cyclase and the lipolytic response to NE were markedly reduced. Data in Table 1 also show that thyroid did not affect the activity of phosphodiesterase.

Discussion.—The scheme outlined below is a convenient one for describing the effects of certain drugs on the mobilization of FFA from adipose tissue *in vitro*.



The rate of lipolysis depends on the extent to which inactive lipase is converted to the active form; this in turn depends on the intracellular level of cyclic 3′,5′-AMP.[17, 11, 12] The nucleotide level at any time reflects the balance between formation by adenyl cyclase and its inactivation by phosphodiesterase.

In the absence of sympathetic function, cyclic 3′,5′-AMP is formed continuously at a low rate.[11] NE increases the level of cyclic 3′,5′-AMP by stimulating the adenyl cyclase system. With increasing concentrations of catecholamine, cyclic

3′,5′-AMP rises until adenyl cyclase is completely activated. Theophylline also increases the steady-state level of the nucleotide by inhibiting the destruction by phosphodiesterase. With increasing concentrations of theophylline there is a progressive rise in the cyclic 3′,5′-AMP level until lipase is completely activated. Since the maximal lipolytic response elicited by theophylline is considerably greater than that evoked by NE, it may be inferred that normally the steady level of cyclic 3′,5′-AMP is the limiting factor in the activation of lipase by the sympathetic system. To this scheme must now be added reactions that describe the effects of thyroid hormones. Our present studies indicate that these hormones do not affect the total lipase activity of adipose tissue, since large concentrations of theophylline produce the same maximum lipolytic activity in adipose tissue from hypothyroid, euthyroid, and hyperthyroid rats. However, adipose tissue from hyperthyroid rats is hyperresponsive to the stimulating action of NE, while adipose tissue from hypothyroid rats is hyporesponsive. This suggests that the amount of adenyl cyclase is increased by thyroid hormones and reduced by thyroidectomy. Direct assay of adenyl cyclase activity in adipose tissue in fact shows that thyroxine more than doubles the adenyl cyclase activity and thyroidectomy reduces it to a very low value. The increased activity of adenyl cyclase present in the hyperthyroid state enables catecholamines to stimulate the formation of cyclic 3′,5′-AMP at a greatly increased rate, while the converse is true after thyroidectomy.

We are now testing the mechanism by which adenyl cyclase activity is increased after thyroid hormones. From preliminary results (this laboratory, unpublished) it appears that it is due to *de novo* synthesis of the enzyme; thus triiodothyronine (10^{-6} *M*) incubated with fat cells for a period of two hours increases the activity of adenyl cyclase by 50 per cent. This increase is prevented by puromycin, a drug that blocks protein synthesis, reverses the hypermetabolism induced in rats by thyroxine, and restores the oxygen consumption of thyrotoxic rats to the euthyroid level.[18]

In conclusion, thyroid hormone appears to induce the formation of additional adenyl cyclase in adipose tissue; in contrast, thyroidectomy causes a reduction in the amount of the enzyme. In neither case is the amount of lipase affected. An increase in the amount of adenyl cyclase would explain the increased mobilization of FFA in the hyperthyroid state. Should these effects in adipose tissue be typical of other thyroid functions, the interplay between the thyroid and sympathetic systems could be explained by a given amount of catecholamine activating a larger amount of adenyl cyclase. As a logical extension of this view, certain actions of thyroid hormones should be diminished by sympathectomy. This might explain why certain sympatholytic agents produce ameliorative effects in patients with hyperthyroidism.

Summary.—Epididymal fat pads from hyperthyroid rats were more sensitive to the lipolytic effects of theophylline than those from control animals, but the maximum response to large concentrations of theophylline was virtually identical in the fat pads from euthyroid, hyperthyroid, and hypothyroid rats. These results indicate that thyroxine did not increase the amount of adipose tissue lipase. The lipolytic response of fat pads from hyperthyroid rats to norepi-

nephrine (NE) in amounts that completely activate adenyl cyclase was two to three times that from control animals but was less than the maximum response elicited by theophylline. In contrast, the response to NE of adipose tissue from thyroidectomized rats was negligible. From these results, it was inferred that the amount of adenyl cyclase might be dependent on the action of thyroid. Direct assay of adenyl cyclase in adipose tissue showed that the increased lipolytic reponse to NE in hyperthyroid rats and the poor response in hypothyroid animals were correlated with changes in the amount of enzyme. These findings suggest that thyroid hormones exert a control on FFA mobilization by regulating the amount of adenyl cyclase in adipose tissue.

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[1] Bacq, Z. M., *Pharmacol. Rev.*, **1**, 1 (1949).

[2] Brewster, W. R., J. P. Isaac, P. F. Osgood, and T. L. King, *Circulation*, **13**, 1 (1956).

[3] Swanson, H. E., *Endocrinology*, **59**, 217 (1956).

[4] Beaven, M. A., E. Costa, and B. B. Brodie, *Life Sci.*, **2**, 241 (1963).

[5] Rich, C., E. L. Bierman, and I. L. Schwartz, *J. Clin. Invest.*, **38**, 275 (1959).

[6] Debons, A. F., and I. L. Schwartz, *J. Lipid Res.*, **2**, 86 (1961).

[7] Felt, V., V. Vrbensky, and P. Benes, *Experientia*, **18**, 315 (1963).

[8] Deykin, D., and M. Vaughan, *J. Lipid Res.*, **4**, 200 (1963).

[9] Wenke, M., E. Mühlbachova, D. Schusterova, K. Elisova, and S. Hynie, *Intern. J. Neuropharmacol.*, **3**, 283 (1964).

[10] Mühlbachova, E., M. Wenke, D. Schusterova, D. Krcikova, and K. Elisova, Intern. *J. Neuropharmacol.*, **3**, 217 (1964).

[11] Hynie, S., G. Krishna, and B. B. Brodie, *J. Pharmacol. Exptl. Therap.*, **153**, 90 (1966).

[12] Weiss, B., J. I. Davies, and B. B. Brodie, *Biochem. Pharmacol.*, **15**, 1553 (1966).

[13] Schusterova, D., D. Krcikova, E. Mühlbachova, S. Hynie, and M. Wenke, Intern. *J. Neuropharmacol.*, **3**, 129 (1964).

[14] Sutherland, E. W., C. F. Cori, R. Haynes, and N. S. Olsen, *J. Biol. Chem.*, **180**, 825 (1949).

[15] Lambert, M., and A. C. Neish, *Can. J. Res. (Sect. B)*, **28**, 83 (1950).

[16] Krishna, G., B. Weiss, and B. B. Brodie, in press.

[17] Butcher, R. W., R. J. Ho, H. C. Meng, and E. W. Sutherland, *J. Biol. Chem.*, **240**, 4515 (1965).

[18] Weiss, W. P., and L. Sokoloff, *Science*, **140**, 1324 (1963).