Rat Liver Adenyl Cyclase Activity in Various Thyroid States

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ABSTRACT Thyroidectomized and euthyroid rats were injected with three doses of triiodothyronine (T_3) or of the diluent over a 6 day period, and liver homogenates were assayed for basal, epinephrine-stimulated, and NaF-stimulated adenyl cyclase activity. Based on NaF-stimulated levels, total adenyl cyclase activity, expressed per milligram of liver protein, was increased after thyroidectomy. Administration of T_3 to either hypothyroid or euthyroid rats, however, had no effect on the NaF-stimulated levels. Basal and epinephrine-stimulated enzyme activities were the same in hypothyroid, euthyroid, and hyperthyroid (euthyroid + T_3) liver homogenates. In contrast, injections of T_3 in hypothyroid rats increased the activities of basal and epinephrine-stimulated adenyl cyclase. In view of the findings in euthyroid and hyperthyroid liver, it is possible that this effect is transient. In general, no correlation was found between the effects of thyroid hormone on respiration and on adenyl cyclase activity of the rat liver. These results imply that the hepatic thermogenic response to thyroid hormone is not mediated by stimulation of adenyl cyclase activity with the possible exception of the early effects of T_3 in the athyroid rat.

INTRODUCTION

In previous studies from this laboratory, increased energy utilization for transmembrane-active Na^+ transport was implicated in the mechanism of thyroid thermogenesis (1). The effect of triiodothyronine (T_3)[1] on the ouabain-sensitive fraction of respiration correlated with changes in $Na^+ + K^+$-dependent adenosine triphosphatase (NaK-ATPase) in rat liver, kidney, and brain (2). The effect of T_3 on NaK-ATPase activity was selective in that the activities of Mg-ATPase and 5′-nucleotidase, two other plasma membrane enzymes, were not significantly altered by administration of T_3 to euthyroid or to hypothyroid rats. Adenyl cyclase, a plasma membrane enzyme involved in many hormone-regulated systems, however, was not assayed in these earlier studies.

Krishna, Hynie, and Brodie (3) attributed the increased lipolysis in adipose tissue of thyroxine-treated euthyroid rats to increased activity of epinephrine-stimulated adenyl cyclase. It is probable that thyroid hormone does not stimulate adenyl cyclase activity in all target tissues, since a number of studies showed no change in myocardial adenyl cyclase activity or of cyclic AMP content of hyperthyroid animals (4–7). Thyroid hormone, however, may alter the sensitivity of adenyl cyclase to stimulation since Levey, Skelton, and Epstein (5) found depressed levels of epinephrine and fluoride-stimulated enzyme activity in hypothyroid, rat heart homogenates.

The present study was intended to assess the effects of thyroid status on basal, and epinephrine and fluoride-stimulated hepatic adenyl cyclase activity with respect to thermogenesis.

METHODS

Preparation of animals. Male, Sprague Dawley rats (170–250 g) fed on Purina chow diet (Ralston Purina Co., St. Louis, Mo.) ad lib. were used in all experiments. The hypothyroid rats were thyroidectomized 3–5 wk before use and maintained on the same diets as the euthyroid group. A few rats from whom the parathyroid glands had inadvertently been removed developed tetany and died within 2–3 days. Other animals exhibiting irritability were not used. Serum protein-bound iodine levels on seven euthyroid and nine hypothyroid rats were determined (Bioscience Laboratories, Van Nuys, Calif.) with the following results: euthyroid = 4.1±0.2 μg/100 ml, thyroidectomized = 1.4±0.2 μg/100 ml.

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[1] *Abbreviations used in this paper:* NaK-ATPase, $Na^+ + K^+$-dependent ATPase; T_3, triiodothyronine.

Pairs of euthyroid rats were injected either with T_3 (Sigma Chemical Co., St. Louis, Mo.) dissolved in 5×10^{-4} M NaOH, 50 μg/100 g body weight or an equal amount of diluent on 3 alternate days and assayed 24–48 hr after the third injection. The animals were killed by decapitation and 1-g portions of liver (1 g) were homogenized in 9 ml of 50 mM Tris, 1 mM ethylene glycol bis(β-aminoethyl ether) *N, N, N′, N′*-tetra-acetic acid (EGTA), 1.3 mM β-mercaptoethanol in a Teflon-glass homogenizer with five strokes at medium speed. The homogenate was diluted 1:5 with the same buffer solution for use in the assay.

QO_2 measurements. Oxygen consumption was determined in liver slices from separate rats in the same group in a Warburg Respirometer as previously described (2).

Adenyl cyclase assay. The enzyme was assayed by a modification of the method of Ramachandran (8). The diluted homogenate (25 μl) was added to 30 μl of incubation solution with or without epinephrine (0.01 mM) or NaF (10 mM). The tubes were preincubated at 37°C for 5 min and the reaction was started by the addition of ATP-α-^{32}P, ATP, and cyclic AMP in 20 μl of 50 mM tris buffer. The final incubation mixtures contained: 30–100 μg of homogenate protein, 2 μCi of ATP-α-^{32}P (~2 Ci/mM, New England Nuclear, Boston, Mass.), 2 mM ATP, 3.4 mM $MgCl_2$, 0.25 mM $KHCO_3$, 0.08% bovine serum albumin, 0.33 mM EGTA, 2 mM cyclic 3′,5′-AMP, 17 μg of creatinine kinase, 30 μg creatine PO_4, 50 mM Tris-HCl, pH = 7.5, in a final volume of 75 μl. The reaction was stopped in 15 min by the addition of 200 μl of 50 mM Tris-HCl buffer, pH = 7.4, containing 14 mM cyclic AMP and 0.16 μCi cyclic AMP-^{3}H, and boiling for 3 min. After the tubes had cooled, the entire contents was pipetted onto a Pasteur pipet column (0.5 × 4.0 cm) composed of $PbSO_4$,[2] 2.0 cm below, and neutral alumina, activity I (Brinkman Instruments, Inc., Westbury, N. Y.) 2.0 cm above. The columns were washed with 2.8 ml of 10 mM Tris-HCl buffer, pH 7.4 and the effluent, containing 80–90% of the cyclic AMP, was collected in counting vials. 15 ml of Bray's dioxane counting solution[3] was then added to the vials and the samples were assayed for ^{3}H and ^{32}P content in a liquid scintillation spectrometer (Mark I, Nuclear-Chicago Corp., Des Plaines, Ill.). The assays were corrected for quenching with an external standard. The blank values were determined by adding ATP-α-^{32}P to incubation mixtures devoid of the labeled nucleotide at the end of the incubation period just before boiling. To calculate the per cent of cyclic AMP in the column effluents, the same quantity of ^{3}H as in the incubation mixtures, devoid of ATP-α-^{32}P, was processed in parallel with the assay tubes, diluted 1:5, and 250 μl was placed directly in counting vials and the 100% value was computed from these counting rates. Similar samples were also assayed for total ^{32}P content in the absence of cyclic AMP-^{3}H. The protein contents were determined by the method of Lowry, Rosebrough, Farr and Randall (9).

[2] Commercial $PbSO_4$ was found to be unsatisfactory for use in these columns. Accordingly $PbSO_4$ was prepared from reagent grade $ZnSO_4$ and $PbNO_3$ in stoichiometric proportions and cleaned by repeated washings with distilled H_2O.

[3] The scintillation solution consisted of 30 g, 2,5-diphenyloxazole (PPO), 1.5 g 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene, 240 g naphthalene in 429 ml xylene, 1284 ml *p*-dioxane, and 1284 ml ethylene glycol monomethyl ether.

TABLE I

QO_2 of Rat Liver Slices from Hypothyroid and Euthyroid Rats: Effect of T_3

Thyroid states	Pairs of rats	(1) QO_2	(2) QO_2	Pairs of rats	(3) QO_2
		μl/mg dry wt. per hr	*μl/mg protein per hr*		*mg protein/ mg dry wt. × 100*
Hypothyroid	10	5.6±0.2	10.5±0.4	6	53.3±2.8
Hypothyroid + T_3	10	11.4±0.6	20.1±1.1	6	56.6±2.6
Euthyroid	14	8.3±0.3	12.8±0.5	15	65.0±4.2
Euthyroid + T_3	14	12.4±0.4	18.0±0.6	15	68.8±3.1

The QO_2 of liver slices from euthyroid and hypothyroid rats with or without previous T_3 treatment was measured as described in the text (column 1). In parallel experiments, ratios of milligrams protein/milligram dry weight were determined on separate rats (column 3), were used to express QO_2 as a function of protein concentration (column 2).

RESULTS

QO_2 in rat liver. The QO_2 of slices of euthyroid and hypothyroid rat liver expressed per milligrams dry weight are presented in Table I, and shows a significant increase in QO_2 after T_3 treatment. Earlier studies (12, 13) showed that on this treatment schedule, the QO_2 reaches a steady level by 48 hr. Thermogenesis of this magnitude should be sufficient to determine whether effects of thyroid hormone on adenyl cyclase activity are involved in this response. The ratio of protein concentration to dry weight increases with T_3 treatment (Table I) and this ratio has been used to express QO_2 as a function of milligrams protein to serve as a basis for comparison with enzyme data.

Adenyl cyclase activity. The effects of T_3 on rat liver adenyl cyclase activity are summarized in Table II. In hypothyroid rats the hormone had no effect on NaF-stimulated activity which suggests that T_3 did not alter maximum enzyme activity. Injection of T_3, however, increased basal activity by 49% ($P < 0.01$) and epinephrine-stimulated activity by 56% ($P < 0.01$). The absolute increase in activity produced by epinephrine (E-B) was 66% greater after T_3 ($P < 0.05$). A comparison of the fractional increase in activity produced by epinephrine (E/B) reveals a twofold increase in all four groups. Thus, T_3 increased basal and epinephrine-sensitive activity proportionately.

In euthyroid rats, T_3 had no significant effect on basal, epinephrine-, or NaF-stimulated activity. It is of interest that total activity in the presence of NaF was considerably higher in the hypothyroid than in the euthyroid rat liver regardless of whether or not T_3 was given.

TABLE II

The Effect of T_3 Treatment In Vivo on Adenyl Cyclase Activity of Rat Liver

Thyroid status	Rat No.	Adenyl cyclase activity (*pmoles cyclic AMP formed/mg protein per 15 min*)				
		Basal	+Epinephrine	+NaF	(E-B)	(E/B)
Hypothyroid	11	34.1±3.5	68.9±5.7	355±20	34.6±4.1	2.09±0.14
Hypothyroid + T_3	11	50.9±5.2*	107.8±11.8*	365±23	57.6±9.5*	2.23±0.24
Euthyroid	11	30.5±3.1	69.7±6.2	246±15	39.2±4.0	2.34±0.15
Euthyroid + T_3	10	35.7±6.8	68.5±12.9	239±32	32.8±6.6	1.94±0.12

Data are expressed as mean ±1 SEM.

* Denotes statistically significant differences ($P < 0.05$) between values in treated vs. untreated rats in the same group; (E-B) denotes the paired differences in activity of the epinephrine (E) and basal (B) states; (E/B) denotes the ratio of epinephrine (E) to basal (B) states.

DISCUSSION

Sutherland, Rall, and Menon (10) proposed that NaF-stimulated activity provides a measure of the total adenyl cyclase (core enzyme) content of a tissue. NaF-stimulated total adenyl cyclase activity was higher in initially hypothyroid than in euthyroid rat liver (Table II). These changes are the converse of those reported by Levey, Skelton, and Epstein (11) of decreased total adenyl cyclase activity in hypothyroid cat myocardium. Both in our studies and those of Levey et al. (11), enzyme activity is expressed per milligrams of protein in the cell fraction. It is possible that divergent changes in average protein content of these tissues could explain the observed differences. The failure of a short course of treatment with T_3 to restore NaF-stimulated activity to that of the euthyroid state may indicate a slow response mechanism. Alternatively, this increase in apparent enzyme activity after thyroidectomy might be a result of effects other than that of circulating levels of T_3 or thyroxine, e.g., extrathyroidal effects of excess thyroid-stimulating hormone (TSH), of calcitonin deprivation, or of subtle hypoparathyroidism. In any case, there was no correlation between total adenyl cyclase activity and the metabolic response to the hormone (see Tables I and II).

Basal and epinephrine-stimulated adenyl cyclase activity may be the relevant quantities with respect to thyroidal influences in vivo. A comparison of these activities reveals no differences in the hypothyroid, euthyroid, and hyperthyroid (euthyroid + T_3) livers (Table II). These results eliminate any simple and direct correlation between effects on adenyl cyclase activity and on respiration (see Tables I and II). This interpretation is in accord with the inference that changes in adenyl cyclase activity do not mediate the thermogenic action of thyroid hormone in the mammalian heart (4–6). The response of adipose tissue to thyroid hormone, however, apparently involves effects on adenyl cyclase activity or on catecholamine stimulation of the enzyme (3). This raises the question of the physiological implications of the finding of a significant increase in basal and epinephrine-stimulated adenyl cyclase activity of liver in T_3-treated, thyroidectomized rats (Table II). The lack of correlation between the effects of T_3 on adenyl cyclase activity and QO_2 in euthyroid rats, however, casts doubt on the role of changes in adenyl cyclase activity in the thermogenic response of thyroidectomized animals. Additional information is needed to resolve this question.

In general, our results support the earlier suggestions that among the population of plasma membrane-bound enzymes, thyroid hormones selectively increase the activity of NaK-ATPase.

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