Rat Liver Adenyl Cyclase Activity in Various Thyroid States

JUDITH K. JONES, FARAMARZ ISMAIL-BEIGI, and ISIDORE S. EDELMAN

From the Cardiovascular Research Institute, and the Departments of Medicine, and of Biochemistry and Biophysics of the University of California School of Medicine, San Francisco, California 94122

ABSTRACT Thyroidectomized and euthyroid rats were injected with three doses of triiodothyronine (T₃) 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 T₃ 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 + T₃ ) liver homogenates. In contrast, injections of T₃ 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 T₃ 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 (T₃) on the ouabain-sensitive fraction of respiration correlated with changes in Na⁺ + K⁺-dependent adenosine triphosphatase (Na⁺-K⁺-ATPase) in rat liver, kidney, and brain (2). The effect of T₃ on Na⁺-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 T₃ 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.
Pairs of euthyroid rats were injected either with T₃ (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) N₄, N₄', 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.

**QO₂ 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 9 min and the reaction was started by the addition of ATP-α⁻³²P, 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⁻³²P (20 Ci/mM, New England Nuclear, Boston, Mass.), 2 mM ATP, 3.4 mM MgCl₂, 0.25 mM KHCO₃, 0.08% bovine serum albumin, 0.33 mM EGTA, 2 mM cyclic AMP, 17 μg of creatine kinase, 30 μg creatine PO₄, 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⁻³⁰P, 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 PbSO₄, 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 was then added to the vials and the samples were assayed for ³⁰P and ³²P 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⁻³²P 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 ³²P as in the incubation mixtures, devoid of ATP-α⁻³²P, 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 ³²P content in the absence of cyclic AMP-³²P. The protein contents were determined by the method of Lowry, Rose-brough, Farr and Randall (9).

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### Table I

**QO₂ of Rat Liver Slices from Hypothyroid and Euthyroid Rats: Effect of T₃**

<table>
<thead>
<tr>
<th>Thyroid states</th>
<th>Pairs of rats</th>
<th>QO₂ (1) μl/mg dry wt. per hr</th>
<th>QO₂ (2) μl/mg protein per hr</th>
<th>Pairs of rats</th>
<th>QO₂ (3) mg protein/mg dry wt. × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td>10</td>
<td>5.6±.0.2</td>
<td>10.5±0.4</td>
<td>6</td>
<td>53.3±2.8</td>
</tr>
<tr>
<td>Hypothyroid + T₃</td>
<td>10</td>
<td>11.4±0.6</td>
<td>20.1±1.1</td>
<td>6</td>
<td>56.6±2.6</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>14</td>
<td>8.3±0.3</td>
<td>12.8±0.5</td>
<td>15</td>
<td>65.0±4.2</td>
</tr>
<tr>
<td>Euthyroid + T₃</td>
<td>14</td>
<td>12.4±0.4</td>
<td>18.0±0.6</td>
<td>15</td>
<td>68.8±3.1</td>
</tr>
</tbody>
</table>

The QO₂ of liver slices from euthyroid and hypothyroid rats with or without previous T₃ 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 QO₂ as a function of protein concentration (column 2).

**RESULTS**

**QO₂ in rat liver.** The QO₂ of slices of euthyroid and hypothyroid rat liver expressed per milligrams dry weight are presented in Table I, and shows a significant increase in QO₂ after T₃ treatment. Earlier studies (12, 13) showed that on this treatment schedule, the QO₂ 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 T₃ treatment (Table I) and this ratio has been used to express QO₂ as a function of milligrams protein to serve as a basis for comparison with enzyme data.

**Adenyl cyclase activity.** The effects of T₃ 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 T₃ did not alter maximum enzyme activity. Injection of T₃, 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 T₃ (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, T₃ increased basal and epinephrine-sensitive activity proportionately.

In euthyroid rats, T₃ 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 T₃ was given.
TABLE II
The Effect of T₃ Treatment In Vivo on Adenyl Cyclase Activity of Rat Liver

<table>
<thead>
<tr>
<th>Thyroid status</th>
<th>Rat No.</th>
<th>Basal (cpm/15 min)</th>
<th>+Epinephrine (cpm/15 min)</th>
<th>+NaF (cpm/15 min)</th>
<th>(E-B)</th>
<th>(E/B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td>11</td>
<td>34.1±3.5</td>
<td>68.9±5.7</td>
<td>355±20</td>
<td>34.6±4.1</td>
<td>2.09±0.14</td>
</tr>
<tr>
<td>Hypothyroid + T₃</td>
<td>11</td>
<td>50.9±5.2*</td>
<td>107.8±11.8*</td>
<td>365±23</td>
<td>57.6±9.5*</td>
<td>2.23±0.24</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>11</td>
<td>30.5±3.1</td>
<td>69.7±6.2</td>
<td>246±15</td>
<td>39.2±4.0</td>
<td>2.34±0.15</td>
</tr>
<tr>
<td>Euthyroid + T₃</td>
<td>10</td>
<td>35.7±6.8</td>
<td>68.5±12.9</td>
<td>239±32</td>
<td>32.8±6.6</td>
<td>1.94±0.12</td>
</tr>
</tbody>
</table>

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 T₃ 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 T₃ 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 + T₃) 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 T₃-treated, thyroidectomized rats (Table II). The lack of correlation between the effects of T₃ on adenyl cyclase activity and QO₂ 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 Na⁺-ATPase.

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