Hyperthyroidism Impairs the Activation of Glycogen Phosphorylase by Epinephrine in Rat Hepatocytes*

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The activation of glycogen phosphorylase by epinephrine was examined in hepatocytes from fed female rats administered 0.25 mg of triiodothyronine per kg of body weight daily for 2 days. Basal cyclic AMP accumulation by the hepatocytes was not altered by the thyroid hormone administration. Basal glycogen phosphorylase a activities were 1.18 ± 0.07 units/mg protein in control and 0.37 ± 0.04 unit/mg of protein in triiodothyronine-treated rat hepatocytes, representing a 70% reduction in the basal activity of this enzyme. Phosphorylase a activity of hepatocytes in response to 10 μM epinephrine increased 1.2 units/mg of protein (100% over basal) in those from control rats, while increasing only 0.2 unit/mg of protein (50% over basal) in hepatocytes from triiodothyronine-treated rats. The basal glycogen phosphorylase activity was reduced 25% with respect to control levels in hepatocytes from rats treated with thyroid hormones, a change of insufficient magnitude to account for the dramatic reduction in the basal phosphorylase activity. To explore further the basis for the reduced basal phosphorylase activity, both the phosphorylase a and phosphorylase phosphatase activities were measured. Thyroid hormone treatment reduced heptocyte phosphorylase a activity 17%, while increasing phosphorylase phosphatase activity by 50%. Although triiodothyronine treatment did not influence the affinity of adrenergic receptors of hepatocyte membrane fractions, it did result in a 70% reduction in the number of α-adrenergic receptor sites per mg of membrane protein. These data suggest that thyroid hormones exert their influence on the hormonal regulation of hepatic glycogenolysis via (i) altering protein phosphorylase activity which regulates phosphorylase and phosphorylase kinase and (ii) modulating the number of α-adrenergic receptor sites which apparently transduce epinephrine binding into α-adrenergic control of hepatic glycogenolysis.

Catecholamines promote glycogenolysis in the rat liver via both α- and β-adrenergic receptor-mediated pathways. The β-adrenergic receptor-mediated activation of glycogen phosphorylase is blocked by propranolol and is presumed to result from an elevation of intracellular cyclic AMP accumulation.

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EXPERIMENTAL PROCEDURES

Animals—Rats (175-225 g fed female Sprague-Dawley DS strain rats from Taconic Farms, Germantown, N.Y.) were rendered hyperthyroid by the subcutaneous administration of triiodothyronine (0.25 mg/kg of body weight) at 48, 24, and 3 h prior to their killing. Control rats were littermates or animals of the same weight as those administered thyroid hormone.

Hepatocyte Preparation—Hepatocytes were isolated according to the procedure of Berry and Friend (13) as described previously (12). Following isolation, the rat hepatocytes were incubated in a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 22 mM glucose for 30 min, then washed, and resuspended in fresh medium for 20 min for the experiments in plastic tubes (17 × 100 mm, Sarstedt No. 55.468) in a total volume of 1 ml and were constantly shaken at 37 °C in an orbital water bath operating at 150 to 200 rpm. Cyclic AMP Accumulation Measurements—To measure cyclic AMP content, the hepatocytes were incubated for 20 min as described above and the incubation was terminated 1 min after the addition of hormones by the addition of 0.1 ml of 1 N HCl. The incubation tubes were then heated in a boiling water bath for 1 min and after cooling were neutralized with NaOH. A 20-μl aliquot was taken from the tube and the cyclic AMP content was assayed by a modification of the method of Gilman (14) using the cyclic AMP binding protein extracted from bovine adrenal gland. The free cyclic AMP was separated from the bound cyclic AMP by adsorption on activated charcoal, as suggested by Brown et al. (15).
Activation of Glycogen Phosphorylase in Hyperthyroidism

Glycogen Phosphorylase Activity—Glycogen phosphorylase activity was measured in tubes incubated with or without hormones. The reactions were terminated after 1 min of incubation with hormones by the addition of 0.1 ml of ice-cold buffer (pH 7.0) containing 100 mM 4-morpholinopropanesulfonic acid, 50 mM sodium fluoride, 50 mM EDTA, and 10 mM dithiothreitol. Upon addition of the ice-cold buffer, the tubes were shaken and immediately frozen in a dry ice/ethanol bath and stored at -20°C. The tubes were thawed in an ice bath and homogenized for 20 s with a Willems Polytron (Brinkmann Instruments) at setting 5. The homogenates were centrifuged at 12,000 x g_{	ext{max}} for 10 min at 4°C. This assay was performed on the supernatant which contained approximately 2-3 mg of protein/ml. Glycogen phosphorylase a was measured using a filter disc assay similar to that of Gilboe et al. (16) as previously described. Caffeine (0.5 mM) was included in the assay to suppress phosphorylase b activity (17). One unit of phosphorylase activity is the amount of enzyme that converts 1 μmol of substrate in a 20-min incubation under conditions as described above.

An index of the "total" glycogen phosphorylase activity can be generated by assay in the presence of high concentrations of AMP and SO_{4}^{2-} (17). This activity is somewhat less than that observed following complete phosphorylation of the enzyme, but is directly proportional to maximal activity (17).

Phosphorylase Phosphatase Assay—Isolated hepatocytes were incubated with or without hormone as described above in the study of phosphorylase activation and then immediately frozen in a dry ice/ethanol bath. Frozen samples of the liver cell suspensions were diluted and thawed with 2 volumes of an ice-cold buffer containing 125 mM KCl, 187.5 mM NaF, 3.75 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 1.875 mM magnesium chloride (pH 7.4), and 0.75 μM/mg of heat-stable protein kinase inhibitor, as described by VanDeWerve et al. (18). Phosphorylase b kinase activity was assayed exactly as described by VanDeWerve et al. (18) except that the activity of phosphorylase a enzyme was determined in the presence of 5 mM caffeine and 10 mM EDTA by the method of Gilboe et al. (16). Phosphorylase b kinase activity was measured at 0-, 2-, 4-, 6-, 8-, and 10-min intervals at 30°C. One unit of phosphorylase kinase is the amount of enzyme that converts 1 unit of rabbit muscle phosphorylase b into phosphorylase a per min under the conditions of this assay.

Phosphorylase Phosphatase Measurements—Phosphorylase phosphatase activity was determined by a modification of the method of Khattri and Soderling (19) as described by Chan et al. (20). Frozen samples of hepatocytes (1-ml volume containing 50-100 mg of cell, wet weight) were diluted and thawed on ice with 1 ml of a buffer containing 80% glycerol (v/v), 10 mM Tris (pH 7.4), 5 mM EDTA and then homogenized in a Polytron homogenizer at setting 5. The homogenate was centrifuged at 1,000 x g for 10 min and the resulting supernatant was used as a source for phosphorylase phosphatase activity. A 20-μl aliquot of the enzyme was added to 70 μl of reaction mixture consisting of 10 mM Tris (pH 7.5), 1 mM dithiothreitol, 0.1 mM MgCl_{2} μM phosphotyrosyl a (2 x 10^{-4} M) and 10 μg of glycogen phosphorylase a (2 x 10^{-4} M) activity was incubated for 1 min at 37°C. The incubation was terminated by vacuum filtration and a 15-ml wash with an ice-cold phosphate buffer. The filters (Whatman G/FC, 2.5 cm) were then dried and counted by liquid scintillation spectrometry.

Materials—Rabbit muscle glycogen phosphorylase b, phosphorylase b kinase, and heat-stable protein kinase inhibitor were obtained from Sigma. Premozin was a kind gift from Pfizer, Inc. The [^{4}H]cyclic AMP, [^{3}H]glucose, and [^{14}C]dihydroergocryptine were purchased from New England Nuclear. The γ-[^{32}P]ATP was purchased from Amersham. All other chemicals were obtained from standard commercial suppliers previously listed (12).

RESULTS

Hyperthyroid status was induced in rats by the administration of 0.25 mg of triiodothyronine per kg of body weight at 48, 24, and 3 h prior to hepatocyte isolation. This dose of triiodothyronine has been shown to reverse the biochemical alterations in the hormonal regulation of glycogenolytic enzymes in hepatocytes (12, 24), lipolysis and cyclic AMP accumulation in rat fat cells (8), and glucose utilization in rat soleus muscle (25) associated with the hypothyroid state to the euthyroid level. The triiodothyronine treatment did not alter the body weight, hepatocyte yield per liver, or the amount of protein per hepatocyte preparation in these studies (Table I).

The ability of various glycoconylcortic hormones to stimulate glycogen phosphorylase activity of hepatocytes isolated from control and triiodothyronine-treated rats was investigated. The data presented in Table II highlight a number of important observations concerning the influence of hyperthyroidism on the hormonal regulation of hepatic phosphorylase. Basal (unstimulated) levels of glycogen phosphorylase a activity were 1.18 ± 0.07 and 0.37 ± 0.04 units/mg of protein in control and thyroid hormone-treated rat hepatocytes, respectively. This reduction in phosphorylase a activity was detected within 18 h of the administration of a single dose of triiodothyronine and was maximal by about 24 h (data not shown). In addition to this 70% reduction in phosphorylase a levels noted in the hyperthyroid state, a marked reduction in the ability of phenylephrine, epinephrine, isoproterenol, and glucagon to activate phosphorylase was apparent (Table II). Epinephrine, at 10 μM, increased phosphorylase activity of control rat hepatocytes by 1.2 units per mg of protein (100% increase over basal), whereas in the hepatocytes isolated from triiodothyronine-treated rats epinephrine was able to increase phosphorylase a activity by only 0.2 unit per mg of protein (50% increase over basal). Thus, the thyroid hormone administration led to an 80% reduction in the increment of phosphorylase activation produced in response to 10 μM epinephrine. The ability of glucagon and isoproterenol to activate phosphorylase was also blunted in hepatocytes from hyperthyroid as compared to euthyroid rats (Table II). The kinetics of the response of hepatocyte phosphorylase to epinephrine stimulation was examined in the hyperthyroid state and was

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Whole body weight, hepatocyte, and protein yield of hepatocyte preparations obtained from control and triiodothyronine-treated rats</th>
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<tbody>
<tr>
<td>Source</td>
<td>Body weight</td>
</tr>
<tr>
<td>Control rats</td>
<td>(26)</td>
</tr>
<tr>
<td>Triiodothyronine- treated rats</td>
<td>(21)</td>
</tr>
</tbody>
</table>

* Difference from control rat values not significant.

Data Presentation—Unless otherwise stated, data are presented as the mean ± S.E. of at least three different hepatocyte preparations.
Hormonal activation of glycogen phosphorylase in hepatocytes isolated from control and triiodothyronine-treated rats

Hepatocytes were isolated from control rats and rats administered 25 μg of triiodothyronine/100 g of body weight at 48, 24, and 3 h prior to hepatocyte isolation. An index of total glycogen phosphorylase activity was determined according to the methods of Stalmans and Hers (17). The data are expressed in units per mg of protein and are given ±S.E. from four separate experiments for both groups.

<table>
<thead>
<tr>
<th>Source of hepatocytes</th>
<th>Glycogen phosphorylase activity</th>
<th>units/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>+Phenylephrine (10⁻⁵ M)</td>
</tr>
<tr>
<td>Control rat (% change)</td>
<td>1.18 ± 0.07 (9%)</td>
<td>1.87 ± 0.13* (58%)</td>
</tr>
<tr>
<td>Triiodothyronine-treated rat (% change)</td>
<td>0.37 ± 0.04 (0%)</td>
<td>0.47 ± 0.05* (28%)</td>
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*p < 0.05 compared to basal values.

Total glycogen phosphorylase activity of hepatocytes obtained from control and triiodothyronine-treated rats

Hepatocytes were isolated from control rats and rats treated with 25 μg of triiodothyronine/100 g of body weight at 48, 24, and 3 h prior to hepatocyte isolation. An index of total glycogen phosphorylase activity was defined as the release of 1 nmol of phosphate per min under the conditions of this assay. Phosphorylase activity in hepatocytes incubated with high concentrations of sulfate ion and 5'-AMP has been demonstrated to be a useful index of total glycogen phosphorylase activity (17). The level of phosphatase activity noted in hepatocytes isolated from control and the triiodothyronine-treated rats was investigated using rabbit muscle [32P]phosphorylase a as the substrate (Table V). The level of phosphatase activity in these hepatocyte supernatant fractions revealed a 50% stimulation, phosphorylase b kinase activity was reduced 17% in hepatocytes from thyroid hormone-treated as compared to control rats (Table IV). Incubation of hepatocytes obtained from control rats with 10 μM epinephrine increased the phosphorylase b kinase activity 1-fold over basal. Incubating hepatocytes from the triiodothyronine-treated rats with 10 μM epinephrine, in contrast, produced only a slight increase in phosphorylase b kinase activity. Thus hyperthyroidism is associated with a reduction in phosphorylase kinase activity and an inability of epinephrine to increase the activity of this enzyme.

Protein phosphatase activity of hepatocytes isolated from the control and the triiodothyronine-treated rats was investigated using rabbit muscle [32P]phosphorylase a as the substrate (Table V). The level of phosphatase activity in hepatocytes from control rats was similar to that reported previously in rabbit liver (26). Study of phosphatase activity in these hepatocyte supernatant fractions revealed a 50% reduction, phosphorylase b kinase activity was reduced 17% in hepatocytes from thyroid hormone-treated as compared to control rats (Table IV). Incubation of hepatocytes obtained from control rats with 10 μM epinephrine increased the phosphorylase b kinase activity 1-fold over basal. Incubating hepatocytes from the triiodothyronine-treated rats with 10 μM epinephrine, in contrast, produced only a slight increase in phosphorylase b kinase activity. Thus hyperthyroidism is associated with a reduction in phosphorylase kinase activity and an inability of epinephrine to increase the activity of this enzyme.

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increase in phosphorylase phosphatase activity in the hyperthyroid state (Table V).

The reduced level of phosphorylase kinase and increased level of phosphatase activity provided some insight as to the basis for the low phosphorylase a activity of hepatocytes from thyroid hormone-treated as compared to control rats. However, the possibility that the poor phosphorylase response of the hyperthyroid rat hepatocytes to epinephrine stimulation may involve other additional factors deserved investigation. The dose-response relationships for activation of phosphorylase by phenylephrine, epinephrine, isoproterenol, and glucagon in hepatocytes isolated from control and triiodothyronine-treated rats. Rat hepatocytes (50 mg of cells wet weight/tube) were isolated from euthyroid (control) and triiodothyronine-treated rats. Purified membranes were prepared from isolated rat hepatocytes from euthyroid (control) and hyperthyroid (triiodothyronine-treated) rats (12). Membranes (approximately 100 μg of protein) were incubated with 5 nM [3H]dihydroergocryptine and various concentrations of the δ-adrenergic antagonist prazosin for 20 min at 20 °C. At the end of the assay, the binding was measured as described by Hoffman et al. (22). The data presented are the mean values obtained from three separate experiments where binding to liver membranes prepared from euthyroid and hyperthyroid rat hepatocytes was measured simultaneously. [3H]dihydroergocryptine binding (prazosin-sensitive) was 1175 and 324 fmol/mg of membrane protein for control and triiodothyronine-treated preparations, respectively.

Previous studies clearly indicate that the activation of phosphorylase in rat hepatocytes by epinephrine is mediated via δ-adrenergic receptors (22, 27). Prazosin, a potent δ-adrenergic antagonist, has been useful in identifying δ-adrenergic receptor sites in liver membranes (22, 27, 28), hepatocyte membranes (22), and many other tissues (for review see Ref. 29). The status of δ-adrenergic receptors in purified membranes obtained from euthyroid, hyperthyroid, and hypothyroid rat hepatocytes was investigated using [3H]dihydroergocryptine as the radioligand and prazosin as the blocking agent to differentiate the δ-adrenergic component of the binding from total binding (22, 27–29). The data are expressed as the mean values of experiments performed on separate occasions for both the euthyroid and hyperthyroid states. Phenylephrine (PHE), epinephrine (EPI), isoproterenol (ISO), and glucagon were present at the concentrations indicated.

**Table VI**

<table>
<thead>
<tr>
<th>Source of purified membranes</th>
<th>Specific [3H]dihydroergocryptine binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>1175 ± 200</td>
</tr>
<tr>
<td>Hypothyroid rats</td>
<td>920 ± 185</td>
</tr>
<tr>
<td>Triiodothyronine-treated rats</td>
<td>325 ± 35*</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with control rats.

![Fig. 1. The activation of glycogen phosphorylase by phenylephrine, epinephrine, isoproterenol, or glucagon in hepatocytes isolated from control and triiodothyronine-treated rats.](image)

![Fig. 2. Inhibition of [3H]dihydroergocryptine binding by prazosin in liver cell membranes prepared from control and triiodothyronine-treated rats.](image)
Although thyroid hormone administration has been shown to modulate catecholamine responsiveness in the heart (9-11) and in adipose tissue (8), only recently has its influence on the hormonal regulation of hepatic glycogenolysis been examined. In the heart, hyperthyroidism has been shown to increase the sensitivity of rat myocardial phosphorylase a to catecholamine activation (9,10). The ability of epinephrine to stimulate cyclic AMP accumulation and lipolysis in fat cells is likewise potentiated in the hyperthyroid state (18). The present study demonstrates that the ability of epinephrine to activate hepatic glycogenolysis, in contrast, is markedly impaired in the hyperthyroid state.

The activation of glycogen breakdown by catecholamines in the rat liver is a response displaying both \( \alpha \) - and \( \beta \)-adrenergic components (1-7). Previously, we demonstrated that hypothyroidism selectively potentiates the \( \beta \)-adrenergic component of phosphorylase activation without altering the ability of either glucagon or phenylephrine to stimulate phosphorylase activation (12). Both the cyclic AMP response of the isolated hepatocytes and the response of the hepatocyte adenylate cyclase to \( \beta \)-adrenergic stimulation are potentiated in the hypothyroid state (12, 24). A 2- to 3-fold increase in the steady state level of \( \beta \)-adrenergic receptors has been identified in the hypothyroid as compared to euthyroid rat hepatocyte preparations (24) and this increase in receptors is a likely explanation for the enhanced \( \beta \)-adrenergic responsiveness.

In contrast to the effects of hypothyroidism, the hyperthyroid state was associated with an impaired ability of the hepatocytes to respond to a variety of glycogenolytic hormones (present study). The activation of phosphorylase in response to isoproterenol, phenylephrine, glucagon, or epinephrine stimulation was blunted in hepatocytes from rats administered thyroid hormone. The number of \( \alpha \)-adrenergic receptor sites was 80% lower in hepatocyte membranes obtained from triiodothyronine-treated as compared to euthyroid rats, although the affinity of these sites for prazosin was not affected (present study). This reduced level of \( \alpha \)-adrenergic receptors would explain, in part, the poor phosphorylase response of hyperthyroid rat hepatocytes to epinephrine stimulation. \( \beta \)-Adrenergic receptors and glucagon receptors are also reduced in the hepatocyte membranes prepared from the hyperthyroid rat, but to a distinctly lesser degree. 1 These data do not support the postulate of Kunos and co-workers (30,31) which suggests that adrenergic receptor interconversion exists in the liver and thyroid hormones mediate this interconversion. Hypothyroidism is associated with an increase in \( \beta \)-adrenergic receptors and no change in \( \alpha \)-adrenergic receptors in the liver (24 and present study), while hyperthyroidism is associated with a substantial reduction (80%) in hepatic \( \alpha \)-adrenergic receptors and a somewhat lesser reduction in the \( \beta \)-receptor.

Of potentially greater significance with respect to the influence of thyroid hormones on the hormonal regulation of hepatic glycogenolysis was the observation that hyperthyroidism resulted in a 70% reduction in the basal level of phosphorylase a activity. Although a 25% reduction in the amount of total glycogen phosphorylase activity was noted in the hyperthyroid state, this reduction was of insufficient magnitude to account for the low basal phosphorylase a activity. Phosphorylase b kinase activity was also reduced, being 17% lower in the hyperthyroid as compared to euthyroid rat hepatocyte. Interestingly, the phosphorylase phosphatase activity of the hyperthyroid rat hepatocytes was examined and found to be increased 50% over the euthyroid level. This increase in phosphatase activity provides a likely explanation for the reduced phosphorylase kinase and phosphorylase a levels.

A broad specificity protein phosphatase activity, capable of reversing both cyclic AMP-dependent and independent protein kinase-catalyzed phosphorylations, has been identified in a number of tissues including the rat liver (32-36). The present study demonstrates that the phosphatase activity, assayed with \(^{32}P\)phosphorylase as the substrate, was 50% greater in hepatocytes from thyroid hormone-treated rats. An increase of this magnitude in phosphatase activity would be expected to dramatically dampen the amplitude of the maximal response achievable in cyclic cascade systems such as hepatic phosphorylase (for a recent review see Chock et al. (37)). Our data support this prediction. An increase in activity of the broad specificity protein phosphatase of liver as noted in the hyperthyroid state would presumably be reflected in the activity of several other enzymes which are substrates for this phosphatase such as hydroxymethylglutaryl-CoA reductase (38), hydroxymethylglutaryl-CoA reductase kinase (38), and acetyl-CoA carboxylase (39). The physiological impact of an increase in the phosphatase activity and its influence on hepatic phosphorylase, hydroxymethylglutaryl-CoA reductase, and acetyl-CoA carboxylase remains to be established.

In summary, our studies indicate that thyroid hormones may exert their marked influence on the hormonal regulation of hepatic glycogenolysis via the modulation of the number of receptors for the glycogenolytic hormones such as epinephrine and more importantly by increasing the phosphatase activity. Definition of the nature of this increased phosphatase activity in hepatocytes from hyperthyroid rats is currently in progress.

REFERENCES

Activation of Glycogen Phosphorylase in Hyperthyroidism