Hormonal Activation of Glycogen Phosphorylase in Hepatocytes from Hypothyroid Rats

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The ability of catecholamines and glucagon to stimulate glycogen phosphorylase activity was examined in hepatocytes isolated from euthyroid and hypothyroid fed female rats. Hypothyroidism did not alter the ability of either phenylephrine or epinephrine to activate glycogen phosphorylase. Activation of glycogen phosphorylase in response to a maximal concentration of isoproterenol, however, was 100% higher in hepatocytes isolated from hypothyroid as compared to euthyroid rats. In the presence of 50 μM methylisobutyl xanthine, hypothyroid rat hepatocytes displayed half-maximal activation of phosphorylase with only 1 nM isoproterenol, whereas euthyroid rat hepatocytes required 33 nM isoproterenol for half-maximal activation. Methylisobutyl xanthine (50 μM) potentiated the ability of isoproterenol to activate phosphorylase in euthyroid rat hepatocytes while potentiating the activation of phosphorylase in response to isoproterenol and to epinephrine in hypothyroid rat hepatocytes. Glucagon activation of glycogen phosphorylase in rat hepatocytes was not altered by hypothyroidism in either the presence or absence of methylisobutyl xanthine.

Cyclic AMP accumulation following epinephrine or isoproterenol stimulation was dramatically higher in hypothyroid as compared to euthyroid rat hepatocytes, in both the absence or presence of 50 μM methylisobutyl xanthine. Glucagon stimulation of cyclic AMP accumulation was not altered by hypothyroidism. The amplified cyclic AMP accumulation and phosphorylase activation of hypothyroid rat hepatocytes in response to isoproterenol could be blocked by propranolol (10 μM) but not phenolamine (10 μM).

Hypothyroidism appears to selectively enhance activation of glycogen phosphorylase and stimulation of cyclic AMP accumulation by β-adrenergic agonists in isolated rat hepatocytes. Thyroid hormones appear to have the opposite effect on β-adrenergic responses in the liver as compared to heart and adipose tissue.

Glucagon and catecholamines elevate adenosine 3':5'-monophosphate levels and promote glycogenolysis in the perfused rat liver (1) and in isolated rat hepatocytes (2–4). These agents have also been shown to stimulate gluconeogenesis in isolated rat hepatocytes (5, 6). In cat and dog liver slice preparations, the elevation of cyclic AMP levels by these hormones appears to be responsible for increased glycogen breakdown (7). Most recent studies on the hormonal regulation of glycogenolysis utilizing isolated rat hepatocytes have emphasized conditions in which activation of glycogen phosphorylase by glucagon results from a rise in cyclic AMP (3, 4, 8, 9). However, the α-adrenergic agonist phenylephrine (3, 4, 8–10), vasopressin (11, 12), and low concentrations of glucagon (10) have been reported to activate glycogen phosphorylase in isolated rat hepatocytes through a cyclic AMP-independent pathway. Biochemical definition of a cyclic AMP-independent mechanism(s) for hormonal activation of glycogen phosphorylase is currently lacking, although a role for calcium (8, 13) through phosphatidylinositol breakdown (14) has been proposed.

Thyroid hormones are well known modulators of the responsiveness of various tissues to catecholamines. Cyclic AMP accumulation and lipolysis in response to epinephrine is severely blunted in fat cells isolated from hypothyroid rats (16–17). Hyperthyroidism, in contrast, enhances epinephrine-stimulated cyclic AMP accumulation and lipolysis in the isolated rat fat cell (17). Thyroid hormones also potentiate β-adrenergic hormone action in the heart (18–20). Hyperthyroidism increases the sensitivity of rat myocardial phosphorylase α to catecholamine activation (18, 19). Fetal mouse hearts incubated in organ culture with 3,3',5'-(−)-triiodothyronine show a selective increase in their sensitivity to the chronotropic effects of β-adrenergic agonists (20). Our combined interests in the study of the hormonal regulation of hepatic glycogenolysis and of thyroid hormone action prompted our investigation of catecholamine and glucagon activation of glycogen phosphorylase in hepatocytes isolated from hypothyroid rats. In addition, we have examined the role of cyclic AMP accumulation in the regulation of glycogenolysis by glucagon and catecholamines in hypothyroid rat hepatocytes. The present study demonstrates that, in contrast to its influence on β-adrenergic action in the heart and adipose tissue, hypothyroidism enhances the β-adrenergic stimulation of cyclic AMP accumulation and activation of glycogen phosphorylase while not modifying glucagon stimulation of those same parameters.

MATERIALS AND METHODS

Sources of the materials used for these studies are the following: (−)-epinephrine, (−)-isoproterenol hydrochloride, (−)-phenylephrine hydrochloride, 4-morpholinepropanesulfonic acid, Tris, 3-(N-morpholino)ethanesulfonic acid, α-D-glucose 1-phosphate (grade V), adenosine 3'5'-monophosphoric acid, and glucagon were obtained from Sigma; 1-methyl-3-isobutyl xanthine was obtained from Aldrich; (−)-propranolol was a gift from AYERST; phenolamine was from Smith, Kline, and French; phentolamine was obtained from CIBA.
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Crude collagenase type II (Lot 45 A145) was obtained from Worthington. All other chemicals were obtained from standard commercial sources.

Rats were rendered hypothyroid by maintaining them on an iodine-deficient diet (U. S. Biochemical Corp.) and drinking water containing 0.00625% propylthiouracil for 10 to 25 days. The euthyroid control rats were fed the same iodine-deficient test diet in which normal iodine has been replaced by the commercial supplier. Hepatocytes were isolated from 175- to 225-g fed female Sprague-Dawley rats (Charles River, CD strain) following the procedure of Berry and Friend (21) as previously described (10). Following isolation the rat hepatocytes were incubated in 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. The liver cells (approximately 7.0 × 10^6 cells/tube) were incubated in plastic tubes (17 × 100 mm, Falcon 19717) in a total volume of 1 ml and were constantly shaken in an orbital water bath operating at 150 to 200 rpm.

To measure total cyclic AMP content the hepatocytes were incubated as described above, the incubation was then stopped 1 min after addition of hormones by the addition of 0.1 ml of 2 N HCl. Stimulation of rat hepatocyte cyclic AMP accumulation by hormones has been shown to be maximal at 1 min following the addition of the hormone (10). 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 (22). The free cyclic AMP was separated from the bound cyclic AMP by charcoal adsorption, as suggested by Brown et al. (23). The above methodology for measuring the cyclic AMP content of hepatocytes was compared with two alternative procedures. The first method terminated the incubation with 1.0 ml of cold absolute ethanol. The cells were then homogenized for 30 s with a Brinkmann Polytron at setting 5. The homogenate was centrifuged at 12,000 × g max for 15 min at 4°C and the resultant pellet was discarded. The supernatant was flash-evaporated, and the residue was brought to 4 ml with distilled water, flash-evaporated again, and finally resuspended in 2 ml of distilled water. The cyclic AMP content of a 20-μl aliquot was measured by the above method. The second method stopped the incubation and extracted the cyclic AMP in 1 N HClO, following which the cyclic AMP was purified on Dowex (AG 1-X8, 200 to 400 mesh, Bio-Rad Laboratories), flash-evaporated to dryness, and brought to 1 ml with distilled water. The cyclic AMP content of a 20-μl aliquot was measured by the method of Gilman (22) described above. All three assays provided equivalent data and the first procedure was adopted for routine determinations.

Glycogen phosphorylase a activity was measured in a separate set of 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-morpholinepropanesulfonic acid, 0.5 M sodium fluoride, 50 mM EDTA, and 10 mM dithiothreitol. Activation of hepatocyte glycogen phosphorylase in response to hormones has been shown to be maximal at 1 min following the addition of the hormone (3, 10). 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 then thawed at room temperature and homogenized for 20 s with a Willems Polytron (Brinkmann Instruments) at setting 5. The homogenates were centrifuged at 12,000 × g max for 5 min at 4°C with the resultant pellets discarded. This assay was performed on the supernatant which contained approximately 10 mg of protein/ml. Glycogen phosphorylase a was measured using a filter disc assay similar to that of Gilboe et al. (24) as previously described (10). Caffeine (0.5 mM) was included with isoproterenol, epinephrine, or phenylephrine. Rat hepatocytes (6.6 to 7.0 × 10^6 cells/tube) were isolated from euthyroid or hypothyroid rats, incubated in a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 22 mM glucose for 30 min, then resuspended in fresh medium for another 20 min for the experiments. The indicated agents were added in the absence of any methyl xanthine. The values are the means of three to four experiments performed on separate occasions and are expressed ± the standard error. Basal glycogen phosphorylase a activities were 1.11 ± 0.2 (n = 4) μmol/mg of protein for euthyroid and 1.12 ± 0.1 (n = 4) μmol/mg for hypothyroid rat hepatocytes.

RESULTS

Basal levels of glycogen phosphorylase activity in isolated rat hepatocytes were not altered by hypothyroidism. Basal phosphorylase activities in the absence of methyl xanthine were 1.11 ± 0.2 (n = 4) μmol/mg of protein for euthyroid and 1.12 ± 0.1 (n = 4) μmol/mg for hypothyroid rat hepatocytes. The ability of phenylephrine, epinephrine, and isoproterenol to activate phosphorylase in euthyroid and hypothyroid rat hepatocytes was examined and the results are shown in Fig. 1 (top panel). In the absence of a methyl xanthine, activation of glycogen phosphorylase by the β-adrenergic agonist phenylephrine was nearly identical in both euthyroid and hypothyroid rat hepatocytes. The activation of phosphorylase by the β-adrenergic agonist isoproterenol, in comparison, displayed an increased sensitivity and higher maximal response in hepatocytes isolated from hypothyroid as compared to euthyroid rats. In the hypothyroid rat hepatocytes 10 μM isoproterenol activated phosphorylase to the same level as 10 μM phenylephrine. In the euthyroid rat hepatocytes, 10 μM isoproterenol could only activate phosphorylase to 50% of the level obtained with 10 μM phenylephrine, an observation consistent with an earlier report (27). Not only was maximal activation of phosphorylase by isoproterenol...
100% higher in the hypothyroid rat hepatocytes than in those isolated from euthyroid rats, but the concentration of isoproterenol providing half-maximal activation was also an order of magnitude lower in the hypothyroid cells. The activation of hepatocyte glycogen phosphorylase by epinephrine, a mixed α- and β-adrenergic agonist, was not altered by hypothyroidism (Fig. 1).

Cyclic AMP levels were measured in isolated hepatocytes from euthyroid and hypothyroid rats in the absence of methyl xanthine. Basal cyclic AMP levels were 1.1 ± 0.1 (n = 4) pmol/10⁶ cells for the euthyroid and 1.4 ± 0.3 (n = 4) pmol/10⁶ cells for the hypothyroid rat hepatocytes. Cyclic AMP levels were also determined in rat hepatocytes from euthyroid and hypothyroid rats in response to varying concentrations of phenylephrine, epinephrine, and isoproterenol in the absence of a methyl xanthine (Fig. 1, bottom panel). The most striking feature of this figure is the dramatic increase in cyclic AMP accumulation of the hypothyroid as compared to euthyroid rat hepatocytes in response to isoproterenol and to epinephrine. Under these conditions, 10 μM isoproterenol stimulated a 600% increase over basal cyclic AMP levels in the hypothyroid rat hepatocytes and only a 50% increase in the euthyroid rat hepatocytes. Phenylephrine was a relatively poor stimulator of cyclic AMP accumulation in hypothyroid rat hepatocytes.

In the presence of 50 μM methylisobutyl xanthine, glycogen phosphorylase activity was 1.77 ± 0.1 (n = 4) μmol/mg of protein in euthyroid and 1.44 ± 0.6 μmol/mg of protein in hypothyroid rat hepatocytes. The activation of glycogen phosphorylase by phenylephrine, epinephrine, and isoproterenol in the presence of 50 μM methylisobutyl xanthine was examined in hepatocytes from euthyroid and hypothyroid rats (Fig. 2, top panel). Phenylephrine activation of phosphorylase was not potentiated by the presence of methylisobutyl xanthine in either euthyroid or hypothyroid rat hepatocytes. The hypothyroid rat hepatocytes displayed a slightly higher level of phosphorylase activation over euthyroid in response to 10 μM phenylephrine when the methylisobutyl xanthine was present.

Activation of glycogen phosphorylase by the β-adrenergic agonist isoproterenol was potentiated in both euthyroid and hypothyroid rat hepatocytes by the presence of 50 μM methylisobutyl xanthine (Figs. 1 and 2, top panels). In the presence of this methyl xanthine, the maximal response of the phosphorylase to isoproterenol in the hypothyroid rat hepatocytes was 25% higher than that of the euthyroid rat hepatocytes (Fig. 2, top panel). Hepatocytes from the hypothyroid rat also displayed a greater sensitivity with respect to phosphorylase activation in response to isoproterenol than did the euthyroid rat hepatocytes. Hypothyroid rat hepatocytes displayed half-maximal activation of phosphorylase with 1 nM isoproterenol as compared to 33 nM isoproterenol for the euthyroid rat hepatocytes under these conditions.

Epinephrine activation of glycogen phosphorylase in euthyroid rat hepatocytes was unaffected by the 50 μM methylisobutyl xanthine (Figs. 1 and 2, top panels). The phosphorylase response of the hypothyroid rat hepatocytes to epinephrine, however, was potentiated by the methyl xanthine, resulting in a small increase in the sensitivity without any apparent change in the maximal phosphorylase response to this catecholamine.

Cyclic AMP levels in the presence of 50 μM methylisobutyl xanthine were 1.8 ± 0.6 pmol/10⁶ cells for euthyroid and 1.3 ± 0.2 pmol/10⁶ cells for hypothyroid rat hepatocytes. The experiments performed with 50 μM methylisobutyl xanthine were performed on separate days with different batches of hepatocytes than those performed in the absence of this methyl xanthine. When performed with the same preparation of hepatocytes, methylisobutyl xanthine alone increased the level of cyclic AMP in both euthyroid and hypothyroid rat hepatocytes (data not shown). Cyclic AMP accumulation in response to epinephrine and to isoproterenol was dramatically increased in euthyroid rat hepatocytes by the presence of 50 μM methylisobutyl xanthine (Fig. 2, bottom panel). Although the methyl xanthine potentiated the rise in cyclic AMP of euthyroid rat hepatocytes in response to epinephrine, it failed to potentiate epinephrine activation of glycogen phosphorylase (Fig. 2, top panel). The 50 μM methylisobutyl xanthine potentiated cyclic AMP accumulation in response to epinephrine and to isoproterenol in the hypothyroid rat hepatocytes (Fig. 2, bottom panel). As noted in the absence of any methyl xanthine, the hypothyroid rat hepatocytes displayed an increased capacity to accumulate cyclic AMP in response to isoproterenol and epinephrine over that of the euthyroid rat hepatocytes (Fig. 2, bottom panel). Epinephrine stimulated cyclic AMP accumulation to a greater extent than isoproterenol in both euthyroid and hypothyroid rat hepatocytes, an
observation similar to that noted in the perfused liver (28). Phenylephrine at 1 \mu M or higher increased cyclic AMP accumulation of hypothyroid, but not euthyroid, rat hepatocytes in the presence of methylisobutyl xanthine.

We next investigated the effects of hypothyroidism on the ability of glucagon to regulate glycogen phosphorylase activity and cyclic AMP accumulation in rat hepatocytes. The glycogen phosphorylase response of hepatocytes obtained from euthyroid and hypothyroid rats to glucagon was studied both in the absence (Fig. 3, top left) and presence (Fig. 3, top right) of 50 \mu M methylisobutyl xanthine. Hypothyroid and euthyroid rat hepatocytes responded in a similar fashion to glucagon with or without methylisobutyl xanthine present. As also shown in Fig. 3 (bottom left), glucagon stimulation of cyclic AMP accumulation was identical in euthyroid and hypothyroid rat hepatocytes. The cyclic AMP response of both euthyroid and hypothyroid rat hepatocytes was potentiated equally by the presence of 50 \mu M methylisobutyl xanthine (Fig. 3, bottom right, please note scale). These data would suggest that the amplified response of hypothyroid rat hepatocytes to isoproterenol does not involve loci in the cyclic AMP-mediated pathway below the point of cyclic AMP generation.

To determine whether activation of \( \alpha \) - or \( \beta \)-adrenergic receptors was responsible for the enhanced isoproterenol response of hypothyroid rat hepatocyte phosphorylase, the effects of the adrenergic antagonists propranolol and phentolamine were examined (Table I). Basal glycogen phosphorylase activities were essentially not affected by either propranolol (10 \mu M) or phentolamine (10 \mu M). Activation of phosphorylase by 1 \mu M phenylephrine was reduced in euthyroid and blocked in hypothyroid rat hepatocytes by 10 \mu M phentolamine. Phentolamine (10 \mu M) was a relatively poor inhibitor of phosphorylase activation by 10 \mu M phenylephrine or 0.1 \mu M and 10 \mu M epinephrine in hepatocytes from euthyroid or hypothyroid rats. Activation of phosphorylase by either 0.1 or 10 \mu M isoproterenol was not blocked by phentolamine. Propranolol partly blocked phenylephrine and epinephrine activation of phosphorylase in hypothyroid rat hepatocytes, but completely blocked activation of 0.1 \mu M isoproterenol in hepatocytes from either euthyroid or hypothyroid rats. At 10 \mu M isoproterenol, 10 \mu M propranolol blocked activation of phosphorylase in euthyroid and hypothyroid rat hepatocytes 50 to 60%. The activation of phosphorylase by 0.5 and 10 nm glucagon in hypothyroid rat hepatocytes was not affected by 10 \mu M propranolol or phentolamine (data not shown). The enhanced response of the hypothyroid rat hepatocyte phosphorylase to 0.1 \mu M isoproterenol would appear to be the result of interaction of this agonist with the \( \beta \) and not an \( \alpha \) receptor.

**Table I**

| Glycogen phosphorylase activity of euthyroid and hypothyroid rat hepatocytes: effects of propranolol and phentolamine on hormonal stimulation |
|---|---|
| **Additions** | **% Stimulation over basal level** |
| | Euthyroid | Hypothyroid |
| Buffer alone | | |
| Buffer + propranolol (10 \mu M) | 10 \pm 2 | 0 \pm 2 |
| Buffer + phentolamine (10 \mu M) | 0 \pm 1 | -7 \pm 2 |
| Phenylephrine (1 \mu M) | 50 \pm 4 | 76 \pm 8 |
| Phenylephrine + propranolol (10 \mu M) | 45 \pm 7 | 43 \pm 12 |
| Phenylephrine + phentolamine (10 \mu M) | 19 \pm 3 | 13 \pm 13 |
| Phenylephrine (10 \mu M) | 90 \pm 2 | 135 \pm 22 |
| Phenylephrine + propranolol (10 \mu M) | 79 \pm 10 | 75 \pm 19 |
| Phenylephrine + phentolamine (10 \mu M) | 38 \pm 12 | 83 \pm 46 |
| Epinephrine (0.1 \mu M) | 127 \pm 9 | 155 \pm 25 |
| Epinephrine + propranolol (10 \mu M) | 114 \pm 20 | 72 \pm 9 |
| Epinephrine + phentolamine (10 \mu M) | 78 \pm 28 | 116 \pm 46 |
| Epinephrine (10 \mu M) | 100 \pm 17 | 166 \pm 9 |
| Epinephrine + propranolol (10 \mu M) | 96 \pm 17 | 114 \pm 22 |
| Epinephrine + phentolamine (10 \mu M) | 75 \pm 26 | 145 \pm 5 |
| Isoproterenol (0.1 \mu M) | 96 \pm 7 | 131 \pm 21 |
| Isoproterenol + propranolol (10 \mu M) | 4 \pm 2 | 2 \pm 1 |
| Isoproterenol + phentolamine (10 \mu M) | 17 \pm 12 | 133 \pm 36 |
| Isoproterenol (10 \mu M) | 51 \pm 4 | 156 \pm 30 |
| Isoproterenol + propranolol (10 \mu M) | 16 \pm 4 | 74 \pm 13 |
| Isoproterenol + phentolamine (10 \mu M) | 44 \pm 3 | 135 \pm 32 |
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Another 20 min for the experiments. The indicated agents were added 1 min before the end of this 20-min incubation. One minute following addition of the hormone, the incubation was stopped and total cyclic AMP was determined. The results are expressed as the mean value of duplicates from a single representative experiment.

The results of similar blocking studies on hormonal stimulation of cyclic AMP accumulation in hypothyroid rat hepatocytes are shown in Table II. Phenylephrine displayed a weak stimulation of cyclic AMP accumulation which could be blocked by propranolol but not phentolamine. This suggests that at 10 μM, phenylephrine possesses sufficient β agonist activity to activate cyclic AMP accumulation in the hypothyroid rat hepatocytes. The enhanced β-adrenergic responsiveness of the hypothyroid rat hepatocytes allows detection of the weak β-adrenergic agonist activity of phenylephrine and in the presence of 50 μM methylisobutyl xanthine phenylephrine stimulated significant increases in cyclic AMP accumulation in these cells (Fig. 2, bottom panel). Epinephrine stimulation of cyclic AMP accumulation was abolished by propranolol but not phentolamine. Isoproterenol stimulation of cyclic AMP accumulation was completely blocked by the β-adrenergic antagonist propranolol. The ability of glucagon to stimulate cyclic AMP accumulation was slightly impaired by propranolol but not by phentolamine (data not shown). These data indicate that the enhanced cyclic AMP accumulation of hypothyroid rat hepatocytes in response to isoproterenol is due to interaction of this agonist with β and not α receptors.

**DISCUSSION**

Murad and Freeland (29) reported that hypothyroidism reduced liver glycogen content in rats. Subsequent studies, however, suggested that the liver glycogen content is unaffected by hypothyroidism (30-32). Takahasi and Suzuki (32) reported that the total glycogen content, cyclic AMP content, glycogen synthase activity, and the activity of glycogen synthase α were not altered in freeze-clamped livers from thyroidectomized rats. The activity of glycogen phosphorylase was reported to be slightly lower in livers from thyroidectomized rats by these same investigators (32). However, little attention has been focused on the influence of thyroid status on the hormonal regulation of glycogenolysis. The present study addresses this question utilizing isolated hepatocytes from hypothyroid rats.

The present study demonstrates that hypothyroidism potentiates the activation of glycogen phosphorylase and the accumulation of cyclic AMP in isolated hepatocytes in response to β-adrenergic agonists, although not influencing the basal levels of these parameters. Epinephrine and isoproterenol stimulation of cyclic AMP accumulation in isolated hepatocytes was markedly potentiated by hypothyroidism. Propranolol, a potent β-adrenergic antagonist, effectively blocked the amplified cyclic AMP accumulation of hypothyroid rat hepatocytes in response to epinephrine and isoproterenol, while phentolamine had little effect. Hypothyroidism did not, however, alter glucagon-stimulated cyclic AMP accumulation or activation of glycogen phosphorylase.

The α-adrenergic agonist phenylephrine activated phosphorylase in euthyroid and hypothyroid rat hepatocytes to a similar extent. Methylisobutyl xanthine did not potentiate phenylephrine action in either euthyroid or hypothyroid rat hepatocytes. Although the methyl xanthine did potentiate cyclic AMP accumulation in response to epinephrine in the euthyroid rat hepatocytes, it did not potentiate epinephrine activation of glycogen phosphorylase. These data support the view that epinephrine primarily activates glycogen phosphorylase through a cyclic AMP-independent mechanism (3.4.8-10). The hypothyroid rat hepatocyte data also suggest epinephrine activates glycogen phosphorylase through an essentially cyclic AMP-independent mechanism(s).

Gumaa et al. (33) reported a small increase in cyclic AMP phosphodiesterase activity measured at 10 and 100 μM substrate in a particulate membrane fraction prepared from hypothyroid rat liver, although total cyclic AMP content in the freeze-clamped liver was unaltered by hypothyroidism. Basal levels of cyclic AMP in isolated hepatocytes were the same in hypothyroid and euthyroid rat preparations in the present study. The observed normal activation of glycogen phosphorylase and cyclic AMP accumulation in response to glucagon in hepatocytes from hypothyroid rats would argue against altered cyclic AMP phosphodiesterase activity in these cells. Furthermore, our studies demonstrate that hypothyroidism enhances the β-adrenergic activation of glycogen phosphorylase. If a modified level of cyclic AMP phosphodiesterase were responsible for this observed change, a reduction in phosphodiesterase activity would be indicated rather than an increase in activity.

Hypothyroidism reduces catecholamine-stimulated cyclic AMP accumulation and lipolysis in fat cells (15-17). Increased low Km cyclic AMP phosphodiesterase activity (15) and reduced hormone receptor-adenylate cyclase “coupling” (17) have been proposed as partial explanations for these effects of hypothyroidism. In the heart, catecholamine activation of phosphorylase α is enhanced by hyperthyroidism (18,19). Similarly, enhanced catecholamine-stimulated cyclic AMP accumulation is demonstrated in fat cells from hyperthyroid rats (17). The hepatocyte would appear to be unique in that it is a system where hypothyroidism enhances rather than depresses a β-adrenergic response. These observations suggest thyroid hormones do not have identical effects on β-adrenergic hormone responsiveness in all tissues. The hepatocyte is clearly different from both the heart and from the isolated fat cell in that β-adrenergic action is enhanced by hypothyroidism, not hyperthyroidism. However, this enhanced β-adrenergic response would probably have a minimal effect physiologically with respect to glycogenolysis since epinephrine appears to exert its influence on phosphorylase through primarily a cyclic AMP-independent mechanism(s).

The enhanced activation of glycogen phosphorylase by hypothyroidism as compared to euthyroid rat hepatocytes is clearly a β-adrenergic response. This amplified response of the hypothyroid rat hepatocytes can be blocked by propranolol, but not phentolamine. In addition, the cyclic AMP accumulation of these same hepatocytes in response to
isoproterenol was similarly blocked by propranolol, but not phentolamine. Methylisobutyl xanthine potentiated both the phosphorylase activation and cyclic AMP response of euthyroid and hypothyroid rat hepatocytes to isoproterenol. The precise mechanism by which hypothyroidism selectively amplifies the β-adrenergic response of hypothyroid rat hepatocytes remains obscure. This increased sensitivity could be due to an increase in the number of β-adrenergic hormone receptors, an increase in the affinity of the β-adrenergic receptor for the catecholamine, an increase in the efficiency of the transmembrane signaling between the β-adrenergic receptor and the adenylate cyclase, or perhaps some complex modulation involving two or more of these loci.

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