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Effect of Adrenergic Amines on Phosphatidylinositol Labeling and Glycogen Synthase Activity in Fat Cells from Euthyroid and Hypothyroid Rats

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SUMMARY

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Adipocytes from hypothyroid rats exhibit a markedly decreased response to β-adrenergic stimulation as reflected by lipolysis, glycogen synthase activity, and cyclic AMP accumulation as compared to fat cells from euthyroid rats. No effect of insulin on glycogen synthase activity was detected in cells from hypothyroid animals. On the other hand, the sensitivity to α_1-adrenergic stimulation was virtually the same in hypothyroid as compared to euthyroid rats as reflected by phosphatidylinositol labeling with [^{32}P]P_i and glycogen synthase activity. It is concluded that thyroid hormones modulate the responsiveness of fat cells to β-adrenergic amines without modifying that to α-catecholamines. The present results do not support the suggestion that thyroid status modulates the interconversion of α- and β-adrenoceptors.

INTRODUCTION

The response of many mammalian tissues to adrenergic amines is influenced by the thyroid status of animals. In fat cells the rise in cyclic AMP and the activation of lipolysis due to β-adrenergic stimulation are markedly reduced in cells from hypothyroid rats (1-4). The role of α-adrenergic activation in the regulation of rat adipose tissue metabolism has received little attention. However, Lawrence and Larner reported that α-adrenergic amines produce an inactivation of glycogen synthase dependent on the presence of extracellular calcium (5, 6). Calcium has been proposed as a second messenger for α-adrenergic stimulation (7). The activation of receptors whose actions seem to be mediated by an increase in cystosol calcium is generally accompanied by an increased incorporation of [^{32}P]P_i into phosphatidylinositol (8). It has been suggested that phosphatidylinositol turnover may be involved in the gating or mobilization of calcium (8). Consistent with this, we observed that α-adrenergic activation of rat fat cells produces a marked increase in the incorporation of [^{32}P]P_i into phosphatidylinositol which does not require the presence of extracellular calcium (9). The present experiments were designed to investigate the effect of hypothyroidism on the α-adrenergic modulation of adipose tissue metabolism.

MATERIALS AND METHODS

Epinephrine, isoproterenol, (±)-propranolol, UDPG, and glycogen were obtained from Sigma Chemical Co., crude collagenase (*Clostridium histolyticum*) was from Worthington Biochemical Corp. (Lot CLS 48N248), bovine serum albumin (Fraction V) was from Armour Pharmaceutical Co. (Lot T13705), [^{32}P]P_i as orthophosphoric acid (carrier free) and UDP-[U-^{14}C]glucose were from New England Nuclear, and enzymes and coenzymes for glycerol determination were obtained from Boehringer Mannheim. (−)-Propranolol was a gift from Ayerst Laboratories.

Female, 175- to 250-g Sprague-Dawley rats (Charles River CD strain) were rendered hypothyroid by maintaining them on an iodine-deficient diet (U.S. Biochemical Corp.) and drinking water containing 6.25 mg/dl propylthiouracil (Nutritional Biochemical Corp.) for 30-45 days. Euthyroid control rats were fed the same diet to which iodine was added by the supplier. Animals had access to water and food *ad libitum*.

White fat cells were obtained by the enzymatic digestion of parametrial adipose tissue according to the procedure of Rodbell (10). Pooled adipose tissue (approx 25 g) from five or six rats was carefully minced with scissors and placed in small plastic bottles. Each bottle, contain-

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ing approximately 13 g of tissue and 20 ml of either Krebs–Ringer Tris buffer or Krebs–Ringer phosphate buffer supplemented with 3% albumin and 1 mg/ml crude collagenase, was incubated for 50 min at 37°C in an orbital water bath shaker at 150 rpm. Krebs–Ringer Tris buffer of the following composition was used in all the experiments except for the assay of glycogen synthase: 120 mM NaCl, 1.4 mM $CaCl_2$, 5.2 mM KCl, 1.4 mM $MgSO_4$, 5 mM Tris supplemented with 3% albumin. For the assay of glycogen synthase, more reproducible results were obtained when cells were digested and incubated in Krebs–Ringer phosphate buffer of the following composition: 120 mM NaCl, 1.4 mM $CaCl_2$, 5.2 mM KCl, 1.4 mM $MgSO_4$, 10 mM Na_2HPO_4 supplemented with 3% albumin.

The incorporation of [^{32}P]P_i into phospholipids was measured as previously described (9). In brief, fat cells were incubated in plastic bottles containing 3 ml of phosphate-free medium and [^{32}P]P_i at a concentration of 10 μCi/ml. Usually 1 ml of packed cells was added to each bottle. After 1 h of incubation the cells were transferred to plastic tubes and centrifuged for 15 s in a clinical centrifuge. The medium was aspirated and lipids were extracted with chloroform/methanol (2:1). Phospholipids were adsorbed with silicic acid granules and removed from the silicic acid granules with methanol. Major phospholipids were separated by one-dimensional thin-layer chromatography on glass plates coated with silica gel H (Merck) containing 1 g of magnesium acetate for each 40 g of silica. The solvent system contained chloroform/methanol/water/28% NH_4OH (130:70:5:5). This procedure was suitable for the separation of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Phosphatidic acid was not separated from phosphatidylserine, which migrated only slightly above the origin. The amount of phosphatidylserine + phosphatidic acid may be overestimated by the presence of some contaminants at the origin; however, nearly all the radioactivity corresponded to phosphatidic acid. The same comment may by applied to phosphatidylinositol due to incomplete separation from sphingomyelin. The incorporation of label into phosphatidylserine or sphingomyelin is less than 10% of that of phosphatidic acid or phosphatidylinositol (9) and there is no effect of α-catecholamines on the labeling of phosphatidylserine or sphingomyelin (9). Lipids were visualized by using iodine-vapor staining. The phosphorus content of phospholipids was determined by a micromodification of the procedure of Bartlett (11) after acid hydrolysis of silica gel scrapings containing each individual phospholipid. Samples of the hydrolysate were counted to determine the amount of radioactivity present in each phospholipid.

For the assay of glycogen synthase activity, cells were incubated with hormones for 2 min, then centrifuged for 15 s in a clinical centrifuge. The medium was removed, 0.3 ml of cold buffer (100 mM Tris, 10 mM EDTA, pH 7.8) was added to the cells, and the cells were immediately homogenized at 0°C for 15 s with a Willems Polytron (Brinkmann Instruments) at setting 6. The homogenates were centrifuged at 12,000*g* for 20 min at 0–4°C. The infranatant was carefully aspirated with a syringe fitted with a long needle and 50 μl was added to each assay tube. Glycogen synthase I was assayed by measuring the incorporation of UDP-[^{14}C]glucose into glycogen. The assay volume was 100 μl and contained 50 mM Tris (pH 7.8), 10 mM EDTA, 12 mg/ml glycogen, and 1 mM UDP[U-^{14}C]glucose (approximately 150,000 cpm), and the incubation time was 60 min at 30°C. Total glycogen synthase was assayed in the same way except that 7.2 mM glucose-6-phosphate was added to the reaction mixture.

Glycerol release was determined at 60 min as previously described (13). Cyclic AMP accumulation was determined at 10 min by a modification of the protein kinase binding procedure (14). The free cyclic AMP was separated from the bound cyclic AMP by charcoal adsorption (15). Each experiment was repeated at least three times and the results are expressed as the mean ± the standard error of the mean.

RESULTS

Lipolysis and cyclic AMP accumulation. Hypothyroid rats have the same body weight as controls but have more parametrial adipose tissue (4). The increase in adipose tissue mass was reflected by an increase in fat cell size.

The addition of epinephrine at a concentration of 1 μM or isoproterenol at a concentration of 0.1 μM (Fig. 1) increased the accumulation of cyclic AMP and lipolysis by fat cells from euthyroid animals. Fat cells isolated from hypothyroid rats did not show any significant increase in cyclic AMP over basal in response to epinephrine even at a concentration of 100 μM and only a slight increase in response to 100 μM isoproterenol. Catecholamine-mediated lipolysis was abolished in cells from hypothyroid rats with the following exceptions: 100 μM isoproterenol produced a slight increase in lipolysis, and a near-normal lipolytic response to 100 μM epinephrine was observed (Fig. 1). It is interesting to note that the increase in lipolysis due to 100 μM epinephrine was much higher than that produced by the same concentration of the pure β-adrenergic agonist isoproterenol in cells from hypothyroid rats (Fig. 1).

Phosphatidylinositol labeling. Fat cells were incubated in the presence of radioactive phosphate and the major phospholipids were separated by thin-layer chromatography. The individual phospholipid fractions were analyzed for total phosphate and for incorporation of radioactivity. The phospholipid composition of adipocytes from hypothyroid and euthyroid rats was the same (Table 1). Similar results have been obtained with fat cell ghosts (16). The uptake of [^{32}P]P_i into the different phospholipids of fat cells was similar in fat cells from euthyroid and hypothyroid rats except for a small but consistent increase in the specific radioactivity of phosphatidylinositol in cells from hypothyroid animals (Table 1).

The effect of epinephrine on the incorporation of [^{32}P]-P_i into phosphatidylinositol was studied in the presence of 30 μM (±)-propranolol to avoid any effects of β-adrenergic activation by epinephrine (9). (±)-Propranolol (30 μM) blocked the β action of epinephrine at concentrations between 0.1 and 10 μM as reflected by lipolysis, i.e., no significant increase in glycerol release was observed. Epinephrine at concentrations between 30 and 100 μM

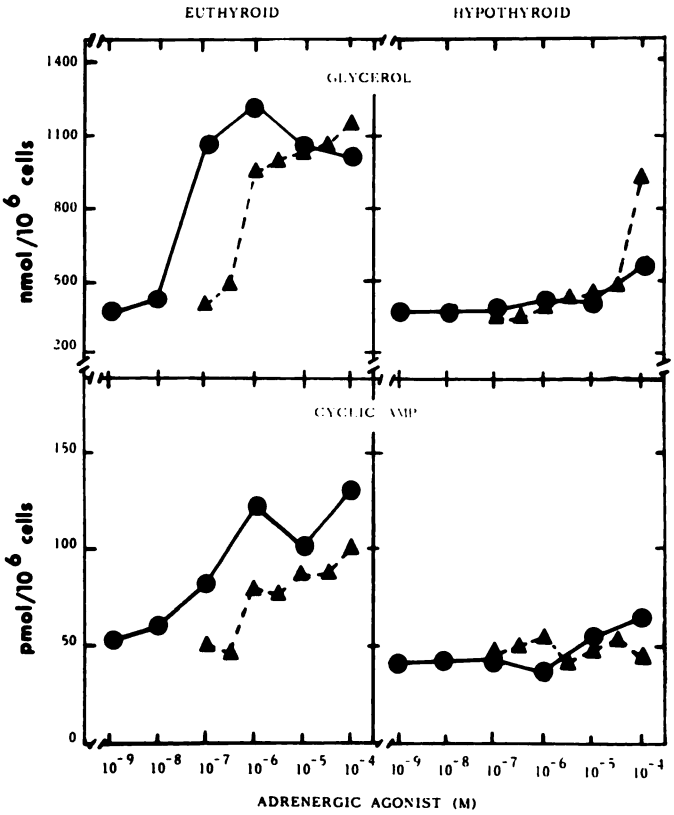

FIG. 1. *Effects of epinephrine and isoproterenol on lipolysis and cyclic AMP accumulation in rat fat cells from euthyroid and hypothyroid rats*

Fat cells (2–3 × 10^5 cells) were incubated in the absence or presence of either isoproterenol (circles, solid line) or epinephrine (triangles, broken line). Cyclic AMP was measured at 10 min and glycerol release at 60 min. Basal values for glycerol release were 319 ± 4 and 357 ± 27 nmol/10^6 cells in adipocytes from euthyroid and hypothyroid rats, respectively. Basal cyclic AMP values were 43 ± 6 and 33 ± 6 pmol/10^6 cells in adipocytes from euthyroid and hypothyroid rats, respectively (means ± SEM of four to eight experiments in each case). Values are presented as the means of four to eight experiments; the standard errors are in the range of 10–15% of the value.

increased lipolysis two- to threefold in the presence of 30 μM propranolol in cells from euthyroid animals. (±)-Propranolol (30 μM) produced a twofold increase in the incorporation of [^{32}P]P_i into phosphatidylinositol (9). This effect of propranolol seems to be related to its local anesthetic properties rather than to its activity as a β-adrenergic antagonist (9), in agreement with reports in other systems (17). The effect of propranolol on this parameter was of a similar magnitude in cells from euthyroid and hypothyroid animals (see legend to Fig. 2).

The incorporation of [^{32}P]P_i into phosphatidylinositol was stimulated by epinephrine to about the same extent in cells from euthyroid or hypothyroid rats except for a slightly decreased response of cells from hypothyroid rats at low agonist concentrations (Fig. 2). Similar results were obtained with the pure α-adrenergic agonist methoxamine (Fig. 3). The α-adrenergic stimulation of phosphatidylinositol labeling produced by epinephrine is blocked by low concentrations of prazosin (9). The stimulation by 100 μM methoxamine was also blocked by concentrations of prazosin as low as 1 μM (Table 2).

Glycogen synthase activity. Glycogen synthase I activity was lower in cells from hypothyroid rats than in cells from euthyroid animals; however, the total glycogen synthase activity was the same (Table 3). The addition of hexoses to the incubation medium increased glycogen synthase I activity to a similar extent in adipocytes from euthyroid or hypothyroid animals (Table 3).

The effects of insulin and adrenergic amines on glycogen synthase I are presented in Tables 4 and 5. In these experiments fat pads were digested and cells incubated in a medium containing 10 mM glucose, because the addition of the hexose to the medium increased the reproducibility of the results. In adipocytes from euthyroid rats, both epinephreine and isoproterenol inactivated glycogen synthase I (Tables 4 and 5). The effect of isoproterenol was blocked by propranolol but not by prazosin (Table 4). In contrast, epinephrine action on glycogen synthase was blocked neither by propranolol nor by prazosin (Table 4), but it was abolished by the addition of both agents (Table 4). In adipocytes from hypothyroid animals, epinephrine decreased glycogen synthase I activity but isoproterenol had no effect (Table 5). Methoxamine inactivated glycogen synthase I to about the same extent in cells from hypothyroid as compared to euthyroid animals (Fig. 3). Methoxamine was a weak inactivator of glycogen synthase I.

The inactivation of glycogen synthase produced by 100 μM methoxamine varied between 15 and 50% from one set of experiments to another. However, this α-adrenergic agonist consistently decreased the activity of the enzyme. The action of methoxamine on glycogen synthase was, as its action on phosphatidylinositol labeling, blocked by 1 μM prazosin but not by 30 μM (±)-propranolol (Table 2). Clonidine, a potent α_2-adrenergic agonist produced no effect on phosphatidylinositol labeling and no significant change in glycogen synthase I activity (Table 2).

TABLE 1

Incorporation of [^{32}P]P_i into major phospholipids of fat cells obtained from euthyroid and hypothyroid rats

Fat cells (1.5–2.2 × 10^6) were incubated for 60 min in a medium containing 10 μCi/ml [^{32}P]P_i. Values are the mean ± SEM of five or six experiments performed on different days.

Phospholipid	Euthyroid		Hypothyroid	
	Amount[a]	Specific activity[b]	Amount[a]	Specific activity[b]
Phosphatidylethanolamine	1.80 ± 0.20	1,410 ± 310	1.75 ± 0.15	1,125 ± 150
Phosphatidylcholine	2.35 ± 0.25	6,760 ± 1,625	2.05 ± 0.20	6,160 ± 515
Phosphatidylinositol	0.85 ± 0.10	4,775 ± 690	0.75 ± 0.10	6,900 ± 1,165
Phosphatidic acid plus phosphatidylserine	1.15 ± 0.10	15,275 ± 2,000	1.05 ± 0.15	14,858 ± 1,615

[a] μg inorganic phosphate.
[b] cpm/μg inorganic phosphate.

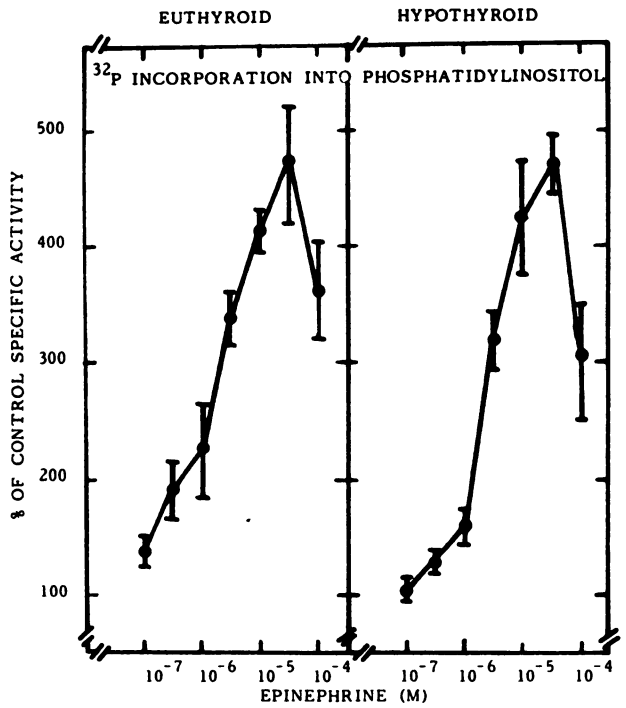

FIG. 2. *Effect of epinephrine in the presence of propranolol on the incorporation of [^{32}P]P_i into phosphatidylinositol in fat cells from euthyroid and hypothyroid animals*

Fat cells (2×10^6) were incubated with 30 μM propranolol in the absence or presence of epinephrine. Basal phosphatidylinositol specific radioactivity was 5120 ± 555 and 7695 ± 1805 cpm/μg inorganic phosphate in fat cells from euthyroid and hypothyroid animals, respectively. Phosphatidylinositol specific radioactivity in the presence of 30 μM propranolol was 11,485 ± 3995 and 14,375 ± 3225 cpm/μg inorganic phosphate in fat cells from euthyroid and hypothyroid animals, respectively (results are the means ± SEM of three determinations). Results are presented as percentages of the specific activity measured in the presence of 30 μM propranolol. Bars represent the SEM of three determinations performed on different days.

The concentration of methoxamine required to produce a 50% inactivation of glycogen synthase I was about one order of magnitude lower than that required to produce half-maximal stimulation of phosphatidylinositol labeling with [^{32}P]P_i in cells from euthyroid rats and slightly more in adipocytes from hypothyroid animals (Fig. 3). Insulin produced a significant increase in the activity of glycogen synthase I in adipocytes from euthyroid animals, but it had virtually no effect in cells from hypothyroid rats (Table 5).

DISCUSSION

Beta-adrenergic stimulation in fat cells activates adenylate cyclase which converts ATP to cyclic AMP. The role of cyclic AMP as a second messenger for β-adrenergic stimulation and as a modulator of lipolysis is well known (18). The present paper confirms that fat cells from hypothyroid animals exhibit a decreased lipolysis in response to submaximal concentrations of β-adrenergic amines and that little accumulation of cyclic AMP is observed even with high concentrations of the adrenergic agonists (1–4). In the present report, the lipolytic response to 100 μM epinephrine was higher than that observed with 100 μM isoproterenol. This is especially interesting since isoproterenol is more potent as a β-adrenergic activator of lipolysis than epinephrine. These results suggest that the activation of lipolysis observed with such a high concentration of epinephrine is not related to its β-adrenergic potency. It may be related to the fact that epinephrine can also activate lipolysis by a cyclic AMP-independent pathway (19).

Recent evidence suggests that both β- and α-adrenergic amines modulate glycogen metabolism in adipocytes (5, 6). Regulation of glycogen metabolism through cyclic AMP-dependent processes in adipose tissue has been extensively studied (20). Beta-adrenergic amines inactivate glycogen synthase I in normal rats (5, 21). This paper adds the observation that no inactivation of glycogen synthase I is produced in adipocytes from hypothyroid animals in response to a pure β-adrenergic agonist such as isoproterenol, which is consistent with the general lack of response of the cyclic AMP-dependent processes in hypothyroidism in fat cells.

Kaslow *et al.* (21) reported that methoxamine had no effect on glycogen synthase and concluded that adrenergic control of glycogen synthase is exclusively through β-adrenergic receptors. However, Lawrence and Larner reported that the pure α-adrenergic agonist methoxamine inactivates glycogen synthase I (6), which we confirmed. Adipocytes from hypothyroid animals are especially useful to show the α-adrenergic regulation of adipose tissue

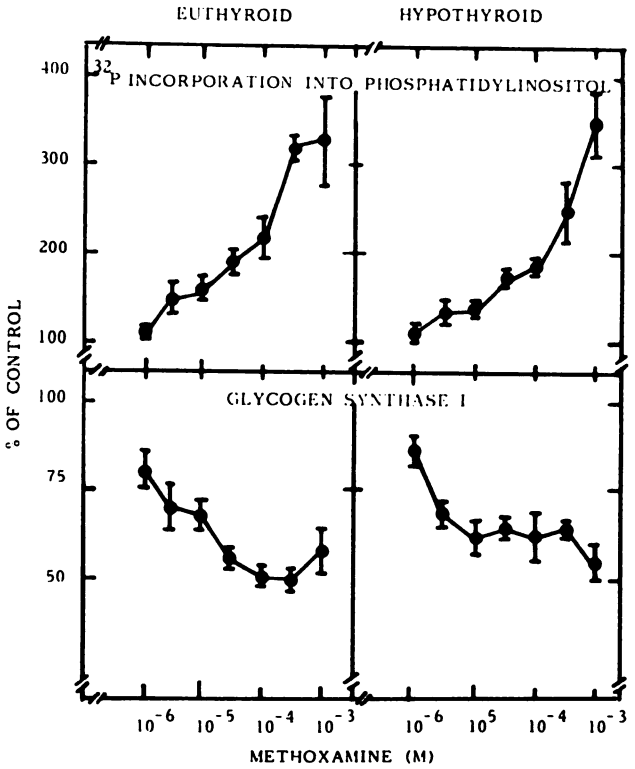

FIG. 3. *Effects of methoxamine on the incorporation of [^{32}P]P_i into phosphatidylinositol and glycogen synthase I activity*

Fat cells ($2–3 \times 10^6$) were incubated in the absence or presence of methoxamine. Basal values for phosphatidylinositol specific activity were 4430 ± 820 and 6100 ± 520 cpm/μg inorganic phosphate in cells obtained from euthyroid and hypothyroid animals, respectively. Basal values for glycogen synthase activity and total glycogen synthase activity are given in Table 5. Bars represent the SEM of at least three determinations performed on different days.

TABLE 2

Effects of α-adrenergic agents on the incorporation of [^{32}P]P$_i$ into phosphatidylinositol and on the activity of glycogen synthase I in isolated fat cells from euthyroid rats

To measure the incorporation of phosphate into phosphatidylinositol, cells were incubated with the agents for 60 min in a medium containing 10 μCi/ml [^{32}P]P$_i$. Basal phosphatidylinositol specific activity was 4585 ± 535 cpm/μg phosphate as the mean ± SEM of three experiments. Glycogen synthase I was assayed in cells incubated in a medium containing 10 mM glucose. Adrenergic antagonists were added 4 min and agonists 2 min before the incubation was terminated. Total glycogen synthase activity was 6.22 ± 0.93 nmol/min × 10^6 cells as the mean ± SEM of four experiments performed in duplicate.

Additions	Phosphatidylinositol specific activity	Glycogen synthase I activity
	% of control	*nmol/min × 10^6 cells*
None	100	0.78 ± 0.01
Methoxamine (100 μM)	250 ± 25*	0.66 ± 0.02*
Methoxamine (100 μM) + (±)-propranolol (30 μM)	673 ± 45**	0.66 ± 0.04****
Methoxamine (100 μM) + prazosin (1 μM)	130 ± 10	0.80 ± 0.03
Clonidine (100 μM)	92 ± 8	0.75 ± 0.03
(±)-Propranolol (30 μM)	275 ± 40***	0.72 ± 0.02
Prazosin (1 μM)	105 ± 5	0.72 ± 0.01

* $P < 0.005$ compared to basal.
** $P < 0.001$ compared to basal.
*** $P < 0.01$ compared to basal.
**** $P < 0.025$ compared to basal.

glycogen synthase I since they are refractory to β-adrenergic activation.

The α-adrenergic actions of epinephrine and methoxamine on phosphatidylinositol labeling and glycogen synthase activity are very sensitive to the selective α_1-adrenergic antagonist prazosin, suggesting that both actions are due to activation of the same type of α adrenoreceptor, i.e., α_1. These data and the fact that the inactivation of glycogen synthase by α-adrenergic amines is dependent on the presence of extracellular calcium (5, 6) are consistent with, although do not prove, the hypothesis that the activation of α_1 adrenoreceptors in adipocytes produces, through phosphatidylinositol turnover, an increase in cytosol calcium that leads to an inactivation of glycogen synthase.

The present data support the hypothesis that α_1 adrenoceptors mediate effects secondary to an elevation of intracellular calcium and involve an increased turnover of phosphatidylinositol (22). In contrast, α_2 adrenoceptors mediate effects secondary to a calcium-independent inactivation of adenylate cyclase (22). There is no α_2-

TABLE 3

Effect of hexoses on glycogen synthase I activity in isolated fat cells from euthyroid and hypothyroid rats

Fat cells were incubated for 2–5 min with hexose. The reaction was terminated and glycogen synthase I assayed. Total glycogen synthase activity was 6.23 ± 0.24 and 5.03 ± 0.36 nmol/min × 10^6 cells in adipocytes from euthyroid and hypothyroid rats, respectively. Values are the mean ± SEM of four determinations in each case.

Hexose	Glycogen synthase I activity			
	Euthyroid		Hypothyroid	
	Activity[a]	% of control	Activity[a]	% of control
None	0.38 ± 0.04	100	0.18 ± 0.02*	100
Glucose (10 mM)	0.56 ± 0.08	147	0.40 ± 0.06**	222
2-Deoxyglucose (10 mM)	1.44 ± 0.02**	379	0.74 ± 0.08**	411

[a] nmol/min × 10^6 cells.
* $P < 0.005$ compared to euthyroid basal activity.
** $P < 0.05$ compared to its respective control activity.

TABLE 4

Effect of adrenergic agonists and antagonists on glycogen synthase I activity of fat cells obtained from euthyroid rats

Fat cells were both obtained by enzymatic digestion of parametrial fat pads and incubated in a medium containing 10 mM glucose. Adrenergic antagonists were added 4 min and agonists 2 min before the incubation was terminated. Total glycogen synthase activity was 7.15 ± 0.72. Values are the mean ± SEM of four experiments performed in duplicate.

Addition	Glycogen synthase I activity	
	nmol/min × 10^6 cells	% of control
None	0.78 ± 0.02	100
Epinephrine (10 μM)	0.49 ± 0.01*	63
Epinephrine (10 μM) + (−)-propranolol (30 μM)	0.61 ± 0.01*	78
Epinephrine (10 μM) + prazosin (1 μM)	0.53 ± 0.03*	68
Epinephrine (10 μM) + (−)-propranolol (30 μM) + prazosin (1 μM)	0.81 ± 0.02	104
Isoproterenol (10 μM)	0.52 ± 0.02*	67
Isoproterenol (10 μM) + (−)-propranolol (30 μM)	0.70 ± 0.04	90
Isoproterenol (10 μM) + prazosin (1 μM)	0.45 ± 0.03*	58
(−)-Propranolol (30 μM)	0.75 ± 0.03	96
Prazosin (1 μM)	0.75 ± 0.02	96
(−)-Propranolol (30 μM) + prazosin (1 μM)	0.77 ± 0.03	99

* $P < 0.001$ compared to basal activity.

TABLE 5

Effect of insulin and adrenergic agonists on glycogen synthase I activity of fat cells obtained from euthryoid and hypothyroid rats

Fat cells were both obtained by enzymatic digestion of parametrial fat and incubated in a medium containing 10 mM glucose. After 2 min with the hormone, the incubation was terminated and glycogen synthase assayed. Total glycogen synthase was 8.18 ± 0.65 and 7.57 ± 0.54 nmol/min × 10^6 cells in adipocytes from euthyroid and hypothyroid rats, respectively. Values are the mean ± SEM of five experiments performed in duplicate.

Addition	Glycogen synthase I activity			
	Euthyroid		Hypothyroid	
	Activity[a]	% of control	Activity[a]	% of control
None	0.79 ± 0.09	100	0.40 ± 0.03*	100
Insulin (2.5 mU/ml)	1.47 ± 0.09**	186	0.44 ± 0.04	110
Isoproterenol (10 μM)	0.47 ± 0.09**	59	0.37 ± 0.03	93
Epinephrine (10 μM)	0.43 ± 0.02**	54	0.23 ± 0.02**	58

[a] nmol/min × 10^6 cells.
* $P < 0.005$ compared to basal euthyroid activity.
** $P < 0.05$ compared to its respective control activity.

adrenergic inhibition of cyclic AMP accumulation in rat adipocytes, in contrast to hamster adipocytes in which there is a marked inhibition of adenylate cyclase by α_2-adrenergic amines (22).

Kunos (23) has suggested that thyroid hormones modulate the interconversion of α and β adrenoreceptors via allosteric transitions of a single basic structure. No direct evidence exists to support this suggestion. Our results do not support this interconversion hypothesis since it was found that α_1-adrenergic sensitivity was not increased in adipocytes from hypothyroid rats. Previously, Malbon *et al.* (24) observed that hypothyroidism increased the sensitivity of hepatocytes to β-adrenergic agonists without modifying that to α-catecholamines. In brief, thyroid hormones appear to modulate β-adrenergic responses in adipocytes and hepatocytes in an opposite fashion without any reciprocal change in α_1-adrenergic responses.

Lawrence *et al.* (25) reported that glucose increased glycogen synthase I activity in isolated rat adipocytes. We confirm this finding and the observation that 2-deoxyglucose is more potent than glucose in stimulating synthase I (26). Since glycogen synthase I activity is decreased in adipocytes from hypothyroid rats compared to fat cells from euthyroid animals while the total activity remains unchanged, it is the state of activation rather than the total amount of enzyme which is affected by the hypothyroid condition.

Glycogen synthase I activity of adipocytes from hypothyroid cells can be increased by hexoses and decreased by α-adrenergic amines, suggesting that the machinery necessary to modulate glycogen metabolism is present. However, insulin was unable to activate the enzyme in these cells. Lawrence *et al.* (25, 26) found that insulin and glucose activate glycogen synthase I and that glucose potentiates the effect of insulin in rat fat cells. The activation of glycogen synthase I by insulin and glucose involves increased glucose transport and phosphorylation (26). In the present experiments glycogen synthase was assayed in cells that had been incubated with glucose, therefore it is not possible to determine if the disappearance of the insulin effect in adipocytes from hypothyroid rats is due to a defect in the insulin receptor, glucose transport, or phosphorylation. In our hands, the activation of glycogen synthase by insulin alone in cells from euthyroid rats has been too small to allow a comparison with that in cells from hypothyroid rats (unpublished data). Further experiments are required to clarify this point.

In conclusion, the response to β-adrenergic amines is markedly decreased in fat cells from hypothyroid rats as reflected by cyclic AMP accumulation, lipolysis, and glycogen synthase I activity. The response to insulin is also decreased in adipocytes from hypothyroid rats as reflected by glycogen synthase I activity. In contrast, α_1-adrenergic action is not affected by hypothyroidism. The activation of α_1 adrenoceptors in fat cells increases the incorporation of $[^{32}P]P_i$ into phosphatidylinositol and inactivates glycogen synthase I.

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