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The Effects of Thyroid Status on the Modulation of Fat Cell β-Adrenergic Receptor Agonist Affinity by Guanine Nucleotides

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SUMMARY

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The influence of thyroid status *in vivo* on the ability of guanine nucleotides to affect the affinity of fat cell β-adrenergic receptors for an (−)agonist is explored in the present study. In euthyroid rat fat cell membranes, 100 μM GTP or Gpp(NH)p induces a reduction in the affinity of specific (−)[^{3}H]dihydroalprenolol binding sites for isoproterenol, but not propranolol. The kinetics of the effect of 100 μM Gpp(NH)p were rapid, achieving near-steady-state levels within 10 min at 22°C. One micromolar Gpp(NH)p or 5 μM GTP (in the presence of a nucleotide regenerating system) induced half-maximal reduction in the affinity of specific (−)[^{3}H]dihydroalprenolol binding sites for isoproterenol. One hundred micromolar concentrations of either guanine nucleotide produced a maximal effect. The ability of Gpp(NH)p to reduce agonist affinity of the binding sites was shown to be readily reversed by simple washing of the membranes. The affinity of specific (−)[^{3}H]dihydroalprenolol binding sites of fat cell membranes for (−)isoproterenol was reduced in the hypothyroid state. Half-maximal inhibition of specific (−)[^{3}H]dihydroalprenolol binding (at 10 nM radioligand) occurred at 7 μM isoproterenol in hypothyroid, as compared to 1 μM isoproterenol in euthryoid, rat fat cell membranes. In hyperthyroid rat fat cell membranes, only 0.4 μM isoproterenol was required to half-maximally inhibit the specific binding of (−)[^{3}H]dihydroalprenolol. In the presence of 50 μM Gpp(NH)p the concentration of isoproterenol required for half-maximal inhibition of specific binding in euthyroid rat fat cell membranes was increased from 1 to 7 μM. Neither 100 μM GTP nor Gpp(NH)p influenced the affinity of specific (−)[^{3}H]dihydroalprenolol binding sites for isoproterenol in fat cell membranes obtained from hypo- or hyperthyroid rats. These data suggest that thyroid hormones can modulate β-adrenergic receptor affinity for agonists (but not antagonists) and the ability of guanine nucleotides to regulate agonist (but not antagonist) affinity in fat cells.

INTRODUCTION

Thyroid hormones modulate catecholamine action in a variety of tissues including heart (1–3), liver (4), and adipose tissue (5–11). In adipose tissue, hypothyroidism impairs catecholamine-stimulated adenosine 3′:5′-monophosphate (cyclic AMP) accumulation and lipolysis (5–11). Cyclic AMP accumulation in response to even maximal concentrations of catecholamines is dramatically reduced in fat cells isolated from either thyroidectomized (9) or chemically induced hypothyroid (11) rats. Examination of adenylate cyclase activity of both fat cell ghosts (11) and purified plasma membranes (9) obtained from hypothyroid rats demonstrated a reduction in the maximal response to catecholamine stimulation yet no change in the amount of fluoride-activatable activity. Although hypothyroidism has been shown to increase the activity of particulate, high-affinity form of cyclic AMP phosphodiesterase in fat cells (8, 10), the phosphodiesterase activity of fat cell ghosts (11) and highly purified membranes (9) prepared from the hypothyroid rat was shown to be equivalent to that of euthyroid rat preparations.

We recently explored the possibility that hypothyroidism may be modulating fat cell catecholamine-stimulated adenylate cyclase via alteration in the number or affinity of β-adrenergic receptors. Our studies demonstrated that the number and affinity of β-adrenergic receptors in fat cell plasma membranes were not altered by hypothyroidism (11). Since no alteration either in the number or the

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affinity of specific (−)[^{3}H]dihydroprenolol binding sites or in the amount of fluoride-activatable adenylate cyclase activity of fat cell membranes by hypothyroidism was apparent, a reduction in the "coupling" efficiency between these components was proposed as an explanation for the blunted catecholamine response (11).

Recognizing that guanine nucleotides and nucleosides were capable of regulating the hormonal activation of adenylate cyclase systems has been an important advance in the understanding of adenylate cyclase systems (for a recent review, see Ref. 12). However, attempts to restore the reduced catecholamine response of the hypothyroid rat fat cell ghosts adenylate cyclase by preincubation with the guanine nucleotide analogue, guanyl-5′-yl-imidodiphosphate (GppNHp), were unsuccessful (11).

Guanine nucleotides have also been shown to specifically decrease the affinity of β-adrenergic receptors for agonists in a number of systems (13–17), including the fat cell (17). The present study explores the influence of thyroid status on the ability of guanine nucleotides to modulate fat cell β-adrenergic receptor agonist affinity. The results presented here demonstrate that thyroid status alters both the affinity of fat cell β-adrenergic receptors for agonists (but not antagonists) and the ability of guanine nucleotides to decrease β-receptor affinity for agonists.

EXPERIMENTAL PROCEDURES

Materials. The (−)[^{3}H]dihydroalprenolol (specific activity, 30–50 Ci/mmol) was obtained from New England Nuclear Corp. Guanyl-5′-yl-imidodiphosphate (GppNHp) and guanosine-5′-triphosphate (GTP) were obtained from Boehringer Mannheim. The (−)-, (+)-, and (±)-propranolol hydrochlorides were gifts from Ayerst. The (−)-isoproterenol and (−)-3,3′,5-triiodothyronine (T_3) were obtained from Sigma. All other materials were obtained from standard commercial suppliers or from sources previously identified (11).

Animals. Female Sprague–Dawley rats (Charles River CD strain) weighing 175–200 g were utilized in the studies described. Rats were rendered hypothyroid by maintenance on an iodine-deficient diet (No. 17700, United States Biochemical Corp.) and drinking water containing 0.00625% 6-*N*-propyl-2-thiouracil for 21 days. Control rats were littermates or rats of the same weight as the experimental rats and were maintained on tap water and the same test diet to which normal iodine had been added by the commercial supplier. Hyperthyroid rats were rats maintained on the control diet and subcutaneously administered 30 μg of T_3/100 g body weight daily for 5 days.

Cell isolation and membrane preparation. White fat cells were obtained by collagenase digestion of parametrial adipose tissue according to the procedure of Rodbell (18). Pooled adipose tissue (about 40 g) from 8–12 rats was minced with scissors and placed in small plastic bottles. Each bottle, containing 8 g of tissue and 8 ml of Krebs–Ringer phosphate buffer containing 3% albumin (Armour Pharmaceutical, bovine serum albumin lot No. P56607) and 1 mg/ml of crude collagenase (*Clostridium histolyticum*, Worthington Biochemical Corp., lot No. CLS 45 A145), was incubated for 45–60 min at 37°C. The Krebs–Ringer phosphate buffer contained 128 mM NaCl, 1.4 mM $CaCl_2$, 1.4 mM $MgSO_4$, 5.2 mM KCl, and 10 mM Na_2HPO_4. The albumin buffer was made fresh daily and the pH adjusted to 7.4 with NaOH after the addition of the albumin. At the end of the incubation, cells were filtered through a single layer of nylon chiffon and washed twice with the albumin buffer.

Fat cell membranes were immediately prepared from the isolated cells according to a modification of the method of Williams *et al.* (19) as previously described (11). In all experiments fat cell membranes were used the same day without prior freezing.

β-Adrenergic receptor binding assay. Binding of ligands to fat cell β-adrenergic receptors was assessed through study of competition for (−)[^{3}H]dihydroalprenolol binding as described elsewhere (11, 19). Specific binding of this radioligand is defined as that component of the (−)[^{3}H]dihydroalprenolol binding which is sensitive to competition by 10 μM (±)-propranolol (11, 19, 20). Approximately 70–80% of the radioligand binding to fat cell membranes was specific (data not shown). All of the binding studies described in this report were performed in a 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$ buffer. This same buffer was used for membrane washing and dilution where indicated.

Protein determination. Protein was assayed according to the method of Lowry *et al.* (21), using crystalline bovine serum albumin (Fraction V) as a standard.

RESULTS

Specific (−)[^{3}H]dihydroalprenolol [(−)[^{3}H]DHA] binding to fat cell membranes (11, 19) or to intact fat cells (22) displays properties consistent with those of physiological fat cell β-adrenergic receptors. As shown in Table 1, the amount of specific (−)[^{3}H]DHA binding inhibited by 0.1 or 1 μM (−)-isoproterenol in fat cell membranes was reduced when the incubation mixture

TABLE 1

Effects of Gpp(NH)p and GTP on the binding of (−)[^{3}H]DHA to fat cell membranes

Total binding, specific binding (binding which is sensitive to competition by 10^{-5} M (±)-propranolol), and binding sensitive to 10^{-7} and 10^{-6} M (−)-isoproterenol were assayed using 10 nM (−)[^{3}H]DHA in the absence or presence of either Gpp(NH)p (10^{-4} M) or GTP (10^{-4} M) as described under Experimental Procedures. The binding assay was performed with 300 μg of membrane protein for a period of 12 min at 37°C. The data are expressed as the mean values ± SEM of triplicate determinations from a single representative experiment.

Agents added	Guanine nucleotide added		
	None	10^{-4} M Gpp(NH)p	10^{-4} M GTP
	(−)[^{3}H]DHA bound to membranes, fmol/mg of protein		
None (total binding)	119 ± 2	112 ± 3	109 ± 4
+ 10^{-5} M (±)-propranolol (specific binding)	28 ± 10 (91)	22 ± 2 (90)	26 ± 3 (83)
+ 10^{-7} M (−)-isoproterenol (% inhibition of specific binding)	93 ± 1 (28%)	102 ± 2 (11%)	106 ± 4 (3%)
+ 10^{-6} M (−)-isoproterenol (% inhibition of specific binding)	73 ± 4 (50%)	96 ± 7 (18%)	112 ± 9 (0%)

included 100 μM GTP. A similar reduction in the ability of isoproterenol to inhibit specific (−)[^{3}H]DHA binding was noted when the incubation was performed in the presence of the guanine nucleotide analogue, guanyl-5′-yl-imidodiphosphate, Gpp(NH)p, at 100 μM (Table 1). No appreciable change in the amount of either total or specific (−)[^{3}H]DHA binding to fat cell membranes was observed in the presence of 100 μM GTP or Gpp(NH)p (Table 1). The affinity of these binding sites for the potent β-adrenergic antagonist (−)-propranolol was likewise unaffected by these guanine nucleotides (data not shown). Interestingly, ATP or ITP (100 μM) failed to reduce the ability of isoproterenol (1 μM) to inhibit specific (−)[^{3}H]DHA binding to fat cell membranes, in either the presence or the absence of a nucleotide regenerating system consisting of 10 mM creatine phosphate and 5 units/ml of creatine phosphokinase (data not shown). These data suggest that guanine nucleotides, such as GTP or Gpp(NH)p, diminish the ability of a β-adrenergic agonist, but not antagonist, to inhibit specific (−)[^{3}H]-DHA binding to fat cell membranes.

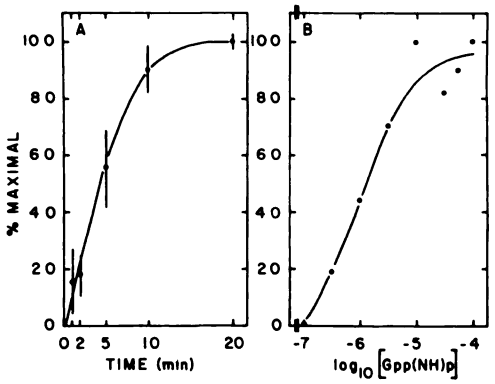

FIG. 1. *Effects of Gpp(NH)p on (−)-isoproterenol (1 μM) displacement of specific (−)-[^{3}H]DHA binding of fat cell membranes*

(A) Kinetics of the ability of 100 μM Gpp(NH)p to reduce displacement of specific (−)[^{3}H]DHA binding of fat cell membranes by 1 μM (−)-isoproterenol. Fat cell membranes (250–300 μg of protein) and 10 nM (−)[^{3}H]DHA were incubated at 22°C for 20 min. Specific (−)[^{3}H]DHA binding had attained steady-state levels under these conditions. Gpp(NH)p (100 μM final assay concentration) was then added to one-half of the tubes and the incubation at 22°C continued. Total and specific (−)[^{3}H]DHA binding and the amount of specific (−)[^{3}H]DHA binding displaced by 1 μM (−)-isoproterenol were assayed in triplicate at the point of addition of the Gpp(NH)p (time = 0 min) and at 1, 2, 5, 10, and 20 min after, in the sets of tubes both containing and lacking 100 μM Gpp(NH)p. The amount of specific (−)[^{3}H]DHA binding displaced by 1 μM (−)-isoproterenol was reduced from 54 ± 9 to 36 ± 5% by the presence of 100 μM Gpp(NH)p at 20 min after addition (maximal effect). Total and specific (−)[^{3}H]DHA bindings were unaffected by the Gpp(NH)p. The data are expressed as the means ± SEM from three separate experiments performed on separate days. (B) Dose–response of the ability of Gpp(NH)p to reduce displacement of specific (−)[^{3}H]DHA binding of fat cell membranes by 1 μM (−)-isoproterenol. Total and specific (−)[^{3}H]DHA binding and the amount of specific (−)[^{3}H]DHA binding inhibited by 1 μM (−)-isoproterenol were assayed in triplicate in tubes containing fat cell membranes (250–300 μg of protein), 10 nM (−)[^{3}H]DHA, and nil or varying concentrations of Gpp(NH)p. Tubes were incubated for 20 min at 22°C, after which period the binding was assayed. The amount of specific binding inhibited by 1 μM (−)-isoproterenol was reduced from 55 to 38% by the presence of 100 μM Gpp(NH)p (maximal effect). The data are expressed as the means of four separate experiments performed on as many days.

The kinetics of this effect of guanine nucleotides on β-adrenergic agonist inhibition of specific (−)[^{3}H]DHA binding to fat cell membranes were examined (Fig. 1A). Since the steady-state level of (−)[^{3}H]DHA binding to fat cell membranes has been shown to decline following 12 min of incubation at 37°C (11, 20), kinetic studies and all subsequent studies were performed at 22°C. At 22°C steady-state binding is achieved within 20 min and maintained for at least another 40 min (20). The kinetics of the effect of 100 μM Gpp(NH)p on isoproterenol (1 μM) inhibition of specific (−)[^{3}H]DHA binding to fat cell membranes were rapid, reaching near-steady-state levels within 10 min at 22°C (Fig. 1A). This effect was maintained with no appreciable decline for an additional 20 min at this temperature (data not shown).

The concentration dependence of this effect of Gpp(NH)p on agonist inhibition of the binding is shown in Fig. 1B. The maximal effect was achieved at approximately 10 μM Gpp(NH)p under these conditions. Half-maximal reduction in isoproterenol (1 μM) inhibition of specific (−)[^{3}H]DHA binding to fat cell membranes was obtained with ~1 μM Gpp(NH)p (Fig. 1B). The concentration of GTP required to achieve half-maximal reduction of agonist inhibition of the binding was approximately 5 μM when performed in the presence of a nucleotide regenerating system (data not shown).

It was of interest to determine if this effect of Gpp(NH)p on the ability of isoproterenol to inhibit specific (−)[^{3}H]DHA binding to fat cell membranes was a reversible process. As shown in Table 2, the ability of 1 μM isoproterenol to inhibit specific (−)[^{3}H]DHA binding was not reduced in membranes which were incubated with 50 μM Gpp(NH)p for 20 min, washed, and then resuspended in fresh buffer for the radioligand binding assay. In addition, exposure of the fat cell membranes previously incubated with Gpp(NH)p, washed, and then resuspended in fresh buffer to 50 μM Gpp(NH)p resulted in a reduction in the amount of specific binding inhibited by 1 μM isoproterenol (Table 2). These data suggest that this action of Gpp(NH)p, reducing the ability of the agonist isoproterenol to inhibit specific binding, is a reversible process.

Thyroid status *in vivo* has been shown to regulate fat cell catecholamine-stimulated adenylate cyclase activity with no apparent alteration in the number or affinity of specific binding sites for (−)[^{3}H]DHA (11) or in the amount of fluoride-activatable adenylate cyclase activity in particulate, broken-cell preparations (9, 11). In fat cell membranes prepared from euthyroid rats, 50 μM Gpp(NH)p reduced the affinity of (−)[^{3}H]DHA binding sites for isoproterenol (Fig. 2, left-hand panel). The guanine nucleotide shifted the dose–response curve for isoproterenol inhibition of specific (−)[^{3}H]DHA binding to the right, with a parallel displacement. Half-maximal inhibition of specific binding by isoproterenol occurred at 1 μM in the absence of Gpp(NH)p and 7 μM in the presence of this guanine nucleotide. What influence, if any, thyroid status might have upon the ability of guanine nucleotides to modulate fat cell β-adrenergic receptor agonist affinity was next explored.

In agreement with our previous findings (11), specific (−)[^{3}H]DHA binding to fat cell membranes was found to

TABLE 2

Gpp(NH)p-induced reduction in the ability of (−)-isoproterenol to inhibit specific (−)[^{3}H]DHA binding to fat cell membranes: Reversibility

Fat cell membranes were prepared and diluted to 4–6 mg of protein/ml in 50 mM Tris–HCl, pH 7.5 at 22°C, 5 mM $MgCl_2$, and incubated with or without 50 μM Gpp(NH)p for 20 min at 22°C. Following this incubation, the fat cell membranes were washed twice by sevenfold dilution with the same buffer and subsequent centrifugation at 10,000*g* for 10 min at 4°C. The final pellets were resuspended in 50 mM Tris–HCl, pH 7.5 at 22°C, 5 mM $MgCl_2$, and the binding of 10 nM (−)[^{3}H]-DHA to these membranes was assayed after 20 min at 22°C. The data are expressed as the mean values ± SEM from four separate experiments performed on separate days. The amount of binding inhibited by 10 μM (±)-propranolol represents specific binding (11). The amounts of binding sensitive to inhibition by 1 μM (−)-isoproterenol or 1 μM (−)-isoproterenol in the presence of 50 μM Gpp(NH)p are provided.

Agent	Fat cell membranes preincubated for 20 min at 22°C with buffer alone	Fat cell membranes preincubated for 20 min at 22°C with buffer containing 50 μM Gpp(NH)p
	Amount of (−)[^{3}H]DHA binding inhibited by the indicated agent, fmol/mg of protein	
10 μM (±)-propranolol	76 ± 11	89 ± 8
1 μM (−)-isoproterenol	44 ± 7	60 ± 9
1 μM (−)-isoproterenol + 50 μM Gpp(NH)p	17 ± 6	27 ± 14

be unaffected by altered thyroid status. Specific binding at 10 nM radioligand in these experiments was 97 ± 8, 88 ± 8, and 81 ± 12 fmol/mg of protein in fat cell membranes obtained from euthyroid, hypothyroid, and hyperthyroid rats, respectively. However, as shown in Fig. 2, the affinity of these (−)[^{3}H]DHA binding sites for isoproterenol as assessed by competition studies was reduced by hypothyroidism, and increased by hyperthyroidism, as compared to the euthyroid state (Fig. 2). Half-maximal inhibition of specific (−)[^{3}H]DHA binding to fat cell membranes was achieved at 1 μM isoproterenol in the euthyroid rat preparation, 7 μM isoproterenol in the hypothyroid rat preparation, and 0.4 μM in the hyperthyroid rat membranes (Fig. 2).

In the presence of 50 μM Gpp(NH)p, the affinity of the specific (−)[^{3}H]DHA binding sites for isoproterenol in fat cell membranes from euthyroid rats was reduced (Fig. 2, left-hand panel); however, in the hypothyroid state, the ability of guanine nucleotides to induce a reduction in agonist affinity was absent (Fig. 2, center panel). Even in the presence of 100 μM Gpp(NH)p, this same condition was noted (data not shown). The curves for concentration-dependent isoproterenol inhibition of specific (−)[^{3}H]DHA binding to fat cell membranes from hypothyroid rats in the absence or presence of Gpp(NH)p were superimposable and were virtually identical to those for isoproterenol inhibition of the binding in euthyroid rat fat cell membranes performed in the presence of this guanine nucleotide (Fig. 2, left-hand and center panels).

The ability of guanine nucleotides to influence the affinity of fat cell membrane (−)[^{3}H]DHA binding sites for isoproterenol was likewise lost in the hyperthyroid state (Fig. 2, right-hand panel). The affinity of specific (−)[^{3}H]DHA binding sites for isoproterenol in fat cell membranes from hyperthyroid rats was the same in both the absence and the presence of 50 μM Gpp(NH)p. In the presence of Gpp(NH)p, the affinity of the receptor sites of hyperthyroid rat fat cell membranes for isoproterenol was 15- to 20-fold higher than that of fat cell membranes obtained from euthyroid or hypothyroid rats (Fig. 2). Additional binding studies performed under the same incubation conditions for an incubation period of 12 min at 37°C yield the same data as that shown for the studies described above which were performed at 22°C for a period of 20 min.

The polyene antibiotic filipin has been shown to reduce the coupling between the β-adrenergic receptor and adenylate cyclase in the frog erythrocyte membranes (23). Similarly, Howlett *et al.* (24) reported that the ability of guanine nucleotides to decrease the affinity of β-adrenergic receptors of S49 lymphoma cell membranes for agonists was lost by treating the membranes with filipin (50 μg/ml). The similarity between the effects of hypothyroidism in the fat cell system and the effects of filipin in the S49 lymphoma cell system on guanine nucleotide induced agonist-specific shifts in β-adrenergic receptor affinity prompted us to investigate the effects of filipin on the fat cell membrane β-receptor. Curiously, specific (−)[^{3}H]DHA binding to fat cell membranes, the affinity of these sites for isoproterenol (as assessed by binding inhibition studies), and the ability of guanine nucleotides to reduce the affinity of specific (−)[^{3}H]DHA binding sites for isoproterenol were each unaffected by exposure of the fat cell membranes to filipin at concentrations as high as 100 μg/ml (data not shown).

DISCUSSION

In agreement with a recent report by Giudicelli *et al.* (17), the present study demonstrates that β-adrenergic receptors of fat cell membranes display a decreased affin-

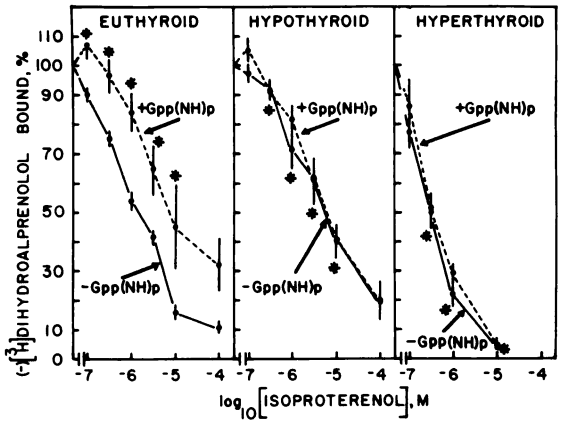

FIG. 2. *Effects of Gpp(NH)p on the ability of (−)-isoproterenol to inhibit specific (−)[^{3}H]DHA binding to fat cell membranes obtained from euthyroid, hypothyroid, or hyperthyroid rats*

Competition for specific (−)[^{3}H]DHA binding sites by (−)-isoproterenol (0.1 to 100 μM) in the absence or presence of 50 μM Gpp(NH)p was assayed with 10 nM radioligand and 250–400 μg of membrane protein at 22°C after 20 min of incubation in triplicate. The data are expressed as mean values ± SEM from four to seven separate experiments performed on as many days with different membrane preparations. (*) $P < 0.05$ versus euthyroid without Gpp(NH)p. Statistical analysis was done by unpaired *t* analysis.

ity for (−)agonists, but not antagonists, in the presence of GTP or Gpp(NH)p. Maguire *et al.* (13, 14) first observed that guanine nucleotides specifically decreased the affinity of β-adrenergic receptors of cultured rat glioma cells and human fibroblasts for (−)agonists. Frog erythrocyte membranes (15) and purified membranes obtained from S49 lymphoma cells (16) also display agonist-specific shifts in affinity of β-adrenergic receptors in the presence of GTP or Gpp(NH)p. The affinity of β-adrenergic receptors of turkey erythrocyte membranes for (−)agonists, in contrast, does not appear to be modulated by guanine nucleotides, suggesting that this effect may not be universal (25).

Several properties of the agonist-specific reduction in affinity of fat cell β-adrenergic receptors induced by guanine nucleotides are identified in the present study. The effect of Gpp(NH)p on the affinity of fat cell β-receptors for isoproterenol was shown to be reversible (Table 2). Ross *et al.* (16) reported that the ability of this GTP analogue to induce an agonist-specific shift in β-adrenergic receptor affinity was reversible in S49 lymphoma cell plasma membranes. The kinetics of this effect of guanine nucleotide on the fat cell β-receptor were moderately fast at 22°C, achieving maximal levels within about 10 min (Fig. 1A). The concentration of guanine nucleotide required for half-maximal reduction of agonist affinity of β-adrenergic receptor in fat cell membranes was about 1 μM for Gpp(NH)p and 5 μM for GTP (Fig. 1B). Concentrations of either ITP or ATP as high as 100 μM failed to mimic the action of guanine nucleotides on agonist affinity of the fat cell β-adrenergic receptors.

Fat cell membranes obtained from hypothyroid, as compared to euthyroid, rats did not display an agonist-specific shift in affinity of β-adrenergic receptors in the presence of Gpp(NH)p (Fig. 2). Binding of (−)agonist to β-adrenergic receptors of hypothyroid rat fat cell membranes in either the presence or the absence of Gpp(NH)p was identical to the binding of (−)agonists to β-adrenergic receptors of euthyroid rat fat cell membranes displayed in the presence of Gpp(NH)p. A reduction in the affinity of β-adrenergic receptors of fat cell membranes obtained from hypothyroid as compared to euthyroid rats for the β-agonist isoproterenol was observed in our previous report (see Fig. 8, Ref. 11). Since this reduction in the affinity of the β-adrenergic receptor for isoproterenol was of insufficient magnitude to explain the severely blunted cyclic AMP response of the hypothyroid rat fat cells to β-agonist stimulation, the significance of this observation was not appreciated (11). Only as the result of the present investigations aimed at exploring guanine nucleotide effects on β-adrenergic receptor affinity of fat cell membranes prepared from animals of altered thyroid states was the significance of this observation clarified.

Fat cell membranes from hyperthyroid rats also failed to display an agonist-specific shift in the affinity of β-adrenergic receptors in the presence of Gpp(NH)p (Fig. 2). The affinity of the β-receptors for isoproterenol was higher in the hyperthyroid, as compared to the euthyroid, state. It would appear that in fat cells, thyroid hormones modulate two aspects of β-adrenergic receptor character. Thyroid hormones can modulate the affinity of fat cell β-adrenergic receptors for an agonist such as isoproterenol, hyperthyroidism increasing and hypothyroidism decreasing this affinity. Second, thyroid hormones appear to modulate the ability of guanine nucleotides to induce agonist specific shifts in β-adrenergic receptor affinity, hyperthyroidism and hypothyroidism leading to an apparent loss of this phenomenon. In the heart, in contrast to adipose tissue, thyroid hormones have been reported to modulate the number of β-adrenergic receptors (26, 27). Perhaps thyroid hormones also modulate the action of guanine nucleotides on the β-adrenergic receptor-coupled adenylate cyclase system of the heart and other tissues.

Haga *et al.* (28) identified a novel variant clone of S49 lymphoma which fails to respond to β-adrenergic agonists, although membranes prepared from these cells display no alteration (as compared to wild type) in basal or NaF-stimulated adenylate cyclase activity or in the amount of β-adrenergic receptor sites. These investigators have designated this novel variant as UNC, for "uncoupled" (28). We speculated earlier that the hypothyroid rat fat cell and UNC cells may be analogous systems (11). Membranes from both variants (UNC and hypothyroid rat fat cells) display altered adenylate cyclase responses to catecholamine stimulation, although possessing the same number of β-adrenergic receptors and amount of fluoride-stimulated adenylate cyclase activity as in their normal counterparts (28, 11).

Several other striking similarities exist between the UNC cells and hypothyroid rat fat cells. Membranes from UNC cells and hypothyroid rat fat cells display an impaired response of adenylate cyclase to activation by Gpp(NH)p as compared to wild-type and euthyroid rat fat cell membranes, respectively (28, 11). β-Adrenergic receptors of membranes from either UNC cells or hypothyroid rat fat cells (unlike those from their wild-type or euthyroid counterparts, respectively) fail to display agonist-specific shifts in affinity in the presence of guanine nucleotides (16, 28, present study). The β-adrenergic receptors of hypothyroid rat fat cell membranes, like those from UNC cell membranes, display agonist binding in the absence of guanine nucleotides which is indistinguishable from that observed in euthyroid rat membranes [or in wild-type membranes with respect to UNC cell membranes (16)] in the presence of guanine nucleotides (Fig. 2). In addition, cholera toxin stimulation of either UNC cell (16) or hypothyroid rat fat cell (29) adenylate cyclase activity appears to be quite similar to that of S49 lymphoma wild-type and euthyroid rat fat cells, respectively. Recent studies of the cholera toxin-catalyzed labeling patterns of plasma membrane proteins, using [^{32}P]NAD^+ as substrate, reveal no differences between UNC and wild-type S49 cell membranes (30) or between hypothyroid and euthyroid rat fat cell ghosts (29). The reduced response of the adenylate cyclase of hypothyroid as compared to euthyroid rat fat cell membranes to epinephrine (9, 11, 29) and guanine nucleotides (11, 29) does not appear to be the result of a deficiency in the M_r 42,000 protein which is specifically ADP-ribosylated in response to cholera toxin (putative GTP-binding protein) (29). Perhaps hypothyroidism produces a modification of other as yet unidentified protein or lipid components of the fat cell membrane which participate

in the interaction between the β-adrenergic receptors and the guanine nucleotide-binding protein(s) which regulate adenylate cyclase activity. Fat cells from the hypothyroid rats would appear, on the basis of these additional data, to be very similar to UNC S49 lymphoma cells, displaying a reduced "coupling" of signal transduction from hormone receptor to adenylate cyclase.

Fat cell membranes prepared from hyperthyroid rats possess β-adrenergic receptors which display a greater affinity for isoproterenol than those of fat cell membranes from the euthyroid rat. Curiously, a guanine nucleotide-induced reduction in receptor affinity for isoproterenol was lost in this hyperthyroid state. Why both hypothyroidism and hyperthyroidism lead to a loss in the guanine nucleotide sensitivity of fat cell β-adrenergic receptor agonist affinity remains obscure. Elucidating the biochemical basis for these effects of thyroid status on β-adrenergic receptor affinity, the receptor-adenylate cyclase coupling process, and the actions of guanine nucleotides will clearly require much further study.

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