Fat Cell Adenylate Cyclase and β-Adrenergic Receptors in Altered Thyroid States*

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The lipolytic sensitivity of fat cells from hypothyroid rats to epinephrine is severely blunted although 100 μM epinephrine stimulates lipolysis to the same level demonstrated by fat cells from euthyroid rats. Hypothyroidism shifts the lipolytic log-dose response curve to epinephrine to the right by a factor of 10. Epinephrine produced no detectable increase in adenosine 3′:5′-monophosphate (cyclic AMP) accumulation in fat cells from hypothyroid rats. In contrast, fat cells from hyperthyroid rats accumulated more cyclic AMP in response to epinephrine than did those from euthyroid rats.

Fat cell ghosts prepared from hypothyroid rats display reduced catecholamine-stimulated adenylate cyclase activity, although the maximal catalytic activity of fat cell ghost adenylate cyclase as measured in the presence of 10 mM sodium fluoride is the same as that of the euthyroid. Fat cell ghosts from hypothyroid rats were incubated with the guanine nucleotide analog, guanylyl-5′-yl imidodiphosphate to attempt to restore the reduced catecholamine-stimulated adenylate cyclase activity. This preincubation with guanylyl-5′-yl imidodiphosphate activated the adenylate cyclase and increased catecholamine-stimulated activity of ghosts prepared from fat cells of both euthyroid and hypothyroid rats but did so to a much greater extent in the fat cell ghosts from euthyroid rats. Fat cell ghosts from hypothyroid rats inactivated in the presence of a maximal concentration of guanylyl-5′-yl imidodiphosphate displayed 37 ± 3% less adenylate cyclase activity than the fat cell ghosts obtained from euthyroid animals. Cyclic AMP phosphodiesterase activity of fat cell ghosts preincubated with or without guanylyl-5′-yl imidodiphosphate, when measured at 0.125 μM cyclic AMP, was the same in ghosts from hypothyroid rats as in those from normal rats. Similarly, 1-methyl-3-isobutyl xanthine (1 mM) did not rectify the reduced response of adenylate cyclase of the fat cell ghosts from hypothyroid rats to catecholamine stimulation.

β-Adrenergic receptors were examined in membranes prepared from isolated fat cells obtained from hyperthyroid, hypothyroid, and euthyroid rats. Putative β-adrenergic receptors were identified with the use of the potent, β-adrenergic antagonist, (-)-[3H]dihydroalprenolol. Specific binding of (-)-[3H]dihydroalprenolol to fat cell membranes was rapid, reversible, and saturated at 50 nM. Scatchard plots of specific binding were curvilinear with upward convexity; Hill plots yield coefficients of 0.7. Competition studies display stereospecificity and a potency order of (−)-agonists (isoproterenol ≫ epinephrine = norepinephrine) indicative of a β-adrenergic receptor. Calculating from membrane binding data, there appear to be approximately 10^6 β-adrenergic receptors/fat cell. Maximum specific binding of (-)-[3H]dihydroalprenolol/mg of membrane protein was the same for fat cell membranes prepared from hypothyroid, hyperthyroid, and euthyroid rats. Scatchard plots of binding studies performed with 1 to 100 nM (−)-[3H]dihydroalprenolol were nearly identical for fat cell membranes prepared from the three different groups, each displaying curvilinearity with an upward convexity. β-Adrenergic agonists compete for (−)-[3H]dihydroalprenol binding with the same potency when measured in fat cell membranes of euthyroid or hypothyroid rats. The total number of β-adrenergic receptors per fat cell was the same for fat cells obtained from hyperthyroid, hypothyroid, and control rats.

These data suggest that thyroid hormones alter neither the maximum catalytic activity of adenylate cyclase nor the number and affinity of putative β-adrenergic receptors of the fat cell. Thyroid hormones, thus, may exert their influence on fat cells by regulating the transduction of information between hormone receptors and adenylate cyclase.

Debons and Schwartz (1) reported that the stimulation by epinephrine of free fatty acid release in vitro was impaired in adipose tissue from rats rendered hypothyroid by 6-N-propyl-2-thiouracil administration in vivo. The reduction in catecholamine-stimulated lipolysis in fat pads, tissue, and isolated cells of hypothyroid rats is now well documented (2-6). Correze et al. (5) reported a reduction in the maximal responses of adenylate cyclase in fat cell membranes to adrenocorticotropin hormone, glucagon, or epinephrine following thyroidectomy.
Armstrong et al. (4), in contrast, reported the activation by hormones and fluoride of adenylate cyclase of fat cell ghost preparations to be the same in hypothyroid and control preparations. These investigators reported, in addition, increased activity of particulate, high affinity forms of adenosine 3':5'-monophosphate phosphodiesterase in fat cells from hypothyroid rats (4, 6).

One mechanism by which thyroid hormones could influence hormone-stimulated lipolysis in the fat cell is via modulation of hormone receptor number or affinity (or both). Clarridge and Mariotti (7) and Williams et al. (8) recently reported increases in the number of β-adrenergic receptors in the heart by thyroid hormone treatment in vivo. The present study was designed to examine the influence of thyroid status on fat cell adenylate cyclase and β-adrenergic receptor number and affinity.

### MATERIALS AND METHODS

Female Sprague-Dawley rats (Charles River CD strain) weighing 175 to 200 g were utilized in this study. Control animals were fed laboratory rodent chow and ad libitum and were rendered hypothyroid as above and then given 30 μg of T₃/100 g, or the enzyme preparation. Reactions were initiated by additions of enzyme to the reaction tubes maintained at 37°C. The incubation period was generally 15 min in length and was terminated by boiling for 1 min. A 0.1-ml aliquot of the boiled samples was then added and the mixture was incubated at 37°C for 10 min. The labeled adenosine thus formed was separated from labeled cyclic AMP by addition of 1 ml of 1:3 (w/w) slurry of washed Dowex 1-X8 (200 to 400 mesh) containing 25 mg/100 mg of adenosine and inosine.

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creasing concentrations of (+) and (−)-propranolol (see Fig. 5). Specific binding of (−)-[3H]DHA (is thus defined as the total binding less binding remaining in presence of 10 μM (±)-propranolol. Non-
specific binding was generally between 20 and 30% of the total
counts bound. Less than 0.5% of the total counts filtered bound to
the filters and this binding was virtually insensitive to competition by
β-adrenergic agonists or antagonists.

The fat cells were counted in aliquots taken from the fat cell
suspension diluted with butter containing albumin. A 0.5-μl aliquot of
the dilute suspension containing 50 to 150 fat cells was placed on a
microscope slide which had a grid pattern etched on the opposite
side. The slide was carefully inverted and the cells counted by visual
inspection with a light microscope. The procedure was repeated two
more times.

(−)-[3H]Dihydralpranolol (specific activity 32.6 Ci/mmol) and
[3H]-labeled cyclic AMP (35 Ci/mmol) were obtained from New
England Nuclear Corp. The (−) and (+) and (±)-propranolol
hydrochlorides were gifts from Ayerst. The (−)-stereoisomers, bitar-
trate form, of norepinephrine, epinephrine, and isoproterenol were
obtained from Winthrop Laboratories. The (−)-stereoisomers, bitar-
trate form, of norepinephrine, epinephrine, and isoproterenol were
obtained from Sigma. The bitartrate had no effect on either total or
specific binding of (−)-[3H]DHA to these membranes (data not
shown). The stock solutions of β-adrenergic agonists and antagonists
were made fresh daily and contained 200 μM metabisulfite to retard
oxidation. Metabisulfite had no effect on either total or specific
binding of (−)-[3H]DHA to these membranes. The guanine nucleo-
tide analog, guanyl-5'-y1 imidodiphosphate, was purchased from
ICN.

RESULTS

Fat cells isolated from hypothyroid rats (maintained on an
iodine-deficient diet and drinking water containing 0.00625%
PTU) demonstrated no detectable stimulation of cyclic AMP
accumulation over basal in response to epinephrine at concon-
trations as high as 100 μM (Fig. 1). Epinephrine-stimulated
lipolysis was clearly blunted in the fat cells from these
animals. The dose dependence for epinephrine-stimulated
lipolysis is shifted to the right by approximately one order of
magnitude, although no cyclic AMP accumulation was noted
even at 100 μM epinephrine.

Fig. 1 further shows that fat cells from hyperthyroid rats
(administered T3), in contrast to the hypothyroid animals,
display an enhanced accumulation of cyclic AMP in response
to epinephrine. The maximal level of epinephrine-stimulated
lipolysis was lower in fat cells of the hyperthyroid rat. Admin-
istration of T3 to hypothyroid animals restored the lipolytic
response of these fat cells to epinephrine. The T3 treatment
actually increased cyclic AMP accumulation in response to
epinephrine beyond the control levels to that displayed by fat
cells from rats administered T3 only. The pool of cyclic AMP
represented by this increased accumulation appears to be
"surplus" cyclic AMP which cannot further increase lipolysis.
Cyclic AMP accumulation in fat cells from hypothyroid ani-
imals was not detectably increased by 100 μM epinephrine.
However, 100 μM epinephrine produced the same lipolytic
response in these fat cells as in those of control animals.

Having confirmed that the in vitro lipolytic response of fat
cells to epinephrine can be modified by altering thyroid status
in vivo, the question as to the role of adenylate cyclase and of
β-adrenergic receptors in this altered catecholamine sensitiv-
ity was addressed. Fat cell ghosts were prepared by hypotonic
lysis of isolated fat cells obtained from hypothyroid and
 euthyroid (control) rats. Fat cell ghosts from hyperthyroid

* Some of the data (including Figs. 3 to 5 and Tables 1 to III) are
presented as a miniprint supplement immediately following the
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money order for $1.35 per set of photocopies.

PTU-treated) display a reduction in the maximal response of
adenylate cyclase to norepinephrine as compared to euthyroid
(control) rats (Fig. 2), although sodium fluoride-stimulated
adenylate cyclase activity was 3.6 nmol/mg of protein/20 min
for the fat cell ghosts prepared from euthyroid rats and 4.1
nmol/mg of protein/20 min for those from hypothyroid rats.
This confirms the observation of Correze et al. (5) that purified
fat cell membranes from thyroidectomized rats display re-
duced maximal stimulation of adenylate cyclase by catecho-
amines, although sodium fluoride-stimulated activity of these
membranes is equal to that of those obtained from euthyroid
rats.

The guanine nucleotide analog Gpp(NH)p activates fat cell
adenylate cyclase (17) and enhances the activation of the
cyclase by catecholamines and other lipolytic hormones (18,
19). Recent reports suggest Gpp(NH)p may increase the "cou-
pling" between β-adrenergic hormone receptor occupancy and
activation of adenylate cyclase (20, 21). We explored the
possibility of restoring the reduced catecholamine-stimulated
adenylate cyclase activity of the hypothyroid rat fat cell ghosts
by prior incubation with Gpp(NH)p. Ghosts prepared from fat
cells of euthyroid and hypothyroid rats were incubated for 60
P-Adrenergic receptors and adenylate cyclase in fat cells

Euthyroid rats with and without prior incubation of 50 

Phosphodiesterase activity measured at 0.125 min at 4° with and without 1, 5, or 50 

Gpp(NH)p. These conditions allow for the lag period noted with Gpp(NH)p activation of adenylate cyclase (18, 19, 22, 23). As further shown in Fig. 2, both activation of adenylate cyclase and the potentiation of norepinephrine-stimulated activity by Gpp(NH)p was reduced in fat cell ghosts from hypothyroid rats when compared to the response of fat cell ghosts from euthyroid rats. Additional experiments were performed in which fat cell ghosts were incubated with a maximal concentration of Gpp(NH)p (100 

M) under the adenylate cyclase assay conditions for a period of 20 min at 37°. The Gpp(NH)p-stimulated adenylate cyclase activity was 38 ± 3% (n = 4) lower in the fat cell ghosts prepared from hypothyroid rats than in those prepared from the euthyroid rats, although sodium fluoride-stimulated activity was identical in both preparations (data not shown).

Low Kₘ cyclic AMP phosphodiesterase activity was measured in the same fat cell ghosts used in the adenylate cyclase assay of Fig. 2 (i.e. incubated at 4° for 60 min with and without Gpp(NH)p). As shown in Table I, cyclic AMP phosphodiesterase activity measured at 0.125 

M substrate was the same in fat cell ghosts prepared from hypothyroid and euthyroid rats with and without prior incubation of 50 

M Gpp(NH)p. As further shown in Table I, 1 mM 1-methyl-3-isobutyl xanthine almost completely inhibited the cyclic AMP phosphodiesterase activity of fat cell ghosts prepared from euthyroid and hypothyroid rats. This same concentration of 1-methyl-3-isobutyl xanthine failed to rectify the disparity in both the norepinephrine-stimulated adenylate cyclase activity and its potentiation by 50 

M Gpp(NH)p between control and hypothyroid preparations (Table II). Corrêa et al. (5) reported that the reduced maximal response of fat cell membrane adenylate cyclase from thyroideactomized rats to epinephrine, adrenocorticotropic hormone, or glucagon could not be normalized to control levels by the presence of theophylline. These data suggest that hypothyroidism reduces lipolytic hormone-stimulation of adenylate cyclase but not its activation by fluoride. In addition, this reduction in hormone-stimulated adenylate cyclase activity is not a reflection of increased cyclic AMP phosphodiesterase activity in purified fat cell membranes from thyroideactomized rats (5) or fat cell ghosts prepared from PTU-treated, hypothyroid rats (present study).

P-Adrenergic receptors were next examined. An in vitro assay for the identification and characterization of P-adrenergic receptors has been reported utilizing the high specific activity labeled P-adrenergic antagonist, [3H]-iodohydroxybenzylpindolol (24) or (-)[3H]dihydralprenolol (25). Williams et al. (15) recently identified and characterized P-adrenergic receptors in fat cell membranes utilizing (-)[3H]DHA. (-)[3H]DHA was used in the present study in an attempt to probe the number and character of putative P-adrenergic receptors in membranes prepared from fat cells obtained from animals with altered thyroid status.

Binding of (-)[3H]DHA to the fat cell membranes was a rapid process at 37° and reached equilibrium within 2 min (Fig. 3A). The binding was saturated at approximately 80 nM (-)[3H]DHA (Fig. 3B). The maximum binding capacity in this experiment was 0.5 pmol of (-)[3H]DHA bound/mg of protein of fat cell membrane. Half-maximal saturation was attained at 19 nM, an approximation of the equilibrium dissociation constant (Kₐ) for this interaction of (-)[3H]DHA with its binding site(s).

Additional saturation studies were performed and analyzed by the method of Scatchard (26) (Fig. 4). Scatchard plots of the binding of (-)[3H]DHA to fat cell membranes were uniformly curvilinear with an upward concavity in each of the 10 separate experiments analyzed by this procedure. Transformation of these data to a Hill plot is shown in the inset to Fig. 4. The Hill coefficient, n_H, was 0.7, less than unity. A simple interpretation of these data would suggest negative cooperative site-to-site interaction among the binding sites or the existence of multiple populations of binding sites of differing affinities. The Kₐ derived from the Hill transformation was 19 nM which is the same value obtained from Fig. 3B. However, the Hill coefficients and curvilinear Scatchard plot indicate these values are, at best, only approximations of the true affinity of this (these) binding site(s) for the radioligand.

As shown in Fig. 5, (-)-propranolol, a potent P-adrenergic antagonist, produced half-maximal inhibition of (-)[3H]DHA binding to fat cell membranes at 50 nM. A final concentration of ~700 nM (+)-propranolol was required to produce this same inhibition, a demonstration of the stereospecificity of the binding process. The specificity of the binding of (-)[3H]DHA to fat cell membrane was further examined by competition studies utilizing P-adrenergic agonists (Fig. 6). The concentration of (+)-isoproterenol required for half-maximal inhibition of (-)[3H]DHA binding was almost 3 orders of magnitude higher than that of (-)-isoproterenol. The potency order of the (-)-P-adrenergic agonists for competition with (-)[3H]DHA binding to fat cell membranes was (+)-isoproterenol >> (-)-norepinephrine = (-)-epinephrine, suggesting, according to Lands et al. (27), a B₁-type adrenergic receptor. These data, confirming the study of Williams et al. (15), suggested that the number and affinity of P-adrenergic receptors in membranes prepared from fat cells of euthyroid, hypothyroid, and hyperthyroid rats could be assessed utilizing this methodology.

As shown in Table III, hypothyroid animals displayed a reduction in mean body weight and a concomitant increase in...
Specific binding of \( (-)^{-}[\text{H}]\text{DHA} \) to freshly prepared fat cell membranes from these animals is also shown in Table III. Specific binding of \( (-)^{-}[\text{H}]\text{DHA} \) measured at 10 and 100 nM expressed in femtomoles bound/mg of protein was statistically the same in fat cell membranes prepared from hypothyroid, hyperthyroid, and euthyroid rats. Calculating specific binding of \( (-)^{-}[\text{H}]\text{DHA} \) on a per cell basis from cell counts and membrane binding data suggests again that neither the \( T_3 \) treatment nor the PTU treatment in vivo significantly alters the total number of \( (-)^{-}[\text{H}]\text{DHA} \) binding sites/fat cell over the control value measured at a saturating concentration of \( (-)^{-}[\text{H}]\text{DHA} \).

Additional binding studies utilizing fat cell membranes prepared and frozen prior to use were performed with \( (-)^{-}[\text{H}]\text{DHA} \) at 12 concentrations yielding from 5 to 70% occupancy under equilibrium conditions. The results of these studies analyzed by the method of Scatchard (26), are shown in Fig. 7. Scatchard plots of specific binding of \( (-)^{-}[\text{H}]\text{DHA} \) to fat cell membranes prepared from hypothyroid (PTU-treated) and hyperthyroid (\( T_3 \)-treated) rats were curvilinear displaying upward concavity. Although the nonlinear nature of these plots prohibit accurate assessment of the total number of \( (-)^{-}\text{DHA} \) binding sites, it is obvious from these data that the apparent density of binding sites is the same over this range of \( (-)^{-}[\text{H}]\text{DHA} \) concentrations shown in Table IV. Fat cell membranes prepared from all three groups display essentially equivalent specific binding capacities for \( (-)^{-}[\text{H}]\text{DHA} \) over this range of concentrations.

**TABLE IV**

Specific binding of \( (-)^{-}[\text{H}]\text{dihydroalprenolol} \) to fat cell membranes prepared from rats of altered thyroid status

Data are taken from separate experiments performed on separate days, two separate paired experiments for all three groups, and an additional separate paired experiment for the control and \( T_3 \)-treated animals. The data are expressed as the mean values ± standard error of the mean for the control and \( T_3 \)-treated groups, and ± one-half the range for the PTU-treated groups. Each separate experiment measured the specific binding at each concentration of ligand in triplicate.

| Final concentration of \( (-)^{-}[\text{H}]\text{dihydroalprenolol} \) in assay (nM) | Specific binding of \( (-)^{-}[\text{H}]\text{dihydroalprenolol} \) from following source of fat cell membranes: | Control (euthyroid) | PTU-treated (hypothyroid) | \( T_3 \)-treated (hyperthyroid) |
|---|---|---|---|---|
| 1 | 20 ± 6 | 16 ± 3 | 28 ± 8 |
| 5 | 52 ± 17 | 34 ± 3 | 59 ± 28 |
| 10 | 71 ± 9 | 67 ± 6 | 78 ± 30 |
| 20 | 108 ± 28 | 75 ± 26 | 91 ± 19 |
| 30 | 95 ± 4 | 124 ± 33 |
| 50 | 151 ± 13 | 131 ± 18 | 148 ± 31 |
| 70 | 213 ± 52 | 210 ± 25 | 332 ± 209 |
| 80 | 180 ± 27 | 212 ± 25 | 263 ± 123 |
| 90 | 227 ± 27 | 240 ± 76 | 231 ± 90 |
| 100 | 222 ± 37 | 236 ± 23 | 261 ± 68 |

This paper confirms the observations that hypothyroidism reduces the lipolytic response of adipose tissue (1-3) and isolated fat cells (4, 6) to submaximal concentrations of catecholamines and that this reduced lipolytic response of fat cells from hypothyroid rats is restored to control levels by administration of \( T_3 \) (6). Cyclic AMP accumulation in isolated fat cells in response to epinephrine could not be detected in the cells from hypothyroid rats and was higher than controls for cells from both the \( T_3 \)-treated groups and the hypothyroid rats subsequently treated with \( T_3 \). Correze et al. (5) reported a reduction in the maximal response of adenylate cyclase to catecholamines in purified fat cell membranes obtained from rats following thyroidectomy. This group of investigators further demonstrated that the amount of fluoride-activatable adenylate cyclase activity of fat cell membranes obtained from thyroidectomized rats was equal to that of preparations obtained from euthyroid rats. We confirmed these observations utilizing chemically induced hypothyroid rats and a fat cell ghost preparation rather than purified fat cell membranes.

Incubating fat cell ghosts obtained from euthyroid rats with Gpp(NH)p and activated adenylyl cyclase and enhancing the stimulation of this enzyme by norepinephrine. However, this treatment failed to restore the reduced catecholamine-stimulated adenylate cyclase activity of fat cell ghosts from hypothyroid rats to the euthyroid level. In addition, incubating fat cell ghosts obtained from hypothyroid rats with a maximal concentration of this guanine nucleotide also failed to stimulate adenylate cyclase activity to the level demonstrated by the fat cell ghosts from the euthyroid rats. Thus, the reduced response of the hypothyroid rat preparations to Gpp(NH)p may be a reflection of an alteration of the guanine nucleotide activating component of the adenylyl cyclase which may, in turn, influence hormone stimulation. Alternatively, it may reflect an alteration at some common point through which both catecholamines and guanine nucleotides activate the fat cell adenylyl cyclase.

It has been suggested that thyroid hormones modulate hormone-dependent lipolysis in fat cells via regulation of the activity of low \( K_a \) microsomal (5) or particulate (4, 6) cyclic AMP phosphodiesterase (or both). Armstrong et al. (4) re-
ported that a particulate low $K_m$ cyclic AMP phosphodiesterase activity was elevated in fat cells from chemically induced hypothyroid rats. In the present study, cyclic AMP phosphodiesterase activity measured at 0.125 $\mu M$ substrate was identical in hypothyroid and euthyroid rat fat cellghost preparations. Thus, the reduced catecholamine-stimulated cyclase activity of the fat cell ghosts from hypothyroid rats was not due to increased phosphodiesterase activity in these preparations. A similar observation was made by Correze et al. (5) using purified fat cell membranes from thyroidectomized rats. Consistent with this, 1 mM 1-methyl-3-isobutyl xanthine, which inhibits phosphodiesterase activity nearly 100%, did not restore the reduced catecholamine-stimulated adenylate cyclase response of the hypothyroid preparations. These data agree with the report that theophylline was ineffective in restoring the reduced response of fat cell membrane adenylate cyclase activity of thyroidectomized rats to epinephrine, glucagon, or adrenocorticotrophic hormone (5). Both the results of the present study and the observations of Correze et al. (5) demonstrate that although fluoride-stimulated adenylate cyclase activity is unchanged by hypothyroidism, hormone-sensitive activity is reduced. These data conflict with observations of Armstrong et al. (4). The reason for this discrepancy is unclear.

Confirming the work of Williams et al. (15) specific binding of (−)[3H]dihydroalprenolol to fat cell membranes is a rapid, saturable process displaying stereospecificity and a potency order of a $\beta_1$-type adrenergic receptor (27). Calculations derived from saturation studies with fat cell membranes suggest there are approximately 100,000 specific binding sites for (−)[3H]dihydroalprenolol per fat cell.

Utilizing (−)[3H]dihydroalprenolol Ciaraldi and Marinietti (7) reported a 2-fold increase in $\beta$-adrenergic receptors in ventricular membranes prepared from the hearts of rats administered 75 $\mu g$ of thyroxine/100 g, body weight, daily for 7 days, and a 30% reduction in $\beta$-adrenergic receptors in membranes prepared from rats administered 0.1% PTU in their drinking water for 10 days. Similarly, Williams et al. (8) reported an increase in the number of $\beta$-adrenergic receptors of myocardiurn from hyperthyroid rats. In the present study, specific binding of (−)[3H]dihydroalprenolol per mg of protein was shown to be the same for fat cell membranes prepared under identical conditions from hypothyroid, hyperthyroid, and euthyroid rats. Assuming the recovery of $\beta$-adrenergic receptors is the same for fat cells obtained from each group, this data would suggest that in fat cells at least, thyroid hormones do not appear to alter the number of $\beta$-adrenergic receptors per mg of membrane protein or per cell.

Scatchard analysis of the binding of (−)[3H]DHA yielded curvilinear plots with an upward concavity for membranes prepared from euthyroid, hypothyroid, and hyperthyroid animals. The density of these binding sites per mg of protein in fat cell membranes was the same in all three groups (Figs. 4 and 7). Since the apparent affinity of the binding sites decreases with increasing occupancy of the binding population, it is not possible to assess the $K_m$ of the different membranes for (−)[3H]dihydroalprenolol. The fact that fat cell membranes of each group display almost identical plots suggests that within the limits of this assay no statistically significant change in affinity or number of putative $\beta$-adrenergic receptors is demonstrable in altered thyroid states.

The possibility exists that antagonists such as (−)[3H]DHA may not reflect change in $\beta$-adrenergic receptors in fat cell membranes. To address this question competition experiments with (−)-isoproterenol were performed on fat cell membranes from euthyroid and hypothyroid rats. The ability of (−)-isoproterenol to compete with (−)[3H]DHA for binding to fat cell membranes was nearly identical in membranes from euthyroid and hypothyroid rats. Although catecholamine-stimulated lipolysis is severely blunted in the hypothyroid animals, within the limits of our assay system, there is no apparent alteration in the number, affinity, or character of (−)[3H]DHA binding sites (putative $\beta$-adrenergic receptors).

In summary, our data show that the influence of thyroid hormones upon catecholamine-stimulated adenylate cyclase of the fat cell do not involve alterations in either the amount of fluoride-activatable adenylate cyclase or the number and affinity of $\beta$-adrenergic receptors. Recent evidence suggest the $\beta$-adrenergic receptor component and the enzyme component of the adenylate cyclase complex are separable macromolecular entities. Limbird and Lefkowitz (28) reported separation of frog erythrocyte membrane $\beta$-adrenergic receptors from the adenylate cyclase enzyme following solubilization of the membrane. Insel et al. (20) have reached a similar conclusion based on genetic manipulations which suggest the $\beta$-adrenergic receptor and adenylate cyclase are products of separate genes. In addition, Haga et al. (30) have recently identified an S49 lymphoma variant clone in which the $\beta$-adrenergic receptor and adenylate cyclase are present but functionally "unequipped." The hypothyroid rat fat cell may be an analogous situation. Thyroid hormones may exert their influence on information transduction between the hormone receptors and adenylate cyclase. This would provide an explanation for the blunted response of fat cells from hypothyroid rats to several other lipolytic hormones, since it has been shown that the maximal response of adenylate cyclase to glucagon and adrenocorticotrophic hormone is also reduced in purified fat cell membranes from thyroidectomized rats (5).

Rajerison et al. (31) proposed a similar role for adrenal steroids and their influence on vasopressin-sensitive adenylate cyclase of the rat kidney medulla. These investigators suggested adrenal steroids regulate the synthesis of a component which is involved in receptor-enzyme coupling. Thus, thyroid hormones may regulate the action of lipolytic hormones in the fat cell by modulating the so-called "coupling" between the hormone receptor and adenylate cyclase.

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