Short-term hyperthyroidism modulates adenosine receptors and catalytic activity of adenylate cyclase in adipocytes

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The effects of short-term hyperthyroidism *in vivo* on the status of the components of the fat-cell hormone-sensitive adenylate cyclase were investigated. The number of β -adrenergic receptors was elevated by about 25% in membranes of fat-cells isolated from hyperthyroid rats as compared with euthyroid rats, but their affinity for radioligand was unchanged. Membranes of hyperthyroid-rat fat-cells displayed less than 65% of the normal complement of receptors for [³H]cyclohexyladenosine. The affinity of the receptors for this ligand was normal. In contrast with the marked increase in the amounts of the α -subunits of the guanine nucleotide-binding proteins G_i (M_r 41 000) and G_o (M_r 39 000) observed in the hypothyroid state [Malbon, Rapiejko & Mangano (1985) *J. Biol. Chem.* **260**, 2558–2564], the amounts of α - G_i , α - G_o as well as α - G_s subunits [M_r 42 000 (major) and 46 000/48 000 (minor)] were not changed by hyperthyroidism. Adenylate cyclase activity in response to forskolin, guanosine 5'-[γ -thio]triphosphate or isoprenaline, in contrast, was decreased by 30–50% in fat-cell membranes from hyperthyroid rats. Fat-cells isolated from hyperthyroid rats accumulated cyclic AMP to less than 50% of the extent in their euthyroid counterparts in the presence of adenosine deaminase and either adrenaline or forskolin, suggesting a decrease in the amount or activity of the catalytic subunit of adenylate cyclase. In the absence of exogenous adenosine deaminase, cyclic AMP accumulation in response to adrenaline was elevated rather than decreased in fat-cells from hyperthyroid rats. The inhibitory influence of adenosine is apparently limited in the hyperthyroid state by the decreased complement of inhibitory R-site purinergic receptors in these fat-cells. Short-term hyperthyroidism modulates the fat-cell adenylate cyclase system at the receptor level (β -receptor number increased, R-site purinergic-receptor number decreased) and the catalytic subunit of adenylate cyclase.

INTRODUCTION

The permissive effects of thyroid hormones are manifest by a change in the sensitivity of many tissues to stimulation by hormones such as insulin and catecholamines. Thyroid hormones regulate catecholamine action in heart (McNiell & Brody, 1968), liver (Malbon *et al.*, 1978c; Malbon & Greenberg, 1982), skeletal muscle (Chu *et al.*, 1985) and fat tissue (Debons & Schwartz, 1961). The biochemical mechanisms through which thyroid hormones elicit their permissive effects on cellular responsiveness to a variety of other hormones is not fully understood. The effect of thyroid hormones on β -adrenergic hormone action has been extensively studied in a variety of systems. Thyroid hormones appear to be capable of regulating β -adrenergic hormone action by regulating the number and function of β -adrenergic receptors (for review see Stiles *et al.*, 1984). The β -adrenergic-receptor-coupled adenylate cyclase system is regulated at other levels also, including G_s (Stiles *et al.*, 1981; Malbon & Greenberg, 1982), G_i (Malbon *et al.*, 1985), and the catalytic moiety (Malbon & Greenberg, 1982).

In addition to modulating receptor-mediated stimu-

latory control of adenylate cyclase, thyroid hormones can also regulate cellular responses to inhibitory hormones. Receptor-mediated inhibitory control of intracellular cyclic AMP concentrations is modulated by α_2 -adrenergic receptors (Steer & Wood, 1979; Aktories *et al.*, 1979; Sabol & Nirenberg, 1979; Jakobs, 1979) and cholinergic muscarinic receptors (Hazeki & Ui, 1981). In rat fat-cells, adenosine and its purine-modified analogues such as N^6 -phenylisopropyladenosine (PIA) or N^6 -cyclohexyladenosine (CHA) act via R-site adenosine receptors to inhibit cyclic AMP accumulation and lipolysis (Fain, 1973; Fain & Weiser, 1975; Trost & Stock, 1977; Trost & Schwabe, 1981). Hypothyroidism has been shown to alter specifically receptor-mediated inhibitory control of adenylate cyclase in the rat fat-cell (Malbon *et al.*, 1985).

Hyperthyroidism is associated with an enhanced stimulation of lipolysis and of cyclic AMP accumulation by adrenaline in adipose tissue and adipocytes (Deykin & Vaughn, 1963; Ichikawa *et al.*, 1971; Malbon *et al.*, 1978a). The effects of thyroid hormones in this system have not been characterized in sufficient detail to allow understanding of the biochemical details underlying this phenomenology. In this paper, we investigate the

Abbreviations used: G_s , the guanine nucleotide-binding stimulatory regulatory binding protein of adenylate cyclase; G_i , the guanine nucleotide-binding inhibitory regulatory protein of adenylate cyclase; G_o , a similar guanine nucleotide-binding protein of unknown function; *cyc*[−], S49 mouse lymphoma mutant cells which lack functional α -subunits of G_s ; CHA, N^6 -cyclohexyladenosine; PIA, N^6 -phenylisopropyladenosine; ICYP, iodoctanopindolol.

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influence of short-term treatment with tri-iodothyronine (hyperthyroidism) on components of the hormone-sensitive adenylate cyclase of the rat fat-cell.

EXPERIMENTAL

Animals

Fed female Sprague-Dawley (SD strain) rats weighing 150–175 g were used in these studies. Rats were rendered hyperthyroid by subcutaneous administration of 25 µg of (–)-3,3',5-tri-iodothyronine/100 g body wt. daily for 5 days. Control rats were littermates or rats of the same weight as the experimental animals.

Isolation of cells

White fat-cells were isolated by collagenase digestion of parametrial adipose tissue by the method of Rodbell (1964) as described previously (Malbon *et al.*, 1978a,b).

Cyclic AMP assay

Cyclic AMP accumulation by rat fat-cells was measured as described by Czech *et al.* (1980).

Preparation of plasma membranes

Fat-cell membranes used in the adenylate cyclase, *cyc*[–] reconstitution and radioligand-binding assays were prepared by the method of Williams *et al.* (1976). Highly purified plasma membranes used in the bacterial-toxin-catalysed labelling were prepared as described by McKeel & Jarett (1970) and stored at –80 °C before use. In all cases, membranes were prepared concurrently from euthyroid- and hyperthyroid-rat fat-cells.

Adenylate cyclase assay

Freshly prepared fat-cell membranes (30 µg) were incubated at 30 °C in a final volume of 0.1 ml containing 50 mM-Hepes (pH 8.0), 25 mM-Tris/HCl, 11 mM-MgCl₂, 10 mM-phosphocreatine, 2 mM-2-mercaptoethanol, 1 mM-cyclic AMP, 0.3 mM-ATP containing 1–2 µCi of [³²P]ATP, creatine kinase (2.0 mg/ml), bovine serum albumin (1.0 mg/ml), 0.4 mM-EGTA, and the indicated concentrations of (–)-isoprenaline or forskolin. The assay was initiated by the addition of membranes and was terminated after 10 min by the addition of 100 µl of a solution containing 2% SDS, 1.4 mM-cyclic AMP and 40 mM-ATP. The cyclic AMP generated was isolated and quantified by the method of Salomon *et al.* (1974). Assays were routinely performed in triplicate.

Assay of functional G_s activity

G_s activity of detergent extracts of fat-cell membranes was measured by its ability to reconstitute the adenylate cyclase system of S49 mouse lymphoma *cyc*[–] mutant cell membranes which lack functional G_s, by the method of Ross & Gilman (1977), as modified by Codina *et al.* (1984). Cholate extracts were treated with *N*-ethylmaleimide to inactivate the catalytic moiety before reconstitution analysis (Malbon *et al.*, 1984a).

Adenosine-receptor binding

Adenosine receptors were measured by radioligand-binding analysis and *N*⁶-cyclohexyl[³H]adenosine. Specific binding of radioligand to R₁ receptors was defined as the binding sensitive to competition by 50 nM-PIA. Membranes (75–110 µg of protein) were incubated and

Fig. 1. Binding of [³H]CHA to fat-cell membranes prepared from euthyroid and hyperthyroid rats

Fat-cell membranes freshly prepared from euthyroid (○) and hyperthyroid (●) rats were incubated for 20 min at 22 °C with increasing concentrations of radioligand. Specific binding is defined as that portion of the total [³H]CHA binding sensitive to competition by 50 nM-PIA. Inset shows Scatchard analysis of the binding data. The data represent mean values ± S.E.M. from three separate experiments performed on as many occasions: *differs significantly from control (*P* < 0.05).

receptor binding was assayed as described previously (Malbon *et al.*, 1985).

Bacterial-toxin-catalysed labelling

Membranes (100 µg of protein) were incubated in the presence of either cholera toxin or pertussis toxin and [³²P]NAD⁺ and then subjected to SDS/polyacrylamide-gel electrophoresis as described by Malbon *et al.* (1985). The gels were fixed and stained with Coomassie Brilliant Blue, destained, dried, and exposed to X-ray film. Electrophoresis and autoradiography were performed as described previously (Malbon *et al.*, 1984a,b).

Quantification of ³²P incorporated into G_s and G_i/G_o

The incorporation of ³²P into G_s and G_i/G_o by bacterial-toxin-catalysed labelling was quantified by the method of Lai *et al.* (1981).

Protein determination

Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Fig. 2. Polyacrylamide-gel analysis of G-proteins after cholera-toxin- and pertussis-toxin-catalysed ADP-ribosylation of membrane proteins from euthyroid- and hyperthyroid-rat fat-cells

Membranes (100 μ g) were incubated in the presence of [32 P]NAD $^{+}$ (0.1 mM) and either activated pertussis toxin (100 μ g/ml) or cholera toxin (100 μ g/ml). The membranes were solubilized and then subjected to SDS/polyacrylamide-gel electrophoresis and autoradiography as described in the Experimental section. Highly purified fat-cell membranes from the euthyroid rat were treated without (lane 1) or with pertussis toxin (lane 3) or cholera toxin (lane 5), and those from the hyperthyroid rat were treated without (lane 2) or with pertussis toxin (lane 4) or cholera toxin (lane 6). The autoradiogram (2 h exposure) is representative of results obtained with at least five separate membrane preparations performed on as many occasions. M_r values of standard proteins are also shown.

Materials

Pertussis toxin was purchased from List Biological Laboratories. All other reagents were obtained as described elsewhere (Malbon *et al.*, 1985).

RESULTS

The effects of short-term hyperthyroidism *in vivo* on the status of adenosine receptors and β -adrenergic receptors were assessed by equilibrium binding techniques using [3 H]CHA and [125 I]iodocyanopindolol ([125 I]ICYP) respectively. Adenosine receptors that mediate inhibition of cyclic AMP accumulation in rat fat-cells are referred to as R_1 , according to the classification

Table 1. Status of G-proteins in altered thyroid states

Status	G_i/G_o^*	$G_s^\dagger$	G_s activity ‡
Euthyroid	193 $\pm$ 31	772 $\pm$ 167	2.7 $\pm$ 0.1
Hyperthyroid	239 $\pm$ 45	831 $\pm$ 183	3.0 $\pm$ 0.4

* Quantification of radiolabelled peptides resulting from pertussis-toxin-catalysed ADP-ribosylation of the α -subunits G_i and G_o , in fmol of ADP-ribose incorporated/mg of membrane protein. Bands of radioactivity were identified and quantified as described in the Experimental section.

† Quantification of cholera-toxin-catalysed ADP-ribosylation of the α -subunits of G_s , in fmol of ADP-ribose incorporated/mg of membrane protein.

‡ Quantification of functional α - G_s in fat-cell membranes. Fat-cell membranes were extracted in cholate, and protein extracts were used to reconstitute the hormone-sensitive adenylate cyclase of membranes from S49 mouse lymphoma *cyc $^{-}$* mutant cells. Details of this reconstitution assay are described in the Experimental section. Adenylate cyclase activity in response to stimulation of 100 μ M-($-$)-isoprenaline and guanosine 5'-[γ -thio]triphosphate is presented as nmol of cyclic AMP/min per mg of protein.

scheme proposed by Londos & Wolff (1977). These sites are activated by the binding of adenosine analogues which require an intact ribose (R) as opposed to a purine (P) ring. Adenosine receptors with these structural requirements have been classified also as P_1 -purinergic receptors (Burnstock, 1981) or as A_1 -receptors (Van Calcar *et al.*, 1979). Fig. 1 displays the binding of the adenosine analogue [3 H]CHA to inhibitory R-site receptors (Londos & Wolff, 1977) of fat-cell membranes prepared from euthyroid and hyperthyroid rats. Maximal specific binding of [3 H]CHA to fat-cell membranes was 0.22 $\pm$ 0.03 and 0.14 $\pm$ 0.02 (mean $\pm$ S.E.M., n = 3) pmol/mg of membrane protein in the euthyroid and hyperthyroid states respectively. As determined by the method of Scatchard (1949), the specific binding was of high affinity (K_D = 2.7 nM; Fig. 1, inset) and unaltered by hyperthyroidism. The P-site adenosine analogue 2',5'-dideoxyadenosine did not displace bound [3 H]CHA at concentrations up to 1.0 μ M (Trost & Schwabe, 1981; results not shown), indicating that the receptors are the R-site adenosine receptors described by Londos & Wolff (1977). An approx. 35% decrease in the number of R_1 -site adenosine receptors was observed in fat-cell membranes from the hyperthyroid rat (Fig. 1). The number of β -adrenergic receptors, in contrast, was observed to increase by 25 $\pm$ 6% (mean $\pm$ S.E.M., n = 3) in the hyperthyroid state, whereas the affinity of these receptors for [125 I]ICYP was unaltered (results not shown).

Receptor-mediated control of adenylate cyclase is transduced via G-proteins (Gilman, 1984). Pertussis toxin catalyses the ADP-ribosylation of the α -subunit of G_i (Katada & Ui, 1982a,b) and G_o (Malbon *et al.*, 1984b) in rat fat-cell membranes and attenuates the responsiveness of fat-cells to inhibitory hormones which regulate cyclic AMP (Moreno *et al.*, 1983; Olansky *et al.*, 1983). Two peptides, of M_r 41 000 (α - G_i) and 39 000 (α - G_o), were ADP-ribosylated in fat-cell membranes in the presence of pertussis toxin and [32 P]NAD $^{+}$ (Fig. 2, lanes 3 and 4). Short-term hyperthyroidism affected neither the electrophoretic mobilities nor the relative amounts of ADP-ribose incorporated into these peptides in the

Fig. 3. Stimulation of adenylate cyclase activity of rat fat-cell membranes by forskolin and (–)-isoprenaline: effects of hyperthyroidism

Membranes prepared from fat-cells isolated from euthyroid (○) and hyperthyroid (●) rats were incubated in the presence of increasing concentrations of forskolin (a) or (–)-isoprenaline (b) and assayed for adenylate cyclase activity as described in the Experimental section. Assays were routinely performed in triplicate with freshly prepared fat-cell membranes. The results shown are mean values from single experiments representative of several experiments (a, $n = 2$; b, $n = 5$) performed on as many separate cell membrane preparations.

presence of 0.1 mg of toxin/ml and 0.1 μ M-NAD⁺ (Table 1).

Cholera toxin catalyses the ADP-ribosylation of the α -subunits of G_s (Gill & Meren, 1978). In the presence of [³²P]NAD⁺, cholera toxin catalysed the radiolabelling of one major M_r -42000 peptide as well as two minor peptides (M_r 46000 and 48000) of fat-cell membranes (Fig. 2, lanes 5 and 6). Neither the electrophoretic mobilities nor the amounts of ADP-ribose incorporated into these three peptides by cholera toxin (0.1 mg/ml) in the presence of 0.1 μ M-NAD⁺ were altered by hyperthyroidism (Table 1). The amount of functional α - G_s was assayed by reconstitution of adenylate cyclase activity of membranes of S49 mouse lymphoma *cyc*[–] mutant cells that lack functional α - G_s (Ross & Gilman, 1977). In agreement with results of cholera-toxin-catalysed labelling, reconstitution studies indicate a normal complement of functional α - G_s in fat-cell membranes of hyperthyroid rats (Table 1).

Forskolin activates adenylate cyclase activity in intact cells and cell membranes (Seamon & Daly, 1981). Forskolin stimulation of adenylate cyclase activity in membranes of fat-cells was dose-dependent and half-maximal at about 1 μ M-forskolin (Fig. 3a). A 50% decrease in forskolin-stimulated adenylate cyclase was observed in the hyperthyroid state. The activity of adenylate cyclase of membranes in response to guanosine 5'-[γ -thio]triphosphate (0.1 mM) and to NaF (10 mM) was decreased by 35 $\pm$ 6% and 37 $\pm$ 6% (mean $\pm$ S.E.M., $n = 5$) respectively in cells isolated from hyperthyroid rats.

Cyclic AMP accumulation in intact fat-cells from euthyroid and hyperthyroid rats was examined in

response to stimulation by the β -adrenergic agonist adrenaline. When incubated in a Krebs–Ringer phosphate buffer (pH 7.4) containing 3% bovine serum albumin (Malbon *et al.*, 1978a), fat-cells from hyperthyroid rats accumulated 30% more cyclic AMP in response to adrenaline than did fat-cells from their euthyroid counterparts (Fig. 4a). Although in agreement with earlier studies, these data were unexpected, in view of the decreased adenylate cyclase response to forskolin as well as to isoprenaline that was observed in fat-cell membranes of hyperthyroid rats (Fig. 3).

The basis for the apparent paradox between membranes exhibiting decreased catalytic activity of adenylate cyclase and cells accumulating greater than normal amounts of cyclic AMP in response to a β -adrenergic agonist was revealed when the incubation mixture for fat-cells included adenosine deaminase (1.0 μ g/ml). Adenosine deaminase eliminates the inhibitory effects on adenylate cyclase produced by adenosine (Fain & Malbon, 1979). In the presence of adenosine deaminase, fat-cells from hyperthyroid rats accumulated 50% less cyclic AMP in response to a maximal concentration of adrenaline than did their euthyroid counterparts (Fig. 4b). Maximal stimulation of cyclic AMP accumulation by adrenaline increased about 7.5-fold in the euthyroid and only 2.2-fold in the hyperthyroid state, suggesting that the hyperthyroid-rat fat-cells were less sensitive to the inhibitory influence of adenosine. Similarly, forskolin-stimulated cyclic AMP accumulation increased only 2.3-fold in hyperthyroid, compared with 3.9-fold in euthyroid, rat fat-cells when adenosine deaminase was included in the incubation mixture (Table 2).

The sensitivity of the fat-cells to the inhibitory effects

Fig. 4. Cyclic AMP accumulation by rat fat-cells in response to stimulation by adrenaline and to inhibition by PIA: effects of hyperthyroidism

Fat-cells from euthyroid (○) and hyperthyroid (●) rats were isolated and incubated in Krebs–Ringer phosphate buffer containing 3% bovine serum albumin in the absence (a) or presence (b) of adenosine deaminase (1.0 $\mu\text{g}/\text{ml}$) and increasing concentrations of adrenaline. In a parallel experiment, fat-cells were incubated in the presence of forskolin (10 μM), adenosine deaminase and increasing concentrations of PIA (c). After a 2 min incubation, the cyclic AMP content of the cells was assayed as described in the Experimental section. These data are means of triplicate determinations of single experiments that are representative of three to five separate experiments performed on as many individual fat-cell preparations.

Table 2. Forskolin-stimulated cyclic AMP accumulation: effects of hyperthyroidism

Fat-cells of euthyroid and hyperthyroid rats were isolated and incubated in Krebs–Ringer phosphate buffer containing 3% bovine serum albumin in the presence or absence of adenosine deaminase (5.0 $\mu\text{g}/\text{ml}$) and forskolin (100 μM). After 2 min the cyclic AMP content of the cells was assayed as described in the Experimental section. The data are means $\pm$ S.E.M. for triplicate determinations of a single experiment, representative of five separate experiments performed on as many fat-cell preparations. Cyclic AMP accumulation is presented in nmol of cyclic AMP/ 10^6 cells.

		Rats	
		Euthyroid	Hyperthyroid
No adenosine deaminase	Basal	1.60 $\pm$ 0.06	1.72 $\pm$ 0.06
	Forskolin	2.71 $\pm$ 0.16	2.87 $\pm$ 0.17
+ Adenosine deaminase	Basal	1.87 $\pm$ 0.06	1.74 $\pm$ 0.18
	Forskolin	10.48 $\pm$ 0.18	6.71 $\pm$ 0.38*

* Differs significantly from euthyroid rats ($P < 0.02$).

of adenosine on cyclic AMP accumulation was investigated. Fat-cells isolated from euthyroid rats incubated in the presence of forskolin (10 μM) and adenosine deaminase (1.0 $\mu\text{g}/\text{ml}$) displayed a dose-dependent inhibition of cyclic AMP accumulation in response to the R-site adenosine analogue PIA. Half-maximal inhibition of forskolin-stimulated cyclic AMP accumulation

occurred at 1.0 nM-PIA (Fig. 4c). A small (3-fold) increase in the concentration of PIA required for half-maximal inhibition of forskolin-stimulated cyclic AMP accumulation was observed consistently in fat-cells from the hyperthyroid rat (Fig. 4c).

DISCUSSION

The permissive effects of thyroid hormones on the action of other hormones is a well-documented but poorly understood phenomenon (for review see Kunos, 1981). Hypothyroidism as well as hyperthyroidism modulate responses of tissues to insulin (Czech *et al.*, 1980), β -adrenergic agonists (Malbon *et al.*, 1978a,c; Malbon & Greenberg, 1982) and other hormones. We have adopted a biochemical approach to the analysis of the hormone-sensitive adenylate cyclase system in cells from animals of altered thyroid status. The availability of defined probes for the analysis of membrane receptors, G-proteins, and the catalytic subunit of adenylate cyclase prompted our studies on the effects of short-term hyperthyroidism *in vivo* on the components of the adenylate cyclase in rat fat-cells.

Hyperthyroidism has been reported to potentiate (Malbon *et al.*, 1978a; Mills *et al.*, 1986), whereas hypothyroidism impairs (Debons & Schwartz, 1961; Correze *et al.*, 1974; Van Inwegen *et al.*, 1975; Malbon *et al.*, 1978a), the lipolytic sensitivity of rat fat-cells to stimulation by catecholamines. Compelling evidence suggests that in hypothyroidism the biochemical basis for the impaired response is at the generation of cyclic AMP (Omri *et al.*, 1984; Malbon *et al.*, 1978a). Impaired activity of the hormone-sensitive adenylate cyclase appears to be a major locus for the permissive effects of

thyroid hormones (Malbon, 1980; Malbon *et al.*, 1978a, 1984a). In addition, the inhibitory regulation of adenylate cyclase is amplified in hypothyroidism (Malbon *et al.*, 1985). Analysis of the inhibitory pathway in these cells reveals a marked increase in the amounts of α -G_i (M_r 41000) and α -G_o (M_r 39000) in the hypothyroid state (Malbon *et al.*, 1984b, 1985).

Our analysis of the status of G_s revealed no differences, qualitative or quantitative, in this G-protein of fat-cell membranes in hyperthyroid rats as compared with euthyroid rats (the present work). Likewise the nature of α -G_i and α -G_o seems to be unaltered by hyperthyroidism; the amount of ADP-ribose incorporated in these peptides in the presence of pertussis toxin and [³²P]NAD⁺ was equivalent. Immunoblot analysis of fat-cell membranes with antisera specific for the M_r -36000 β -subunit common to G_s, G_i and G_o indicated no change in the nature or abundance of this subunit in hyperthyroidism (P. J. Rapiejko, C. C. Malbon & J. K. Northup, unpublished work).

Most remarkable was the influence of short-term hyperthyroidism on the catalytic activity of adenylate cyclase. Although there is no defined probe for the catalytic subunit, the 50% decrease in the adenylate cyclase activity of membranes from the hyperthyroid rat in response to stimulation by guanine nucleotides, F⁻ ion and forskolin indicates a marked decrease in the amount of catalyst or its expressed activity. Adenylate cyclase activity in response to isoprenaline was also impaired in fat-cell membranes from hyperthyroid rats. The inclusion of adenosine deaminase in the reaction mixture does not alter significantly the adenylate cyclase response to guanine nucleotides, F⁻ ion, forskolin or isoprenaline, or the relationship between the euthyroid and hyperthyroid states (results not shown).

The impaired response of the membrane adenylate cyclase to activators including isoprenaline was not observed in intact cells. Rather, an enhanced cyclic AMP response was observed in cells from hyperthyroid rats when stimulated with β -adrenergic agonist. The basis for this apparent paradox was illuminated by our studies of the effects of the presence of adenosine deaminase on the cyclic AMP responses of fat-cells. In the absence of exogenous adenosine deaminase in the incubation media, adenosine and its inhibitory effects on cyclic AMP accumulation dictate the biology. The decrease in adenosine R₁-receptors in hyperthyroidism may lead to an attenuation of the inhibitory effects of adenosine and permit the expression of an enhanced β -adrenergic response. A lower concentration of adenosine generated in suspensions of rat fat-cells from hyperthyroid rats, as compared with euthyroid rats, may contribute also to a decreased inhibitory input to the adenylate cyclase system. However, previously we reported on adenosine release and metabolism in fat-cells isolated from euthyroid rats as compared with hypothyroid rats, and found there to be no significant differences (Fain & Malbon, 1979). The increase in β -receptor number also may contribute to this enhanced response.

When adenosine deaminase was added to fat-cell incubation mixtures, the inhibitory input from adenosine acting at its R₁-site was abolished. In the absence of adenosine, the cyclic AMP response of the fat-cells from hyperthyroid rats reflected the results obtained from the analysis of the components of adenylate cyclase in membranes from these cells. In this situation the amount

or activity of the catalytic subunit of adenylate cyclase dictates the biology. Previously we observed thyroid-hormone regulation of both G_s and catalytic activity in the liver (Malbon & Greenberg, 1982). Stimulation of cyclic AMP accumulation in response to β -agonists is impaired in the hyperthyroid state, just as the adenylate cyclase response of the membranes from hyperthyroid-rat fat-cells in response to β -agonists was impaired. Whether short-term hyperthyroidism *in vivo* alters the amount, as compared with the expressed activity, of the catalytic subunit cannot be established with the probes currently available. However, these data do identify the catalyst as a possible locus for the expression of the permissive effects of thyroid hormones.

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