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Thyroid-Hormone Modulation of the Number of β-Adrenergic Receptors in Rat Fat-Cell Membranes

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Adipocytes from thyroidectomized rats contain 3 times less [^{3}H]dihydroalprenolol-binding sites (β-adrenergic receptors) than adipocytes from euthyroid animals. This alteration is not solely due to cell-size differences, but also to a thyroidectomy-induced defect in β-adrenergic receptor density per adipocyte surface area, a defect that is furthermore corrected by tri-iodothyronine treatment.

It is now well established that the adrenergic control of tissue functions is markedly affected in altered thyroid states (Waldstein, 1966). In particular, lipolytic responses of isolated fat cells to catecholamines are potentiated in the hyperthyroid state and attenuated in the hypothyroid state (Krishna *et al.*, 1968). Corrèze *et al.* (1974) reported a decrease in the maximal response of adenylate cyclase in fat-cell membranes to adrenaline after thyroidectomy, whereas, in contrast, Armstrong *et al.* (1974) found no difference in catecholamine-induced adenylate cyclase activation in hypothyroid and control fat-cell 'ghosts'.

One mechanism by which thyroid hormone could influence catecholamine-induced physiological responses is via modulation of the adrenergic-receptor number and/or affinity in the cell. Although an increase in the number of β-adrenergic receptors has been reported in rat heart after thyroid-hormone treatment (Williams & Lefkowitz, 1977), Malbon *et al.* (1978) were unable to detect any significant modification in rat fat-cell β-adrenergic receptors after induction of either hyper- or hypo-thyroidism. As the influence of thyroidectomy was not investigated in the latter study and because of the modifications of catecholamine-stimulated adenylate cyclase activity reported by Corrèze *et al.* (1974) in adipocytes from thyroidectomized rats, the present study was undertaken to compare the β-adrenergic receptors of fat cells from thyroidectomized and control rats treated with or without tri-iodothyronine.

Materials and Methods

Three-months-old Sprague–Dawley male rats (Charles Rivers) weighing 320–350g (euthyroid) or 150–160g (thyroidectomized) were divided into four groups. Thyroidectomized (thyroidectomy was carried out on the 21st day of life) or euthyroid rats were injected subcutaneously with 60μg of tri-iodothyronine per 100g of body wt. on alternate days for a total of five doses given subcutaneously. The other two groups (thyroidectomized and euthyroid rats) received 0.9% NaCl in place of tri-iodothyonine. Animals were killed 24h after the last injection and their epididymal fat pads rapidly removed. In each experiment, epididymal fat pads (10–20g) from seven to ten rats were pooled and isolated fat cells prepared by a modification (Giudicelli *et al.*, 1975) of the method of Rodbell (1964). After filtration through a nylon screen, fat cells were washed 3 times, suspended in 15ml of medium I (0.25M-sucrose, 1mM-EDTA, 10mM-Tris/HCl, pH7.4) and disrupted by six aspirations through a swinny filter holder fitted with a stainless-steel phot-etched support (XX 30 01200; Millipore, Bedford, MA, U.S.A.), as described by Avruch & Wallach (1971). After breakage, the suspension was immediately centrifuged at 20000*g* for 3min at 20°C in a Sorvall RC 5 centrifuge with an SM-24 rotor. The floating fat cake and the infranatant were discarded and the resulting pellet was redispersed in 10ml of medium I. After centrifugation at 20000*g* for 20min at 4°C, the pellet was resuspended in medium II (10mM-$MgCl_2$, 50mM-Tris/HCl) and recentrifuged at 20000*g* for 20min at 4°C. The final pellet ('crude' membranes) was resuspended in medium II, resulting in a suspension containing 3–4mg of protein/ml, which was stored under liquid N_2 until assayed. Under these conditions, no significant changes in binding were detectable on storage up to 2 weeks.

Recovery of crude-membrane protein was 20–22% of the whole protein content of the fat cell and was unaffected by thyroidectomy and tri-iodothyronine treatment.

Protein was determined by the method of Lowry *et al.* (1951).

Binding assays were carried out by a modifi-

cation of the method of Williams *et al.* (1976*a*). Membrane (150–175 μg) was usually incubated with 15 nM-(−)-[^{3}H]dihydroalprenolol in a total volume of 75 μl of incubation buffer (medium II) for 8 min with shaking at 37°C. Incubations were terminated by adding 1 ml of ice-cold incubation buffer, followed by a rapid vacuum filtration of the suspension through a Whatman GFC glass-fibre filter. Filters were rapidly washed with 2 × 2.5 ml of ice-cold buffer. Under these conditions, filtration and washing procedures required less than 30 s. Filters were dried and added to 5 ml of scintillation 'cocktail' (Instagel; Packard, Downer's Grove, IL, U.S.A.) and counted for radioactivity in a Kontron MR 300 spectrometer.

Non-specific binding (background non-specific adsorption to filters and non-specific adsorption to membrane protein) was determined by measuring the radioactivity retained on filters when incubations were carried out in the presence of a large excess (20 μM) of (±)-alprenolol. Non-specific adsorption to the filter was less than 0.4% of total counts filtered and was not affected by the presence of high concentrations of unlabelled ligands. The non-specific adsorption to membrane protein averaged 15–20% of the counts specifically bound to adipocyte membranes. The binding values reported refer to specific binding determined by subtracting the non-specific binding from the total counts bound.

Cell numbers were calculated by the method of Hirsch & Gallian (1968) from the mean fat-cell size (Goldrick, 1967) and the lipid content of a known volume of the fat-cell suspension. Mean fat-cell diameter and cell lipid dry weight were determined as previously described (Giudicelli *et al.*, 1976).

(−)-[^{3}H]Dihydroalprenolol (sp. radioactivity 39 Ci/mmol) was prepared by The Radiochemical Centre (Amersham, Bucks., U.K.) by catalytic reduction of (−)-alprenolol (Hässle, Mölndal, Sweden) with tritium gas; radiochemical purity, monitored by t.l.c., was greater than 98%. (±)-Alprenolol was a gift from Laboratoires Lematte et Boinot (Paris, France). Other compounds used in this study were: crude bacterial collagenase type CLS 45 DO 06, specific activity 157 units/mg (Worthington Biochemical Corp., Freehold, NJ, U.S.A.), where 1 unit of activity is the amount of enzyme required to produce 1 μmol of amino acid in 5 h with bovine achilles tendon collagen as substrate; bovine serum albumin (fraction V, fatty acid poor; Calbiochem Inc., Los Angeles, CA, U.S.A.) and tri-iodothyronine (sodium salt; Serva, Heidelberg, Germany). All other chemicals were of analytical grade.

Results

(−)-[^{3}H]Dihydroalprenolol, a potent β-adrenergic antagonist, has been used to identify β-adrenergic receptors in membrane preparations from several tissues (Mukherjee *et al.*, 1975; Alexander *et al.*, 1975; Williams *et al.*, 1976*a*,*b*). In rat adipocytes, [^{3}H]dihydroalprenolol binds to sites that are almost entirely localized in the plasma membrane and that have the affinity and specificity expected of β-adrenergic receptors (Williams *et al.*, 1976*a*).

By using these direct binding methods, the number and affinity of (−)-[^{3}H]dihydroalprenolol-binding sites in adipocyte membranes from control and thyroidectomized rats were assessed by Scatchard analysis (Scatchard, 1949; Fig. 1). The negative reciprocal of the slope provides an estimate of the equilibrium dissociation constant K_D for the interaction of (−)-[^{3}H]dihydroalprenolol with the binding sites, whereas the intercept on the abscissa of the plot indicates the maximal number of binding sites. Since fat-cell size was markedly different in control (mean diameter 79 μm) and thyroidectomized rats (mean diameter 55 μm) and because we have recently shown that the number of adipocyte membrane [^{3}H]dihydroalprenolol-binding sites increases proportional to the fat-cell surface in normal rats (Giudicelli & Pecquery, 1978), the maximal number of binding sites has been expressed not only per mg of membrane protein, but also per 10^6 cells. As shown in Fig. 1, thyroidectomy failed to alter the affinity of the receptors for [^{3}H]dihydroalprenolol. In fact, the dissociation constants found in thyroidectomized and control membranes were 9 and 12 nM respectively,

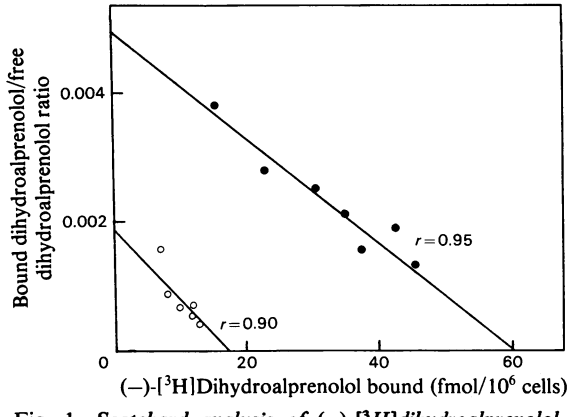

Fig. 1. *Scatchard analysis of* (−)-[^{3}*H*]*dihydroalprenolol binding as a function of* (−)-[^{3}*H*]*dihydroalprnolol concentration in adipocyte membranes of thyroidectomized and euthyroid rats*

Crude adipocyte membranes from euthyroid (●) and thyroidectomized rats (○) (mean fat-cell diameter 79 and 55 μm respectively) were incubated in the presence of different concentrations of [^{3}H]dihydroalprenolol (5 to 30 nM) for 8 min at 37°C and specific binding was determined as described in the Materials and Methods section. Each point is the mean for six determinations from two separate experiments.

values that are in good agreement with those already reported in rat fat-cell membranes (Williams *et al.*, 1976*a*; Malbon *et al.*, 1978). In contrast, the maximal number of [^{3}H]dihydroalprenolol-binding sites was about 3-fold less in membranes from thyroidectomized rats (152fmol/mg of protein or 18fmol/10^6 cells or 11000 sites/cell) than in membranes from euthyroid animals (454fmol/mg of protein or 60fmol/10^7 cells or 36000 sites/cell; Fig. 1).

As shown in Table 1, the low [^{3}H]dihydroalprenolol-binding capacity found in fat cells from thyroidectomized rats was not only due to the lower size of these fat cells (Giudicelli & Pecquery, 1978), but also to an impairment in the density of binding sites per fat-cell surface area. In fact, assuming that the fat cells are spherical, the calculated maximal densities of [^{3}H]dihydroalprenolol-binding sites were 1.9×10^6 sites/mm^2 and 1.1×10^6 sites/mm^2 in control and thyroidectomized fat cells respectively.

Occurrence of such an impairment in the binding-site density is furthermore supported by the data in Table 1 showing that the administration of tri-iodothyronone to thyroidectomized rats, although decreasing the cell size, resulted in a 2-fold increase in the number of β-adrenergic receptors per cell, as well as in the restoration to the normal value of the receptor density per fat-cell surface area (Table 1). On the contrary, administration of tri-iodothyronine to euthyroid rats did not change significantly the maximal number of β-adrenergic receptor binding sites (Table 1).

Discussion

In a recent report, Malbon *et al.* (1978) postulated that thyroid hormones had no influence on the number and affinity of β-adrenergic receptors of the fat cells. This conclusion was drawn from experiments carried out on rats rendered hypo- or hyper-thyroid by short-term treatments. Although the same conclusion could be drawn from our data concerning euthyroid animals treated with tri-iodothyronine, it is clear, however, that thyroid hormones play an important role in the regulation of the number of adipocyte β-adrenergic receptors. This is supported both by the low [^{3}H]dihydroalprenolol-binding capacity found in adipocytes from thyroidectomized rats and by the tri-iodothyronine-induced normalization of this binding capacity, taking into account the small size of these cells (Giudicelli & Pecquery, 1978).

The change in adipocyte β-adrenergic receptor number caused by tri-iodothyronine in thyroidectomized rats might be caused by an effect of tri-iodothyronine on the receptor synthesis, a mechanism that is supported by recent studies. Lo *et al.* (1976) found that tri-iodothyronine administration is able to induce the synthesis of $(Na^+ + K^+)$-dependent-adenosine triphosphatase-associated [^{3}H]ouabain-binding sites in the renal cortex. More recently, Kempson *et al.* (1978) showed that tri-iodothyronine has a dual increasing effect *in vitro* on the number of β-adrenergic receptors of isolated heart ventricle slices, one acute involving a non-nuclear process and one chronic involving protein synthesis and possibly enhanced synthesis of new β-adrenergic receptors.

Another possible mechanism by which thyroid hormones could influence the number of adipocyte β-adrenergic receptors would be an increase in receptors owing to the thyroid-induced decrease in plasma catecholamine concentration (Staffer *et al.*, 1973). In fact, we have recently observed that the adipocyte β-adrenergic receptors are, like those of other catecholamine target cells, decreased in number after exposure to catecholamine (Giudicelli, 1978). Thus, if thyroid hormones decrease plasma catecholamine (Staffer *et al.*, 1973), the number of adipocyte β-adrenergic receptors might increase.

Regardless of the mechanism of this thyroid-induced receptor increase, the fact that results

Table 1. *Effect of tri-iodothyronine (T_3) administration on the specific (−)-[^{3}H]dihydroalprenolol binding to crude adipocyte membranes of thyroidectomized and euthyroid rats*

T_3-treated rats were injected with 60μg of T_3/100g body wt. on alternate days for a total of five subcutaneous injections. Specific [^{3}H]dihydroalprenolol-binding data are the means ± S.E.M. for five determinations obtained from five to six rats in each group with a [^{3}H]dihydroalprenolol concentration of 15nM. The maximal number of binding sites were determined by Scatchard analysis as described in Fig. 1. *Significance tested against results for euthyroid animals; ** significance tested against results for thyroidectomized animals.

Animals	Mean fat-cell diameter (μm)	$10^{-3}\times$ Mean fat-cell surface (mm^2)	Specific [^{3}H]dihydroalprenolol binding (fmol/mg of protein)	(fmol/10^6 cells)	P	Maximal binding capacity (fmol/mg of protein)	(fmol/10^6 cells)	(sites/mm^2 of cell surface)
Euthyroid	75	17.67	228 ± 22	30 ± 3		424	56	1.9×10^6
Euthyroid+T_3	70	15.39	206 ± 34	26 ± 4	<0.05*	392	49	1.9×10^6
Thyroidectomized	56	9.85	82 ± 13	10 ± 2	<0.001*	152	18	1.1×10^6
Thyroidectomized+T_3	51	8.17	150 ± 9	18 ± 1	<0.001*,**	229	28	2.0×10^6

similar to ours were recently reported in heart membranes from thyroidectomized rats (Banerjee & Kung, 1977) suggests that the present studies on the effects of thyroidectomy and tri-iodothyronine treatment in adipocytes could be of general significance for the physiological and pathological regulation of β-adrenergic receptors in other catecholamine target cells.

Note Added in Proof (Received 28 September 1978)

Since the submission of the present paper, Ciaraldi & Marinetti (1978) have reported a 2-fold increase in the number of β-adrenergic receptors of rat fat-cell membranes after thyroxine treatment for 7 days.

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