Biochimica et Biophysica Acta, 731 (1983) 397–405
Elsevier

BBA 71703

CHARACTERIZATION OF THE β-ADRENERGIC RECEPTORS OF HAMSTER WHITE FAT CELLS

EVIDENCE AGAINST AN IMPORTANT ROLE FOR THE α_2-RECEPTOR SUBTYPE IN THE ADRENERGIC CONTROL OF LIPOLYSIS

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(Received January 4th, 1983)

Key words: Adrenergic receptor; Binding site; Lipolysis; (Hamster white adipocyte)

The binding characteristics of the β-adrenergic antagonist, [^{3}H]dihydroalprenolol, to hamster white adipocyte membranes were studied. This binding occurred at two classes of sites, one having high affinity (K_d = 1.6 ± 1.3 nM) but low capacity (32 ± 17 fmol/mg membrane protein) and one having low affinity but high binding capacity. While the binding at the high-affinity sites was competitively and stereoselectively displaced by both β-antagonists and β-agonists, competition at the low-affinity sites occurred only with β-antagonists and was non-stereoselective. Thus, the β-agonist (−)-isoproterenol was further used to define nonspecific binding. Under these conditions, saturation studies showed a single class of high-affinity (K_d = 1.6 ± 0.5 nM) binding sites with a binding capacity of 53 ± 13 fmol/mg membrane protein (corresponding to 4000 ± 980 sites per cell), and independent kinetic analysis provided a K_d value of 1.9 nM. Competition experiments showed that these binding sites had the characteristics of a β_1-receptor subtype, yielding K_d values in good agreement with the K_{act} and the K_i values found for agonist-stimulation and for antagonist-inhibition of adenylate cyclase in membranes and of cyclic AMP accumulation and lipolysis in intact cells. Furthermore, the ability of β-agonists to compete with this binding was severely depressed by p[NH]ppG. These results thus support the contention that the specific [^{3}H]dihydroalprenolol binding sites defined as the binding displaceable by (−)-isoproterenol represent the physiologically relevant β-adrenergic receptors of hamster white adipocytes. Finally, studies of the lipolytic response of these cells to (−)-norepinephrine showed that the inhibitory effect of the α_2-component of this catecholamine was apparent only when the effects of endogenous adenosine were suppressed, a result which argues against an important regulatory role for the α_2-receptors in the adrenergic control of lipolysis in hamster white adipocytes.

Introduction

In rat white adipocytes, the effects of catecholamines on lipolysis result in a stimulation which is initiated by the interaction of these catecholamines with β-adrenergic receptors only [1]. Biochemical and pharmacological studies have further shown that these receptors belong to the β_1-adrenergic receptor subtype [2–4].

Contrary to the situation found in rat adipocytes, the control of lipolysis by catecholamines in white fat cells from other mammalian species, such as man [5,6], dog [7], rabbit [8] and hamster [9,10], is exerted through two opposite effects, one termed α-adrenergic which is inhibitory and one termed β which is stimulatory. Recently, binding studies

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have demonstrated that both α_1- and α_2-adrenergic receptors were present in hamster [11–13] and human fat cells [14,15] and that the antilipolytic response which is observed following exposure to α-agonists was due to the predominant α_2-adrenergic receptor subtype [12–16]. Moreover, using a similar experimental approach, it has been recently suggested that the β-adrenergic receptor population of human white fat cells, though being predominantly of the β_1-subtype [17], may also include some receptors of the β_2-subtype.

These considerations have led us to determine the precise nature of the β-adrenergic receptors of hamster white adipocytes.

We report here the presence of specific [^{3}H]dihydroalprenolol binding sites which fulfil the biochemical, physiological and pharmacological criteria expected of true β_1-adrenergic receptors.

Material and Methods

Chemicals. (−)-[^{3}H]Dihydroalprenolol (spec. act. 30–40 Ci/mmol) and cyclic [^{3}H]AMP were from the Radiochemical Centre (Amersham), bitartrate of (−)-isoproterenol, (−)-norepinephrine and (−)-epinephrine, (−)-propranolol, ATP (A2383), creatine phosphate and creatine kinase were from Sigma, adenosine deaminase, GTP, theophylline and reagents for glycerol determination from Boehringer Mannheim, (+)-isoproterenol from Sterling Winthrop, phentolamine from Ciba-Geigy and collagenase from Worthington.

Preparation of isolated fat cells and ghosts. Male golden hamsters (100–110 g), maintained at 22°C, were fed ad libitum before being killed. Adipocytes were isolated from the epididymal fat following the procedure described by Hittelman et al. [9] with minor modifications with regard to the composition of the isolation medium, which was a Krebs-Ringer phosphate buffer (buffer 1) (pH 7.4 at 37°C) containing collagenase (0.5 mg/ml) and bovine serum albumin (3%), and the time of incubation, which was 30 min (70–80 strokes per min). After filtration, the isolated cells were washed three times with either the buffer 1 (in studies performed on membranes) or with buffer 1 containing 2% albumin (in studies performed on isolated cells).

Fat cell ghosts, prepared essentially as described by Birnbaumer et al. [18] by hypotonic lysis of the cells in the presence of ATP (3 mM), were suspended in $KHCO_3$ (1 mM) and used either fresh or after storage in liquid nitrogen for no longer than 1 week.

β-Adrenergic receptor binding studies. Binding of [^{3}H]dihydroalprenolol was studied as follows: ghosts (100–200 μg protein) were incubated at 30°C in 50 mM Tris-HCl (pH 7.4)/10 mM $MgCl_2$/10 μM phentolamine/0.8 mM ascorbate and various concentrations of (−)-[^{3}H]dihydroalprenolol in a final volume of 150 μl. After 8 min, incubations were stopped by addition of ice-cold buffer followed by rapid vacuum filtration through Whatman GF/C glass-fiber filters. Filters were rapidly washed three times with 5 ml of ice-cold buffer, then dried, added to 10 ml PCS (Amersham) and counted with a 40% efficiency. Nonspecific binding was determined in parallel by measuring the radioactivity remaining on the filters when incubations were performed with either 5 μM (−)-isoproterenol or 10 μM (−)-propranolol.

Scatchard plots [19] of [^{3}H]dihydroalprenolol binding were analysed by graphical fitting of the experimental data on computer-generated figures based on the theoretical model of one ligand–two binding sites according to Feldman [20]. Competition binding curves were analysed in the same way using computer-generated curves based on the two-site competitive binding equation of Hulme et al. [21] that provides the dissociation constants K_d and the proportion of each of the binding sites. These computer analyses were performed on a Commodore CBM computer. Pseudo-Hill numbers (n_H) were calculated by regression analysis from the log-logit plots derived from the competition data.

Assays of adenylate cyclase. Adenylate cyclase activities were assayed at 30°C by incubating ghosts (10–15 μg protein) in 25 mM Tris-HCl (pH 7.4)/2 mM $MgCl_2$/1 mM theophylline/creatine kinase (10 U/ml)/10 mM phosphocreatine/0.2 mM ATP/albumin (0.1%)/1 μM GTP and, in some experiments, adenosine deaminase (50 mU/ml), in a final volume of 50 μl. After 10 min, reaction was stopped by addition of 0.25 M EDTA followed by immersion in a boiling water bath for 2 min. Cyclic AMP produced was determined in an aliquot of the supernatant by ra-

diocompetition as previously described [22].

Cyclic AMP accumulation and lipolysis. In these experiments, intact cells (40 000–80 000 cells) were incubated at 37°C in buffer 1 containing adenosine deaminase (50 mU/ml), 0.1 mM theophylline and albumin (2% for cyclic AMP and 3% for lipolysis). After 10 min (cyclic AMP) or 60 min (lipolysis), incubations were stopped and the total cyclic AMP produced (cells + medium) and the glycerol released into the medium were determined as previously described [22].

Other determinations. Protein was determined according to Lowry et al. [23] and cell numbers as previously described [24].

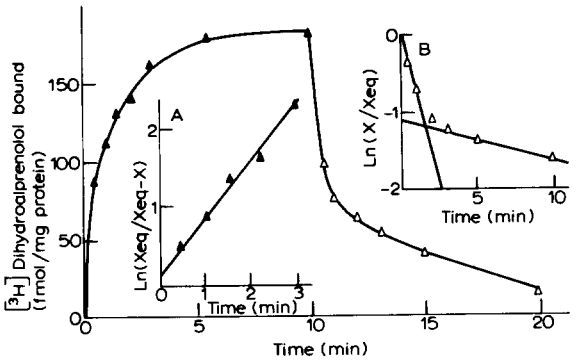
Results

Kinetics of [^{3}H]dihydroalprenolol binding

Using the conditions commonly employed [3,4,24,25] to determine [^{3}H]dihydroalprenolol specific binding to rat adipocyte membranes (binding displaceable by 10 μM propranolol), the kinetics of association of [^{3}H]dihydroalprenolol specific binding was very rapid at 30°C (half time 40 s), equilibrium being reached within 5 min and remaining stable at least during 10 min (Fig. 1). Calculation of the pseudo-first-order rate constant (K_{obs}) yielded the value of 0.667 min^{-1}. Under the same conditions, [^{3}H]dihydroalprenolol binding was also reversible upon addition of an excess of (−)-alprenolol, and a semi-logarithmic representation of these data yielded two apparent straight lines with slopes equal to 0.769 and 0.059 min^{-1}. From these data, which suggest dissociation from two different sites, kinetically derived estimates of the dissociation constant K_d yielded the value of 1.07 nM when the slow dissociation rate constant was used.

Equilibrium studies of [^{3}H]dihydroalprenolol binding

Fig. 2 shows the steady-state binding of [^{3}H]dihydroalprenolol (binding displaceable by 10 μM propranolol) as a function of labeled ligand



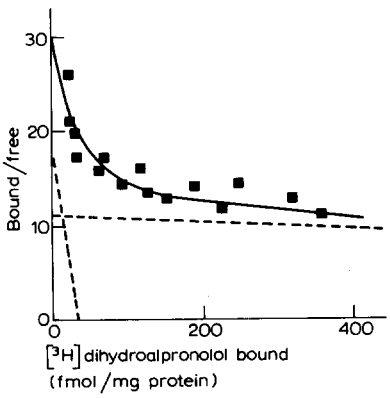


Fig. 1. (left-hand figure) Kinetics of association and dissociation of [^{3}H]dihydroalprenolol binding to hamster white fat cell membranes. Association: membranes were incubated at 30°C for the indicated times with [^{3}H]dihydroalprenolol (8 nM), in the absence or presence of 10 μM (−)-propranolol. Binding data, expressed as the difference between binding in the absence and binding in the presence of (−)-propranolol were plotted as $\ln(Xeq/Xeq - X)$ vs. time (inset A) and the slope of the line, K_{obs} was calculated by regression analysis. Dissociation: after incubation at equilibrium (10 min), the dissociation reaction was initiated by the addition of 10 μM (−)-propranolol and the decrease of binding was measured at the indicated times. Data are expressed as the difference between total binding and binding in the presence of (−)-propranolol. A semilogarithmic representation of these data ($\ln X/Xeq$ vs. time) yielded two straight lines with slopes equal to 0.769 and 0.059 min^{-1} (inset B). Data shown are averages of triplicate determinations for one representative experiment repeated twice.

Fig. 2. Scatchard plot of the concentration-dependence curve for [^{3}H]dihydroalprenolol binding to hamster white fat cell membranes. Membranes were incubated at 30°C for 8 min in the absence or presence of 10 μM (−)-propranolol. Binding data represent the difference between binding occurring in the absence of propranolol and binding in the presence of propranolol. These data were plotted according to Scatchard, and computer analysis was consistent with the existence of two binding components (indicated by the dotted lines). Data points represent the mean value of triplicate determinations from one representative experiment repeated five times. K_d = 2 nM; B_{max} = 37 fmol/mg protein.

concentration (0.2–100 nM). Scatchard analysis [19] of these binding data yielded curvilinear plots, suggesting either negatively cooperative interactions among one class of binding sites or the existence of binding sites of different affinities.

The first of these two possibilities seemed unlikely, since the time course of [^{3}H]dihydroalprenolol dissociation induced by a 100-fold dilution with buffer was unchanged whether the buffer contained propranolol or not (data not shown). On the other hand, the existence of heterogeneous binding was supported by computer analysis of the Scatchard plots which indicated that the binding data were, in fact, compatible with dihydroalprenolol binding to two different affinity sites: one having high affinity ($K_d = 1.63 \pm 1.30$ nM) but low binding capacity ($B_{max} = 32 \pm 17$ fmol/mg protein) and the other one having low affinity ($K_d = 137 \pm 54$ nM) but high binding capacity ($B_{max} = 930 \pm 430$ fmol/mg protein) (mean ± S.D. of five separate experiments).

Displacement of [^{3}H]dihydroalprenolol binding by isoproterenol and propranolol

Competitive displacement of the total amount of [^{3}H]dihydroalprenolol bound at 5 nM by (−)-propranolol and (−)-isoproterenol is shown in Fig. 3. As can be seen, the corresponding curves were quite different. In fact, the (−)-propranolol-competition curve was clearly biphasic (Hill number 0.40). Moreover, computer analysis of these curves indicated interaction of (−)-propranolol with two different classes of [^{3}H]dihydroalprenolol binding sites: one having high affinity for (−)-propranolol ($K_d = 3.3$ nM) representing 31% of the labeled sites and which is stereospecific, and one having lower affinity and which is apparently non-stereospecific, as shown by the equipotency of the (+)- and (−)-propranolol stereoisomers to compete with these sites (respective K_d values 800 and 1100 nM). In contrast, the (−)-isoproterenol competition curves were shallow (Hill number 0.70), yielding an apparent K_d value of 27 nM, and were steeper in the presence of p[NH]ppG ($n_H = 1.02$, $K_d = 146$ nM), a property commonly shared by agonist-binding to true β-receptors [4,26–28]. Moreover, displacement by isoproterenol was stereospecific, the (+)-stereoisomer being 60-times less potent than the (−)-stereoisomer. Finally, maximal displacement by (−)-isoproterenol corresponded to the occupancy of only 30% of the total binding sites, a proportion which is equivalent to the proportion of sites displaced with high affinity by (−)-propranolol. These results thus strongly suggest that the displacement of

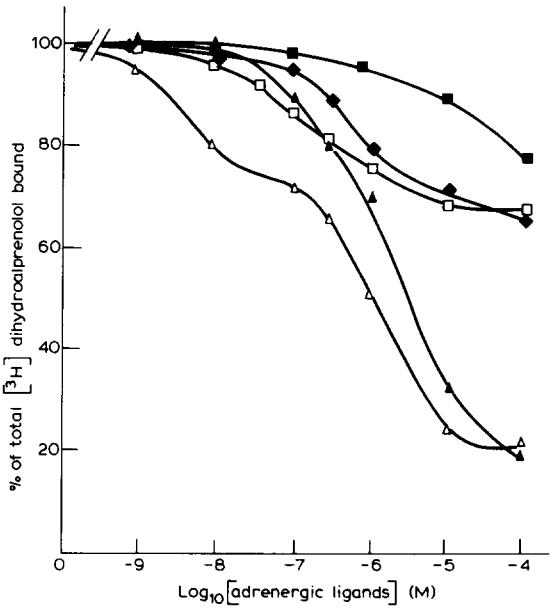


Fig. 3. Displacement of total [^{3}H]dihydroalprenolol binding to hamster white fat cell membranes by the stereoisomers of propranolol and isoproterenol. Influence of p[NH]ppG. Membranes were incubated with 5 nM [^{3}H]dihydroalprenolol and various concentrations of the (+) and (−)-stereoisomers of propranolol and isoproterenol at 30°C for 8 min after which the amount of ligand remaining bound was determined. In some experiments, a fixed concentration of p[NH]ppG (100 μM) was added together with (−)-isoproterenol. Each point represents the mean of three different experiments performed in triplicate. Data given below represent the computer-modelled parameters of the interaction of propranolol and isoproterenol with [^{3}H]dihydroalprenolol binding, where n_H is the pseudo-Hill number and K_d the calculated dissociation constant for the competitors at the high- and low-affinity sites.

	High affinity		Low affinity		n_H
	K_d	%	K_d	%	
(−)-Propanolol (△)	3.3	31	1100	69	0.40
(+)-Propanolol (▲)	—	—	800	100	—
(−)-Isoproterenol (□)	27.0	30	—	—	0.70
(−)-Isoproterenol + p[NH]ppG (◆)	—	—	146	30	1.02
(+)-Isoproterenol (■)	—	—	1620	30	—

[^{3}H]dihydroalprenolol binding by (−)-propranolol concentrations higher than 100 nM reflects the displacement of dihydroalprenolol bound to unspecific and non-receptor sites. Therefore, in all the following experiments, (−)-isoproterenol (5 μM) was used instead of (−)-propranolol (10 μM) to assess the amount of [^{3}H]dihydroalprenolol nonspecific binding.

Kinetics and equilibrium studies of isoproterenol-displaceable [^{3}H]dihydroalprenol binding

Under these experimental conditions, the kinetics of [^{3}H]dihydroalprenolol association and dissociation yielded the following values: $k_{obs} = 0.808$ min^{-1}, $k_1 = 1.37 \cdot 10^8$ M$^{-1} \cdot$ min^{-1} and $k_2 = 0.260$ min^{-1}, and the dissociation data were consistent with dissociation from one binding site only. From these results, the kinetically derived estimates of the dissociation constant K_d yielded the value of 1.9 nM, which is in good agreement with the K_d value calculated for the high-affinity site depicted in Fig. 2.

Under the same conditions, reinvestigation of the equilibrium binding of [^{3}H]dihydroalprenolol yielded linear Scatchard plots (Fig. 4), consistent

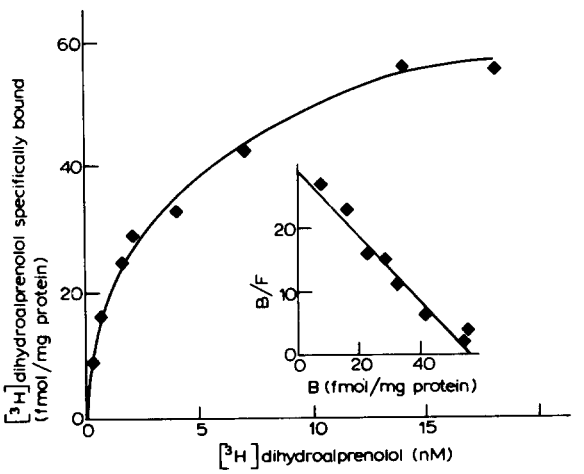


Fig. 4. Concentration-dependence curve for specific binding of [^{3}H]dihydroalprenolol to hamster white fat cell membranes. Membranes were incubated with increasing concentrations of [^{3}H]dihydroalprenolol at 30°C for 8 min in the absence (total binding) and presence of 5 μM (−)-isoproterenol (nonspecific binding) after which specifically bound radioactive ligand was determined. Data points represent the mean value of triplicate determinations from one representative experiment repeated four times. Inset: Scatchard plot of the same experiment.

TABLE I

COMPARISON OF THE β-RECEPTOR DISSOCIATION CONSTANTS FOR CATECHOLAMINES WITH THE CATECHOLAMINE-ACTIVATION CONSTANTS FOR CYCLIC AMP ACCUMULATION IN HAMSTER WHITE FAT CELLS

The K_d values were calculated [30] from three separate competition binding experiments performed as described in the text; the K_{act} values represent the concentration causing half-maximal cyclic AMP accumulation and were obtained from three experiments performed as described under Fig. 5B.

	(−)-Isoproterenol	(−)-Norepinephrine	(−)-Epinephrine
K_d values (nM)	27 ± 10	48 ± 25	240 ± 67
K_{act} values (nM)	35 ± 4	129 ± 30	335 ± 54

again with the existence of a single class of binding sites with a K_d value of 1.56 ± 0.51 nM, which is in good agreement with the value derived kinetically (see above). The maximal number of sites calculated from these experiments was 53 ± 13 fmol/mg protein, which is similar to the calculated binding capacity of the apparent high-affinity [^{3}H]dihydroalprenolol binding sites shown in Fig. 2 and which represents 4000 ± 980 binding sites per adipocyte.

β-Adrenergic specificity of [^{3}H]dihydroalprenolol binding

As shown in Table I, the order of potency of β-adrenergic agonists to displace the [^{3}H]dihydroalprenolol specific binding was (−)-isoproterenol > (−)-norepinephrine > (−)-epinephrine, which is characteristic for the β_1-receptor subtype of adrenergic receptors [29]. Calculation [30] of the relative K_d values for these agonists yielded the values 27, 48, 240 nM for (−)-isoproterenol, (−)-norepinephrine and (−)-epinephrine, respectively. Finally, the potent α_2-agonist clonidine [31] was unable to displace [^{3}H]dihydroalprenolol binding at concentrations up to 10 μM.

Relationship between [^{3}H]dihydroalprenolol binding and β-adrenergic regulation of adenylate cyclase, cyclic AMP accumulation and lipolysis

Adenylate cyclase was studied in ghosts, in the

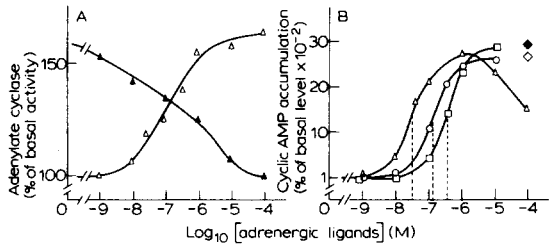


Fig. 5. A. Dose-response curve of (−)-isoproterenol-stimulated adenylate cyclase activity (△) in hamster adipocyte membranes and its inhibition by (−)-propranolol (▲). Incubations were performed as described in the text. Each point is the mean value of three different experiments performed in triplicate and is expressed as percentage of basal value (1480 ± 285 pmol/mg protein per 10 min). Adenylate cyclase inhibition by propranolol was studied in the presence of 10 μM (−)-isoproterenol. B. Dose-response curve of catecholamine-stimulated cyclic AMP accumulation in hamster white adipocytes. Fat cells were incubated as described in the text, in the presence of theophylline (0.1 mM) and adenosine deaminase (50 mU/ml) with (−)-isoproterenol alone (△), (−)-isoproterenol + 100 μM phentolamine (◇), (−)-isoproterenol + 100 μM yohimbine (◆), (−)-norepinephrine + 100 μM phentolamine (○) or (−)-epinephrine + 100 μM phentolamine (□). Each point is the mean value of triplicate determinations from one representative experiment repeated three times and is expressed as the percentage of the basal cyclic AMP level (755 ± 140 pmol/g lipid per 10 min).

presence of GTP, which is required to observe hormonal stimulation of this enzyme in hamster adipocytes [32]. As shown in Fig. 5A, with 1 μM GTP (optimal concentration, data not shown), half-maximal stimulation of adenylate cyclase by (−)-isoproterenol occurred at 86 ± 12 nM ($n = 3$), a K_{act} value which is in between the isoproterenol K_d values found in the competition experiments performed in the absence and presence of p[NH]ppG (see Fig. 3). Moreover, calculation [30] of the K_i value for the inhibition of isoproterenol maximally stimulated adenylate cyclase by (−)-propranolol yielded 3.6 nM, which is in good agreement with the (−)-propranolol K_d value derived from binding experiments.

As shown in Fig. 5B, the cyclic AMP accumulation in intact adipocytes in response to increasing concentrations of adrenergic agonists resulted, in the case of (−)-isoproterenol, in a bell-shaped curve with a peak at 1 μM. The inhibition found at higher concentrations was likely to be due to the interaction of isoproterenol with the α_2-adrenergic receptors [13] since, as shown in Fig. 5B, this inhibition was prevented by phentolamine (a mixed α_1- and α_2-antagonist) or by yohimbine (a pure α_2-antagonist). Paradoxically, this inhibitory effect of high isoproterenol concentrations was not observed on adenylate cyclase (Fig. 5A), although α-adrenergic antagonists were not present in the incubation. However, this is not surprising, considering that high concentrations of sodium, which are required for adenylate cyclase inhibition by α_2-adrenergic agonists [33], were not present in these experiments. As also shown in Fig. 5B, the order of potency of catecholamines (in the presence of phentolamine) to increase cyclic AMP accumulation was typical for a β_1-receptor-mediated process, and the data in Table I indicate that the corresponding K_{act} values for the catecholamines were very similar to their K_d values derived from competition experiments.

Data in Fig. 6A compare the lipolytic responsiveness of hamster adipocytes to increasing concentrations of (−)-isoproterenol, (−)-norepinephrine and (−)-epinephrine, in the absence of theophylline and adenosine deaminase. As can be seen, half-maximal lipolysis stimulation occurred at concentrations similar to the K_{act} values derived from the stimulation of cyclic AMP accumulation by these catecholamines. Moreover, the magnitude of the maximal lipolytic effect in-

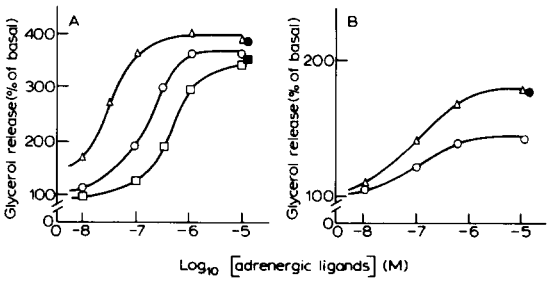


Fig. 6. Dose-response curve of catecholamine-stimulated lipolysis in hamster white adipocytes: influence of theophylline and adenosine deaminase. Fat cells were incubated, as described in the text, with increasing concentrations of (−)-isoproterenol (△), (−)-norepinephrine (○), (−)-norepinephrine + 100 μM phentolamine (●), (−)-epinephrine (□) or (−)-epinephrine + 100 μM phentolamine (■), in the absence (A) or presence (B) of theophylline (0.1 mM) and adenosine deaminase (50 mU/ml). Each point is the mean value of triplicate determinations from one representative experiment repeated twice, and is expressed as the percentage of the basal glycerol release (in A, 4.23 ± 0.38; in B, 13.80 ± 1.05 μmol/g lipid per 60 min).

duced by (−)-norepinephrine and (−)-epinephrine was similar to that seen with (−)-isoproterenol and was unaffected by the addition of phentolamine. However, when the same experiments were repeated in the presence of theophylline (0.1 mM) and adenosine deaminase (50 mU/ml), (−)-norepinephrine was clearly less potent than (−)-isoproterenol (Fig. 6B), a difference which was suppressed by the addition of phentolamine to the incubation medium.

Discussion

It is now well-established that hamster white fat cells exhibit, like the human adipocytes [5,6], a mixed α- and β-adrenergic responsiveness [9,10,34]. Therefore, these cells have been widely used as a model to investigate the adrenergic regulation of lipolysis. However, though physiological studies [34] have suggested and binding experiments [11–13] demonstrated the coexistence of both α_1- and α_2-adrenergic receptor subtypes in these cells, no attempt has been made before to characterize the nature of the β-receptor of hamster white adipocytes. The aim of this study was to probe the existence of such receptors, by identifying specific binding sites for the β-antagonist [^{3}H]dihydroalprenolol and by comparing their properties with the β-adrenergic responsiveness of adenylate cyclase, cyclic AMP accumulation and lipolysis.

As shown here, [^{3}H]dihydroalprenolol specifically labels a single homogeneous class of binding sites, provided that the specific binding is defined as the binding displaceable by the β-agonist (−)-isoproterenol, and not, as usually done in such studies, by the β-antagonist (−)-propranolol. In fact, as shown in Fig. 3, (−)-propranolol displaced nonspecific binding, even when used at the recommended concentration [35] (i.e., 100-times the K_d value of the binding sites displaced with high affinity by (−)-propranolol), here 330 nM. According to the recent suggestion of Dax and Partilla [36] who reported, as have others with erythrocytes [37], the same phenomenon in rat adipocyte [38] and hepatocyte membranes [39], these different behaviours of (−)-isoproterenol and (−)-propranolol in competing with dihydroalprenolol binding might be due to the lipophilic character of dihydroalprenolol and propranolol [40] as compared to the hydrophilic character of isoproterenol.

Moreover, this study shows that the sites specifically labeled by [^{3}H]dihydroalprenolol entirely fulfil the binding criteria expected of true physiological β-receptors. In fact, the binding of [^{3}H]dihydroalprenolol was rapid, reversible and saturable, displaying a high affinity for this ligand, which is similar to the β-receptor affinities reported in other mammalian cells, including rat adipocyte [25], human adipocyte [14,15,17] and hamster brown adipose tissue [41]. This binding was also stereospecific and consistent with the β_1 nature of the catecholamine-induced cyclic AMP accumulation in the intact cell. Moreover, the affinity of the [^{3}H]dihydroalprenolol specific binding sites towards (−)-isoproterenol was decreased by guanine nucleotides, a property which is commonly shared by the binding of catecholamines to β-adrenergic receptors [4,26–28]. Finally, the abilities of (−)-isoproterenol to displace [^{3}H]dihydroalprenolol binding and to stimulate adenylate cyclase activity in membranes and cyclic AMP accumulation in the intact cells were comparable. It can thus be concluded that the sites specifically labeled by [^{3}H]dihydroalprenolol represent the true β-adrenergic receptors of hamster white adipocytes.

A peculiar feature of hamster epididymal adipocytes is their number of β-adrenergic receptors (53 fmol/mg protein), which is rather low compared to the density reported in other species for white adipocytes of the same size, 280 fmol/mg protein in the rat [4] and 320 fmol/mg protein in human subcutaneous adipose tissue [14,15]. Moreover, this number of β_1-receptors appears extremely low compared to the density of α-adrenergic receptors reported in the same cells (800–1100 fmol/mg protein) [11,13], a situation which is not encountered in human omental [15] and subcutaneous fat [15,16], in which the β_1/α-adrenergic receptor number ratio is close to one. From these data and from the similarities between the respective affinities of the β- and α_2-receptors [13] towards norepinephrine in hamster adipocytes, one would predict that the α_2-inhibitory component of catecholamines would prevail over the β_1-stimulatory component on the lipolytic response of these cells. However, considering the dose-response

curves of lipolysis to norepinephrine and epinephrine, it is clear that the β_1-adrenergic stimulatory effect is predominant. This is particularly obvious when lipolysis is studied in the absence of theophylline and adenosine deaminase in the incubation, a condition under which no marked difference was observed between the magnitude of the maximal lipolytic response induced by the rather pure β-agonist isoproterenol and by the mixed α/β-agonists norepinephrine and epinephrine. However, confirming the observations of Schimmel [42], when theophylline and adenosine deaminase were added to the incubation system, the α_2-adrenergic antilipolytic effect of norepinephrine was clearly revealed, as shown by the rather weak ability of norepinephrine to stimulate lipolysis in comparison with the lipolytic potency of isoproterenol observed under the same conditions. A likely explanation for this amplification of the α_2-antilipolytic effect of catecholamines is that, because adenosine deaminase and theophylline suppress the accumulation and the binding of adenosine to its receptors [43], respectively, addition of these agents should prevent the competition, which normally occurs in the cell under physiological conditions, between the inhibitory effects of endogenous adenosine [44,45] and α_2-agonists on both adenylate cyclase and lipolysis [5,33,46]. Therefore, this raises the question of whether the α_2-adrenergic component of catecholamines plays any significant role in the in vitro adrenergic regulation of lipolysis in hamster adipocytes, when adenosine metabolism is unimpaired. The same question may also be raised in vivo, all the more so as the adenosine level in adipose tissue has been reported to be increased by sympathetic nerve stimulation [47]. Further studies on the turnover of adenosine in adipose tissue would probably provide an answer to these questions.

Finally, comparison of our present data on lipolysis in hamster epididymal adipocytes with those reported for the human abdominal subcutaneous adipose tissue [48] indicates that, in spite of differences in the respective proportion of α- and β-adrenergic receptors between these two tissues, their lipolytic responsivenesses to catecholamines are similar. The hamster white adipocyte may thus be an appropriate model for studies on the adrenergic regulation of lipolysis in, at least, the human abdominal subcutaneous fat.

Acknowledgements

This work was supported by grants from the INSERM (CRL 824005) and from the Université René Descartes (Paris V). We thank Miss. B. Vincent for the preparation of this manuscript.

References

1 Giudicelli, Y., Thotakura, N.R., Lacasa, D., Pecquery, R. and Agli, B. (1981) Biochem. Biophys. Res. Commun. 100, 621–628
2 Fain, J.N. (1973) Pharmacol. Rev. 25, 67–118
3 Williams, L.T., Jarett, L. and Lefkowitz, R.J. (1976) J. Biol. Chem. 251, 3096–3104
4 Giudicelli, Y., Lacasa, D. and Agli, B. (1982) Biochim. Biophys. Acta 715, 105–115
5 Burns, T.W., Langley, P.E. and Robison, G.A. (1971) Ann. N.Y. Acad. Sci. 185, 115–128
6 Ostman, J. and Efendic, S. (1970) Acta Med. Scand. 187, 471–476
7 Berlan, M. and Dang Tran, L. (1978) J. Physiol. Paris, 74, 601–608
8 Lafontan, M. and Agid, R. (1976) C.R. Acad. Sci. Paris, 282, 117–1120
9 Hittelman, K.J., Wu, C.F. and Butcher, R.W. (1973) Biochim. Biophys. Acta 304, 188–196
10 Giudicelli, Y., Agli, B., Brulle, D. and Nordmann, R. (1977) FEBS Lett. 83, 225–230
11 Pecquery, R. and Giudicelli, Y. (1980) FEBS Lett. 116, 85–90
12 Garcia-Sainz, J.A., Hoffman, B.B., Li, S.Y., Lefkowitz, R.J. and Fain, J.N. (1980) Life Sci. 27, 953–961
13 Pecquery, R. and Giudicelli, Y. (1982) Biochim. Biophys. Acta 714, 14–25
14 Jacobsson, B., Vauquelin, G., Wesslau, C., Smith, V. and Strosberg, A.D. (1981) Eur. J. Biochem. 114, 349–354
15 Burns, T.W., Langley, P.E., Terry, B.E., Bylund, D.B., Hoffman, B.B., Tharp, M.D., Lefkowitz, R.J., Garcia-Sainz, J.A. and Fain, J.N. (1981) J. Clin. Invest. 67, 467–475
16 Berlan, M. and Lafontan, M. (1980) Eur. J. Pharmacol., 67, 481–484
17 Engfeldt, P., Arner, P., Wahrenberg, H. and Ostman, J. (1982) J. Lipid Res. 23, 715–719
18 Birnbaumer, L., Pohl, S.L. and Rodbell, M. (1969) J. Biol. Chem. 244, 3468–3476
19 Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660–672
20 Feldman, H.A. (1972) Anal. Biochem. 48, 317–338
21 Hulme, E.C., Birdsall, N.J.M., Burgen, A.S.V. and Mehta, P. (1978) Mol. Pharmacol. 14, 737–750
22 Giudicelli, Y., Pecquery, R., Provin, D., Agli, B. and Nordmann, R. (1977) Biochim. Biophys. Acta 486, 385–398
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275

24 Giudicelli, Y. and Pecquery, R. (1978) Eur. J. Biochem. 90, 413–419
25 Giudicelli, Y., Lacasa, D. and Agli, B. (1982) Eur. J. Biochem. 121, 301–308
26 Maguire, M.E., Van Arsdale, P.M. and Gilman, A.G. (1976) Mol. Pharmacol. 12, 335–339
27 Giudicelli, Y., Agli, B. and Lacasa, D. (1979) FEBS Lett. 104, 431–436
28 Kent, R.S., De Lean, A. and Lefkowitz, R.J. (1980) Mol. Pharmacol. 17, 14–23
29 Lands, A.M., Arnold, A., McAuliff, J.P., Luduena, F.P. and Brown, T.G. (1967) Nature 214, 597–598
30 Cheng, Y. and Prusoff, W.H. (1973) Biochem. Pharmacol. 22, 3099–3108
31 U'Prichard, D.C., Bechtel, W.D., Rouot, B.M. and Snyder, S.H. (1979) Mol. Pharmacol. 16, 47–60
32 Aktories, K., Schultz, G. and Jakobs, K.H. (1981) Biochim. Biophys. Acta 676, 59–67
33 Aktories, K., Schultz, G. and Jakobs, K.H. (1979) FEBS Lett. 107, 100–104
34 Schimmel, J.R., Serio, R. and McMahon, K.K. (1980) Biochim. Biophys. Acta 632, 544–552
35 Chamness, G.C. and McGuire, W.L. (1975) Steroids 26, 538–542
36 Dax, E.M. and Partilla, J.S. (1982) Mol. Pharmacol. 22, 5–7
37 Andre, C., Vauquelin, G., Debacker, J.P. and Strosberg, A.D. (1981) Biochem. Pharmacol. 30, 2787–2796
38 Dax, E.M., Partilla, J.S. and Gregerman, R.I. (1982) J. Lipid Res. 23, 1001–1008
39 Dax, E.M., Partilla, J.S. and Gregreman, R.I. (1981) J. Receptor Res. 2, 267–283
40 Mendel, C.M. and Almon, R.R. (1978) Gen. Pharmac. 10, 31–40
41 Svoboda, P., Svartengren, J., Snochowski, M., Houstek, J. and Cannon, B. (1979) Eur. J. Biochem. 102, 203–210
42 Schimmel, R.J. (1980) Biochim. Biophys. Acta 629, 83–94
43 Trost, T. and Schwabe, U. (1981) Mol. Pharmacol. 19, 228–235
44 Fain, J.N. and Malbon, C.C. (1979) Mol. Cell. Biochem. 23, 1–27
45 Londos, C., Cooper, D.M.F. and Wolff, J. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2551–2554
46 Tsai, B.S. and Lefkowitz, R.J. (1979) Mol. Pharmacol. 16, 61–68
47 Fredholm, B.B. and Sollevi, A. (1981) J. Phyisol. 313, 351–367
48 Berlan, M., Lafontan, M. and Dang Tran, L. (1980) J. Physiol. Paris, 76, 133–146