Human fat cell beta-adrenergic receptors: beta-agonist-dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta$_1$-antagonists

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Abstract Beta-adrenergic receptors were characterized in human fat cell membranes using ^{125}I-labeled cyanopindolol (^{125}I-labeled CYP) and highly selective beta 1-antagonists. The iodinated radioligand bound saturably and specifically to a single class of high affinity binding sites. The number of binding sites determined with ^{125}I-labeled CYP closely agreed with that determined with two other tritiated radioligands: [^{3}H]dihydroalprenolol and [^{3}H]CGP-12,177. Since ^{125}I-labeled CYP does not discriminate between beta 1- and beta 2-adrenoceptors, the densities of the two receptor subtypes were determined from the competition curves of ^{125}I-labeled CYP by highly selective beta 1-antagonists (bisoprolol, ICI-89,406, CGP-20,712A, and LK-204,545). Moreover, in order to enable correlation with binding data, the regulation of adenylate cyclase activity and of lipolysis was tested with various beta-agonist and antagonist compounds. The results obtained on fat cell membranes from abdominal subcutaneous adipose tissue demonstrated the following. *1*) ^{125}I-labeled CYP represents a valuable tool for the quantification and the delineation of beta-receptor subtypes. *2*) The presence of sodium ions in binding buffers causes a modification of the affinity of beta-sites for some beta-antagonists. *3*) The human fat cell beta adrenergic receptor population defined by nonselective radioligands is composed of two subtypes that can be interpreted in terms of classic beta 1- and beta 2-adrenergic receptor subtypes as assessed by competition studies with highly selective antagonists; beta 2-sites are predominant (60–70% of ^{125}I-labeled CYP sites) in the adipocytes of slightly overweight women. *4*) Results support the idea that beta 1- as well as beta 2-adrenergic receptors are coupled with adenylate cyclase and involved in the induction of lipolysis. *5*) The results focus on the interest in some beta 2-agonist drugs (zinterol, clenbuterol) as partial inductors of lipolysis, with the lipolytic efficacies of these compounds being well correlated with their efficacies at ^{125}I-labeled CYP sites. — **Mauriege, P., G. De Pergola, M. Berlan, and M. Lafontan.** Human fat cell beta-adrenergic receptors: beta agonist-dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta 1-antagonists. *J. Lipid Res.* 1988. **29:** 587–601.

Supplementary key words adipocytes • beta 1-/beta 2-adrenergic receptors • beta-2 agonists • lipolysis • human adipose tissue • clenbuterol • zinterol • bisoprolol • CGP-20,712A

Catecholamines are the major lipolytic agents in human fat cells; their action is mediated by stimulation of beta- and alpha 2-adrenergic receptors (1–3). Our recent investigations have focused on the interplay existing between beta- and alpha 2-adrenergic receptors in the regulation of lipolysis in human adipocytes (4). The strength of these results would certainly be extended by definition of the stoichiometry of beta- and alpha 2-site coupling to adenylate cyclase and also by the determination of the number of receptors actually involved in the generation of the effects (i.e., the number of functional and spare receptors for each kind of receptor site). In order to be clarified, the two last steps require a better definition of adrenergic receptor properties in human fat cells and the optimization of the binding approaches for accurate receptor evaluation.

Abbreviations, formulas, and terminology: (−)[^{3}H]CGP-12177 (^{3}H-CGP), 4-(3-tertiarybutyl-amino-2-hydroxypropoxy)-benzimidazole-2-one hydrochloride; [^{3}H]DHA, [^{3}H]dihydroalprenolol; bisoprolol hemifumarate, bis[1-(4-[2-isopropylethoxy]-methyl)-phenoxy]-3-isopropyl-amino-2-propanol; ICI-118,551 hydrochloride, [*erythro*-DL-1-(7-methylindan-4-yloxy)-3-isopropyl-aminobutan-2-ol]; ICI-89,406, [1(2-cyanophenoxy)-3β-(3-phenylureido)-ethylamino-2-propanol; KRBA, Krebs Ringer bicarbonate-albumin buffer; ^{125}I-labeled CYP, ^{125}I-labeled cyanopindolol.

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This report will focus on human fat cell beta-adrenoceptors. Examination of various data from the literature reveals that the nature of the beta-adrenergic receptor of white fat cells of various species (rat, hamster, pig, dog) is quite unclear as compared with beta-adrenergic receptor subtypes of heart, lung, smooth muscle, lymphocytes, or hepatocytes (1). On the basis of the orders of potency of epinephrine and norepinephrine in their initiation of lipolytic response, they appear to be mainly of the beta 1-subtype. When synthetic agonists or antagonists were used with the aim of improving the characterization, the classification became very unclear. For example, some investigators proposed that the beta-adrenoceptor of the most commonly used species, the rat, was an atypical beta-adrenoceptor (1, 5–7). The adipocytes of other species have generally been less explored (1, 3, 8).

The human fat cell beta-adrenergic receptor, which is of prime interest for our understanding of fat cell function and disease, is still poorly defined. Previous data obtained by various groups from in vivo experiments or functional studies on isolated human fat cells or membranes suggested that it exhibited some beta 1-specificity (1). However, this definition was based on the use of a limited amount of pharmacological tools of rather poor selectivity. For the moment, it is not clearly settled whether the beta-adrenoceptor site that mediates catecholamine-induced lipolysis in human fat cell is a typical beta 1-site or whether a mixture of beta 1- and beta 2-sites coexists in the human fat cell as shown in a variety of other tissues and cell lines. In addition, the existence of a beta-receptor that does not fit in the standard beta 1-/beta 2-classification cannot be entirely excluded for the moment.

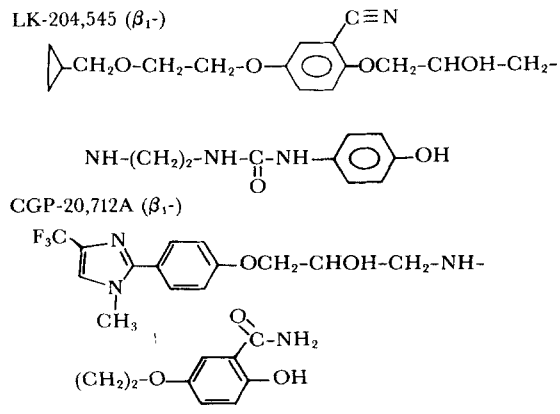
The beta-adrenergic receptor can be quantified in a wide variety of tissues by use of appropriate tritiated or iodinated radioligands. In intact adipocytes and human fat cell membranes, beta-sites have essentially been identified with [^{3}H]dihydroalprenolol ([^{3}H]DHA) (4, 9–11). This radioligand is not suitable for the identification of the receptors on intact cells, probably because of its strong lipophilicity. Some attempts of beta-site identification have also been made on intact human adipocytes with a hydrophilic radioligand [^{3}H]CGP-12,177 ([^{3}H]CGP) which appears to be the most suitable compound for studies on fat cells (12).

In the present study carried out on human fat cell membranes, we tried to extend and validate the use of different radioligands for beta-receptor identification and for the exploration of their involvement during lipolytic stimulation. We quantified the beta receptors using three radioligands ([^{3}H]DHA, [^{3}H]CGP, and ^{125}I-labeled CYP) (13) as beta-receptor probes. Moreover, since these radioligands are not subtype-selective, we also used various recently synthesized highly selective beta 1- and beta 2-antagonists such as ICI-89,406 (14, 15), LK-204,545 (16), CGP-20,712A (17), and bisoprolol (18) to explore the possible existence of beta 1- and beta 2-adrenoceptor subtypes in human fat cell membranes. Functional studies on lipolysis and cyclase activation were also used to define the validity and the limits of the binding data. The interest of the different tools will also be discussed.

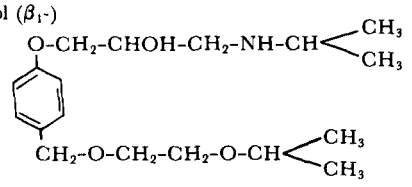
MATERIALS AND METHODS

Materials

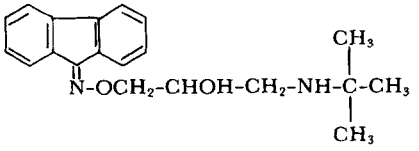
[^{3}H]CGP-12,177 (40 Ci/mmol), [^{3}H]dihydroalprenolol (90 Ci/mmol), ^{125}I-labeled cyanopindolol (2200 Ci/mmol), [8-^{3}H]cyclic AMP (25 Ci/mmol), and [α-^{32}P]ATP (30 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England; (−) and (+) isoproterenol hydrochloride, ascorbic acid, catechol, pargyline, isobutyl methyl xanthine, and bovine serum albumin, fraction V, were obtained from Sigma Chemical Co. (St. Louis, MO). The following compounds were provided as gifts: (−) and (+) propranolol, ICI-118,551, ICI-89,406, practolol, and atenolol from ICI Ltd. (Alderly Park, England); salbutamol from Glaxo Group Research (Ware, England); betaxolol from Synthelabo (Paris, France); phentolamine methane sulfonate, metoprolol, terbutaline, CGP-12,177, and CGP-20,712A from Ciba Geigy Corporation (Basel, Switzerland); pindolol and LK-204,545 from Sandoz Ltd. (Basle, Switzerland); bisoprolol from Merck Ltd. (Darmstadt, FRG); zinterol from Mead-Johnson and Co. (Evansville, IN); clenbuterol from Boehringer (Ingelheim, FRG); acebutolol from Specia (Paris, France); and dobutamine hydrochloride from Lilly Research Labs. (Indianapolis, IN). Collagenase and adenosine deaminase came from Boehringer Mannheim (Mannheim, FRG). All other chemicals were of the highest purity commercially available. IPS-339 was kindly provided by Dr. G. Leclercq (Strasbourg, France). The structures of the recently available beta-antagonists used in the present work are as follows.



bisoprolol (β_1-)



IPS-339 (β_2-)



Isolation of human adipocytes and preparation of fat cell ghosts

Abdominal subcutaneous adipose tissue samples were obtained from premenopausal women (25 to 45 years of age) undergoing elective surgical lipectomy. Although the mean body mass index (ratio: weight(kg)/height(m)) was 26.3 ± 1.3, n = 30, the patients were otherwise healthy and none had any identified metabolic or endocrinological disorders. Subjects fasted overnight before tissue removal; general anesthesia was induced with an association of pentothal, droleptan, and fentanyl. The patients did not receive drugs active on the autonomic nervous system or modifying catecholamine levels. After surgical excision, the adipose tissue was quickly transported to the laboratory in cooled, sterile physiological saline (0.9% NaCl; 5 mM HEPES; pH 7.4) and used within 20 min after removal. According to the method of Rodbell (19), isolated adipocytes were obtained by collagenase digestion of adipose fragments in Krebs-Ringer bicarbonate buffer containing albumin (3.5 g/100 ml) (KRBA) and glucose (0.6 mmol/100 ml) at pH 7.4 and 37°C under gentle shaking at around 60 cycles/min. At the end of incubation, the fat cells were filtered through a silk screen and washed three times with KRBA buffer to eliminate collagenase. Then, the isolated adipocytes were washed four times in a hypotonic lysing medium composed of 2.5 mM $MgCl_2$, 1 mM $KHCO_3$, 2 mM Tris-HCl (pH 7.5), and containing several protease inhibitors: benzamidine (100 μM), phenylmethylsulfonyl fluoride (100 μM), leupeptine (1 μg/ml), and EGTA (3 mM).

Crude adipocyte ghosts were pelleted by centrifugation (40,000 *g*, 15 min) at 4°C, washed twice in the lysing buffer supplemented with 2 μg/ml adenosine deaminase, and pelleted under similar conditions. At the end of the washing procedure, they were resuspended in the same buffer and immediately frozen. The membrane preparation was stored at −80°C and generally used within 1–2 weeks.

Binding assays with crude membranes

Thawed frozen membranes were rehomogenized with four pestle strokes in a Potter apparatus immediately before use and washed once with the buffer commonly used in binding studies (10 mM $MgCl_2$; 50 mM Tris-HCl; pH 7.5). Binding studies with both beta-antagonist radioligands, [^{3}H]DHA and [^{3}H]CGP, were conducted in a final volume of 400 μl as previously reported (20) with only minor modifications. Incubations were carried out at 37°C in a water bath for 20 min under constant shaking at 140 cycles/min.

When using ^{125}I-labeled CYP, the incubation mixture consisted of 50 μl of radioligand solutions ranging in final concentration from 5 to 400 pM and 50 μl of the membrane suspension (0.5–1 mg/ml protein) made up to a final volume of 200 μl with 25 mM Tris-HCl, 120 mM NaCl, 10 mM $MgCl_2$, 1.1 mM ascorbic acid (pH 7.5). Binding assays were performed at 37°C for 45 min under conditions previously described (20). Nonspecific binding was evaluated in the presence of 10 μM (−) propranolol. Generally, at the end of incubation, the reaction was stopped by the addition of 4 ml of ice-cold incubation buffer followed by rapid filtration under reduced pressure through Whatmann GF/C glass-fiber filters placed on a Millipore manifold. The filters were then washed twice with 10-ml portions of ice-cold incubation buffer. For ^{125}I-labeled CYP binding, the radioactivity retained on the wet filters was measured in a Packard gamma counter at an efficiency of 75%.

For [^{3}H]DHA and [^{3}H]CGP binding, the filters were placed in minivials containing 4 ml of liquid scintillation mixture (Ready-Solv. MP, Beckman) and counted in a Packard scintillation spectrometer at an efficiency of 35–40%. Specific binding was defined as the total binding minus the nonspecific binding. Specific binding was directly proportional to the protein concentration at the concentrations of ligand and membrane used. The protein content was determined according to the method of Lowry et al. (21), using bovine serum albumin as standard.

Lipolysis measurements

Isolated fat cells obtained after collagenase treatment were incubated in 1 ml of KRBA (pH 7.4) containing glucose (6 mM) at 37°C under a gas phase of 95% O_2:5% CO_2 with gentle shaking (60 cycles/min) in a water bath, as previously described (20). Pharmacological agents at suitable dilutions were added to the cell suspension just before the beginning of the assay in 10-μl portions to obtain the desired final concentration. After 90 min of incubation, the plastic minivials were placed in an ice bath, and 200-μl aliquots of infranatant were removed for enzymatic determination of glycerol released in the incubation medium, which was taken as the index of fat cell lipolysis. Total lipid was evaluated gravimetrically after extraction. Ascorbic acid (0.1 mM) was included in the incubation medium in order to prevent catecholamine degradation. The lipolytic activity of the fat cells was tested with isoproterenol (full beta-agonist), clenbuterol, zinterol,

salbutamol, terbutaline (beta 2-agonists), and dobutamine (beta 1-agonist). For testing beta-adrenergic blocking agents, the incubation medium was supplemented with isoproterenol (1 μM) to promote an increment of lipolysis. Some experiments were conducted with several selective beta 1-adrenergic antagonists (betaxolol, metoprolol, CGP-20,712A, atenolol, practolol, acebutolol, bisoprolol, and LK-204,545). Other studies were performed with non-selective beta-adrenergic antagonists such as (−)propranolol, (−)pindolol, (−)CGP-12,177, and two selective beta 2-blocking drugs (ICI-118,551 and IPS-339).

Adenylate cyclase assays

Adenylate cyclase assays were performed in duplicate in a final volume of 50 μl consisting of 10 μl of membrane suspension (20–30 μg of protein), 20 μl of buffer or effectors at the suitable concentration, and 20 μl of concentrated incubation mixture containing the following agents to reach the final composition: 40 mM Tris-HCl, 2 mM $MgCl_2$, 1 μM GTP, 100 μM EGTA, 0.5 mM IBMX, 0.5 mM cAMP, 20 μM ATP, 0.5 μCi of [α-^{32}P]ATP, 5 mM creatine phosphate, 0.2 mg/ml creatine kinase, 0.2 mg/ml bovine serum albumin, and 2 μg/ml adenosine deaminase, pH 7.4. Assays were conducted for 20 min at 30°C. The reaction was initiated with 10 μl of thawed membranes washed once and then resuspended in 5 mM Tris-HCl, 0.5 mM $MgCl_2$, pH 7.5, and containing antiprotease compounds: leupeptin, 5 μl/ml; pepstatin, 2 μg/ml; soybean trypsin inhibitor, 10 μg/ml; and L-1-chloro-3-(4-tosylamido)4-phenyl-2-butanon (TPCK), 0.05 mM. The reaction was stopped by addition of 100 μl of a solution containing 2% lauryl sulfate, 40 mM ATP, 1.4 mM cAMP, and trace amounts of [8-^{3}H]cAMP (7,000–8,000 counts/min). [^{32}P]cAMP was isolated by Dowex 50 WX 8 and neutral alumina chromatography as described by Salomon, Londos, and Rodbell (22) with a 75–80% yield, and measured for the radioactivity with an LKB liquid scintillation spectrometer.

Data analysis

The values presented are means ± SEM. The significance of differences was tested by Student's paired *t*-test. All experiments were performed in duplicate and repeated 4–10 times unless otherwise specified. The equilibrium dissociation constants (or affinities) of the radioligands used (K_D) and the maximal number of binding sites (B_{max}) were analyzed according to the method of Scatchard with a computerized method. Half-maximal inhibitory concentrations (EC_{50} values) and slope factors (pseudo-Hill coefficients) for each competitor were calculated from computer-iterative curve fitting to one- or two-site models (giving the relative beta 1-/beta 2-ratio as well as K_DH and K_DL values), using LIGAND, a nonlinear least square computer program (23).

RESULTS

Binding of (−)^{125}I-labeled cyanopindolol to human fat cell membranes

The radioiodinated ligand has never been used for beta-adrenoceptor identification on human fat cell membranes. The first part of our investigations was devoted to the characterization of its binding properties. The characteristics of ^{125}I-labeled CYP binding to crude fractions of human fat cell membranes were explored in a standard buffer (Tris-Mg^{2+}) containing 50 mM Tris-HCl and 10 mM $MgCl_2$, pH 7.5, and in a buffer supplemented with Na^+ ions (Tris-Mg^{2+}-Na^+) composed of 25 mM Tris-HCl, 10 mM $MgCl_2$, and 120 mM NaCl, pH 7.5. Specific binding of ^{125}I-labeled CYP was defined as binding displaceable by 10 μM (−)propranolol or 10 μM (−)isoproterenol. Both drugs gave equivalent values for nonspecific binding which reached a good plateau as shown later in competition studies.

Specific binding (**Fig. 1**) was saturable and of high affinity. Scatchard analysis indicated a homogeneous population of binding sites with an equilibrium dissociation constant (K_D) of 115 ± 15 pM (n = 7) and a maximum binding (B_{max}) of 175 ± 20 fmol/mg of protein (n = 7). Hill coefficients were not significantly different from unity (0.95 ± 0.02), indicating a lack of cooperativity in binding.

Association of ^{125}I-labeled CYP to its sites (four separate experiments) was rapid with equilibrium conditions reached within 15 min and maintained for at least 60 min. The initial rate constants (k_{ob}) for the association reaction were 0.090–0.116 min^{-1}. The dissociation was determined at 37°C by incubating adipocyte membranes with ^{125}I-labeled CYP to equilibrium and then adding 10^{-5} M propranolol and measuring residual specific binding at subsequent time intervals. Dissociation was rather slow but led to monophasic dissociation curves as revealed by computerized treatment of experimental data; the first order dissociation rate constant k_2 was ca. 0.010 min^{-1} and calculation of the second order dissociation rate constants k_1 yielded values from 0.180 to 0.226 $min^{-1}M^{-1}$. The equilibrium dissociation constant (K_D values) estimated from four different analyses from the ratio k_2/k_1 of kinetic data (60–80 pM) correlated well with K_D values obtained by analysis of saturation curves on the same batches (90–160 pM).

Since ^{125}I-labeled CYP has been described to possess some intrinsic activity in rat fat cells (24), saturation experiments were also performed in the presence and absence of 100 μM GppNHp (a nonhydrolyzable analogue of GTP). The compound had no significant influence on the binding parameters.

Concerning the influence of buffer composition, in both buffers ^{125}I-labeled CYP was found to label a homogene-

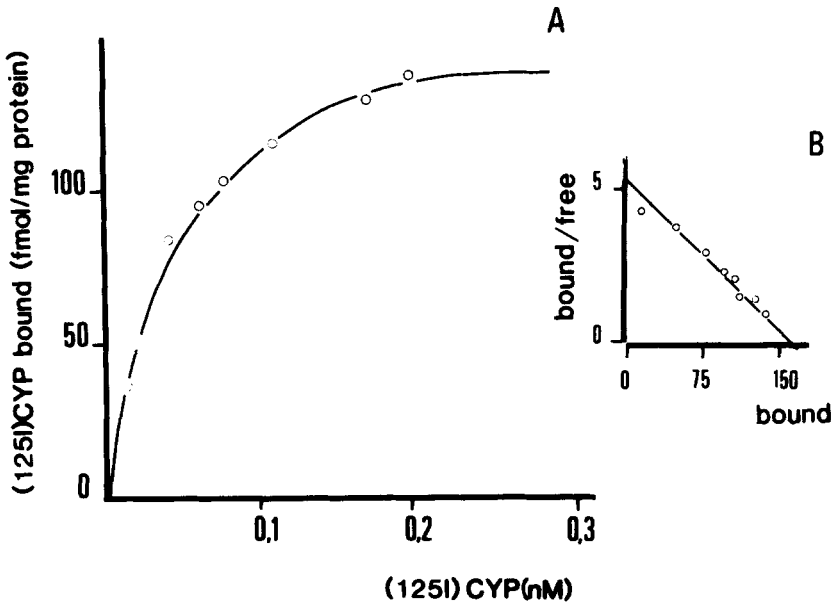


Fig. 1. Specific binding of ^{125}I-labeled CYP to human fat cell membranes from one representative experiment. Panel A: As a function of increasing concentrations of ^{125}I-labeled CYP. Human fat cell membranes were incubated with the indicated concentrations of ^{125}I-labeled CYP for 45 min at 37°C and specific binding was determined as described in Materials and Methods. Nonspecific binding was defined with 10^{-5} M unlabeled propranolol. Panel B: Scatchard plot of specific binding of ^{125}I-labeled CYP derived from the saturation curves (panel A). Final parameter estimates were obtained by a computerized analysis as described in Materials and Methods.

ous population of sites as indicated by the linearity of Scatchard transformation of saturation curves and also through computer-modelling, which gave no statistical significance for a model more complex than a single-site model. In addition, Hill coefficients were again not significantly different from unity. The effect of buffer composition was tested on the same batches of membranes to avoid discrepancies linked to variations in the total number of sites in the various fat samples used. The maximum density of ^{125}I-labeled CYP binding sites was significantly ($P < 0.05$) lower in Tris-Mg^{2+} buffer, i.e., 129 ± 7 fmol/mg protein versus 165 ± 15 fmol/mg protein in Tris-Mg^{2+}-Na^{+} buffer. K_D values were unaffected by the buffer composition (115 ± 15 pM in Tris-Mg^{2+} versus 120 ± 25 pM in Tris-Mg^{2+}-Na^{+} buffer).

Comparative study with tritiated and iodinated radioligands

In order to validate the accuracy and reliability of the data obtained with ^{125}I-labeled CYP, quantification of beta-sites was carried out at 37°C on seven different batches of human fat cell membranes with the hydrophilic radioligand [^{3}H]CGP-12,177 (12) and with the more lipophilic ligands [^{3}H]DHA and ^{125}I-labeled CYP; the two latter drugs had an equivalent partition coefficient in octanol/binding buffer (not shown). In equilibrium binding experiments, the number of binding sites (B_{max}) for all three ligands was not statistically different (**Table 1**). The Scatchard transformation of saturation curves was linear and Hill coefficients were not different from unity.

Displacement studies with selected compounds interacting with beta-adrenergic receptor sites

Stereospecificity. An important criterion for receptor identification is that the receptor should exhibit stereoselectivity, i.e., the ability of competing drugs to inhibit binding is usually higher for (−) isomers than for (+) isomers. **Fig. 2** represents typical competition curves obtained with (−) and (+) propranolol (antagonist) and (−) and (+) isoproterenol (agonist). Clearly, (−) isomers are much more effective competitors than (+) isomers; ^{125}I-labeled CYP sites offer the stereoselectivity expected for true beta-adrenergic receptors.

Nonselective beta-antagonists. Three different compounds were used: (−) propranolol, (−) pindolol, and (−)CGP-12,177. They gave steep displacement curves in competing with ^{125}I-labeled CYP or [^{3}H]DHA (not shown) with Hill coefficients of 1. The curves are adequately modelled by a single homogeneous class of binding sites (see Fig. 3D). This is to be expected since the compounds are considered to have equal affinity for beta 1- and beta 2-adrenergic receptors.

Subtype-selective beta-adrenergic antagonists. To rigorously define the relative proportion of beta 1- and beta 2-adrenergic receptor sites in human fat cell membranes,

TABLE 1. Comparison of beta-adrenergic receptor binding site number from simultaneous determinations of ^{125}I-labeled CYP, [^{3}H]DHA,and [^{3}H]CGP binding in human fat cell membranes of abdominal subcutaneous tissue at 37°C

Radioligand	n	B_{max}[a]	K_D[b]	n_{Hill}[c]
		fmol/mg protein	*nM*	
^{125}I-labeled CYP	7	175 ± 19	0.116 ± 0.014	0.98 ± 0.02
[^{3}H]DHA	7	150 ± 20	1.5 ± 0.4	0.97 ± 0.05
[^{3}H]CGP	7	154 ± 20	0.9 ± 0.1	1.00 ± 0.03

Parameters were determined from equilibrium studies by the computer-aided (23) transformation of the saturation binding data obtained under the binding conditions described in Materials and Methods. The data are expressed as means ± SEM of (n) separate experiments.

[a] B_{max} is the total number of binding sites.

[b] K_D is the equilibrium dissociation constant of binding.

[c] n_{Hill}, slope factors (pseudo-Hill coefficients) defined as described in Materials and Methods.

experiments were carried out with various compounds presenting clear-cut differences in their chemical nature and in their subtype selectivity. Cardioselective compounds such as metoprolol, atenolol, betaxolol, and acebutolol were tested with the most highly selective drugs recently synthesized: bisoprolol (18), CGP-20,712A (17), and LK-204,545 (16). The beta 2-selective drugs were ICI-118,551 and IPS-339.

All the compounds used produced a concentration-dependent inhibition of ^{125}I-labeled CYP binding and were able to completely displace all specifically bound radioligand. The results are summarized in **Fig. 3;** the order of potency for displacement of ^{125}I-labeled CYP was: CGP-20,712A > betaxolol > ICI-118,551 > bisoprolol > LK-204,545 > atenolol > acebutolol. This rank order is not typical for a beta 1-adrenoceptor. All the competition curves were shallow and complex, indicating more than one subtype population; slope factor values (pseudo-Hill coefficients) for the displacement of ^{125}I-labeled CYP were significantly different from unity indicating the presence of a heterogeneous population of binding sites in each membrane preparation. Assuming the presence of two classes of beta-adrenoceptors, the competition curves with beta 1-selective antagonists were subjected to a computer-aided curve-fitting technique. The results are depicted in **Table 2;** they indicate that beta 1-adrenoceptors represent about 40% of the total number of ^{125}I-labeled CYP binding sites. It is noticeable that the five subtype-selective beta 1-antagonists used here gave beta 1-/beta 2-ratio values which were not statistically different.

Surprisingly, when the analysis was conducted with the two beta 2-selective antagonists, the relative proportion of beta 1- and beta 2-sites derived from computer analysis of binding data gave a ratio that was significantly different (Table 2). This result is not easily explainable although some discrepancies have been reported with ICI-118,551 on intact human adipocytes (12). To assess this observation, a similar analysis was also carried out with another radioligand, [^{3}H]DHA. A similar kind of result was

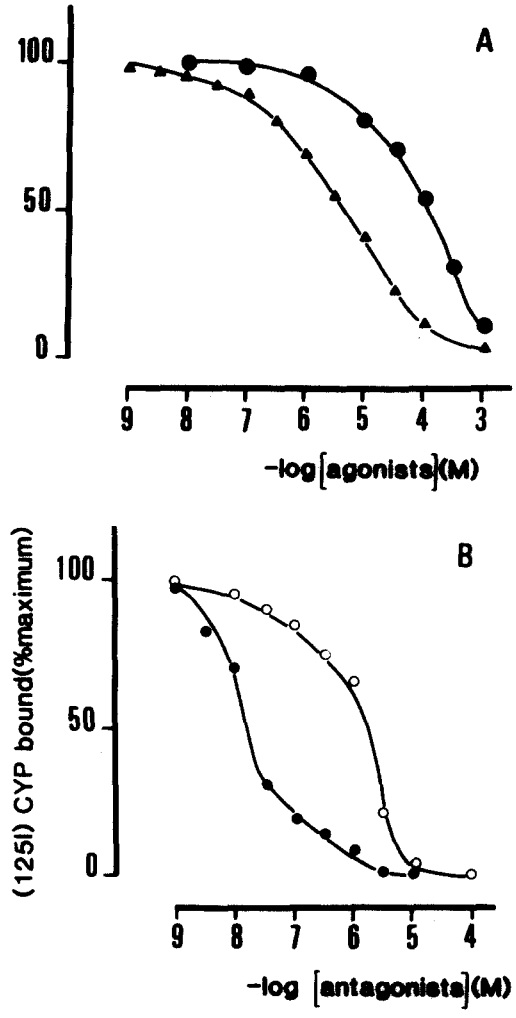


Fig. 2. Inhibition of specific ^{125}I-labeled CYP binding to human fat cell membranes by (+) (●) and (−) (▲) isoproterenol (panel A) or (+) (○) and (−) (●) propranolol (panel B). Binding was studied at 37°C after a 45-min incubation period in the presence of increasing concentrations of competing agents as reported in Materials and Methods. The results are expressed as percentages of ^{125}I-labeled CYP specifically bound.

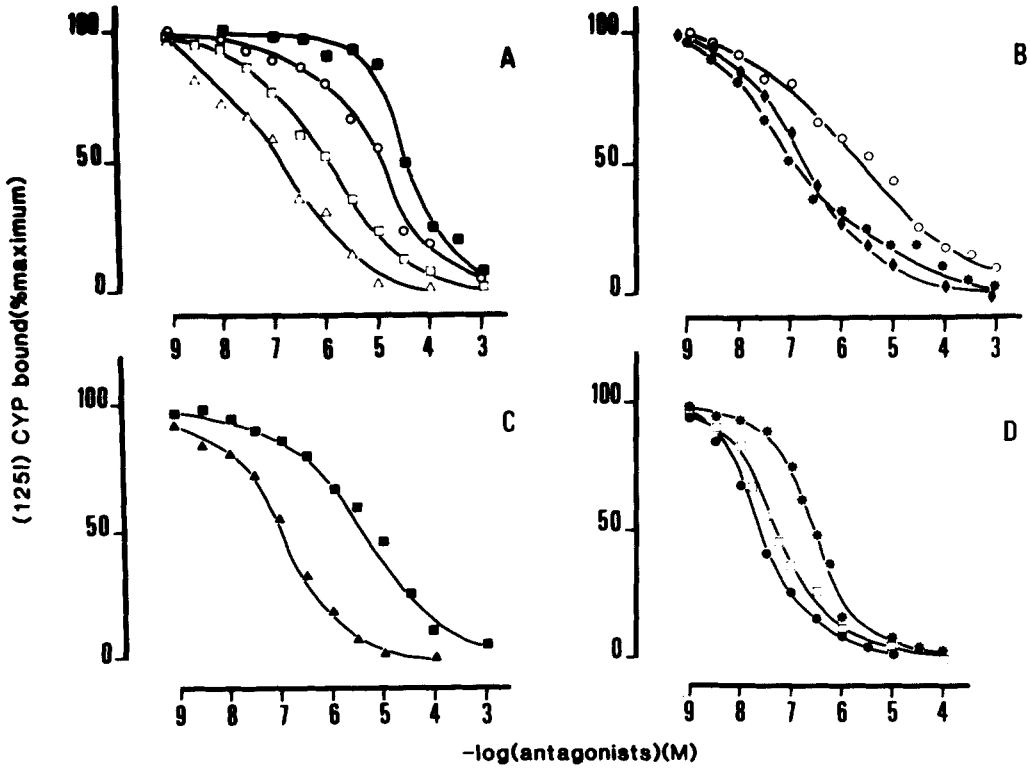


Fig. 3. Inhibitory effects of adrenergic antagonists on the binding of ^{125}I-labeled CYP to human fat cell membranes. Binding was studied at 37°C after a 45-min incubation period in Tris-Mg^{2+}-NaCl buffer as described in Materials and Methods, in the presence of various competing agents. Beta 1-selective antagonists were used; panel A: (○) atenolol, (■) acebutolol, (△) betaxolol, (□) metoprolol; and panel B: (♦) bisoprolol, (*) LK-204,545, (○) CGP-20,712A as well as beta 2-selective (panel C: (■) ICI-118,551, (▲) IPS-339) and nonselective (panel D: (●) propranolol, (□) CGP-12,177, (*) pindolol) adrenergic antagonists. The results are expressed as percentages of the ^{125}I-labeled CYP specifically bound. Points are the means of five to eight experiments done in duplicate. Standard errors of the means were deleted for clarity. Data given by computer-assisted analysis of the curves obtained with selective antagonists in each tissue of the different patients are summarized in Table 2. Mean K_i values for nonselective antagonists were 7.5 ± 0.5 nM for (−)propranolol; 6.0 ± 1.2 nM for CGP-12,177; and 237 ± 90 nM for pindolol.

noticed; the discrepancies in the relative ratios obtained with beta 1- and beta 2- antagonists was still observed although the studies were realized on batches of fat cell membranes different from those used for ^{125}I-labeled CYP displacement studies (Table 2).

Another kind of discrepancy was observed in these studies; 120 mM NaCl affected antagonist interaction with the ^{125}I-labeled CYP binding sites. Comparison of the antagonist dissociation constant values for beta 1- and beta 2-adrenergic receptor antagonists obtained in the two different buffers revealed some interesting results reported in **Fig. 4** and **Table 3.** K_i values were strikingly modified by the inclusion of 120 mM NaCl in the binding buffer. K_i values were increased for LK-204,545, decreased for CGP-20,712A and ICI-118,551, and unchanged for betaxolol, bisoprolol, and ICI-89,406. The heterogeneity of sites (as attested by Hill coefficients) was unchanged and the relative proportion of beta 1- and beta 2-sites defined by computer-modelling of the curves was also not significantly altered. Thus, the affinity of human fat cell beta sites for beta adrenoceptor antagonists was variably modified depending on the nature of the incubation buffer utilized.

Lipolytic potencies of various beta-agonists

In order to provide physiological correlation to our binding data, we studied the regulation of lipolysis on isolated fat cells with several compounds used in the binding analyses.

Firstly, our attention was focused on the synthetic agonists, since we recently reported an extended analysis of the effects of the physiological amines on human fat cells (4, 25). **Fig. 5A** shows the lipolytic response initiated by various beta-agonists expressed as a percent value of the maximal effect initiated by 100 μM isoproterenol. The K_a values given in **Table 4** were calculated on the basis of the maximal effect initiated by each lipolytic agent (Fig. 5B). The relative order of potency in the initiation of the maximal lipolytic activity was isoproterenol > terbutaline = dobutamine > zinterol > clenbuterol > salbutamol, the

TABLE 2. Relative concentration of beta 1- and beta 2-adrenergic receptors in human fat cell membranes: characteristics of competition between various beta 1- and beta 2-selective antagonists and ^{125}I-labeled CYP or [^{3}H]DHA for binding to beta 1- and beta 2-adrenoceptor subtypes

	n	Beta-1[a]		Beta-2	
		%	K_D	%	K_D
			nM		*nM*
^{125}I-labeled CYP binding					
Betaxolol	12	38 ± 4	4 ± 1	62 ± 4	440 ± 120
LK-204,545	6	35 ± 3	1 ± 0.4	65 ± 3	180 ± 50
CGP-20,712A	7	45 ± 5	4 ± 1.5	55 ± 5	1020 ± 230
Bisoprolol	4	32 ± 5	4 ± 1	68 ± 5	1360 ± 580
ICI-89,406	4	42 ± 4	2 ± 1.5	58 ± 4	420 ± 90
IPS-339	3	59 ± 14	165 ± 26	41 ± 14	0.8 ± 0.4
ICI-118,551	9	61 ± 4	712 ± 170	39 ± 4	4.6 ± 0.2
[^{3}H]DHA binding					
Betaxolol	8	28 ± 3	0.2 ± 0.1	72 ± 4	450 ± 280
LK-204,545	9	43 ± 9	2.4 ± 1.4	57 ± 9	180 ± 50
CGP-20,712A	3	30 ± 10	0.6 ± 0.3	70 ± 10	1500 ± 600
ICI-118,551	8	72 ± 4	710 ± 170	28 ± 3	4.6 ± 2

[a]Values (the percentages and affinities for beta 1- and beta 2-adrenergic receptors) were obtained from computer analysis of direct ^{125}I-labeled CYP/ or [^{3}H]DHA/competing drug displacement curves. The competition curves of specific ^{125}I-labeled CYP binding were determined for each drug at 10 to 12 different concentrations as indicated in Fig. 3. Nonlinear analysis of the competition curves yielded the two dissociation constants and the percentage of receptor distribution. Each value is the mean ± SEM of (n) separate experiments that were carried out on corresponding fat cell batches when possible or on different batches. Using beta 1-selective antagonists, the sites of recognition of high affinity are considered as being of the beta 1-type, while the sites of low affinity correspond to the beta 2-sites; an inverse proposal is considered for the beta 2-selective antagonists.

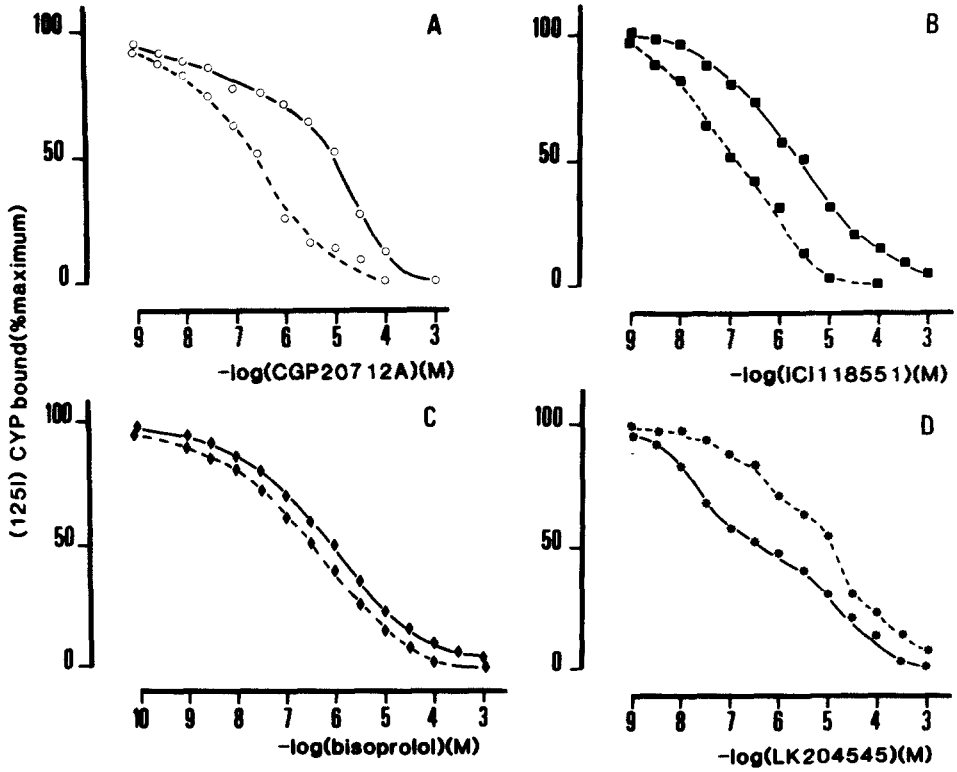


Fig. 4. Influence of NaCl on ^{125}I-labeled CYP competition by beta-antagonists in human fat cell membranes at 37°C. ^{125}I-Labeled CYP binding (250 pM) to human fat cell membranes was measured in a Tris-Mg^{2+} buffer (see Materials and Methods) in the absence (----) and presence (—) of 120 mM NaCl at increasing concentrations of beta-antagonists. The assay conditions were identical to those in Fig. 3. The results are expressed as percentages of ^{125}I-labeled CYP specifically bound. Points are means of four to seven experiments done in duplicate. Data given by computer-assisted analysis of the curves obtained in each tissue are summarized in Table 3.

TABLE 3. Dissociation constants (K_i) and slope factor values (n_{Hill}) calculated from displacement of ^{125}I-labeled CYP from human fat cell membranes in a buffer with 120 mM NaCl (Tris-Mg^{2+}-NaCl) or without (Tris-Mg^{2+})

Competitor	n	Tris-Mg^{2+}		Tris-Mg^{2+}-NaCl	
		K_i[a]	n_{Hill}[b]	K_i	n_{Hill}
		nM		*nM*	
Betaxolol	7	100 ± 20	0.5 ± 0.1	70 ± 20	0.5 ± 0.1
CGP-20,712A	6	75 ± 30	0.5 ± 0.1	2700 ± 900[c]	0.5 ± 0.1
LK-204,545	7	1620 ± 700	0.4 ± 0.1	110 ± 40[c]	0.4 ± 0.1
ICI-118,551	5	110 ± 40	0.6 ± 0.1	3200 ± 1500[c]	0.6 ± 0.1
Bisoprolol	4	240 ± 80	0.5 ± 0.1	140 ± 50	0.6 ± 0.1
ICI-89,406	3	100 ± 40	0.6 ± 0.1	60 ± 10	0.4 ± 0.1

Values are the mean ± SEM of (n) separate experiments performed on corresponding batches when possible.
[a] K_i values were calculated from the median effective concentration values (EC_{50}) according to the general formula of Cheng and Prusoff: $K_i = EC_{50}/[1 + (L)/K_D$ eq.] where (L) is the concentration of ligand used in the assay (250 pM) and K_D eq. is the equilibrium dissociation constant as determined by Scatchard analysis.
[b] n_{Hill}, Slope factor (pseudo-Hill coefficient) values were calculated using an iterative sigmoidal curve fitting program as described in Materials and Methods.
[c] Significantly different from result obtained in Tris-Mg^{2+} buffer ($P < 0.05$) by Student's paired *t* test.

latter being weakly efficient under our working conditions.

Neither compound was as active as isoproterenol; dobutamine, which is classified as a selective beta 1-receptor agonist by virtue of its ability to produce cardiac stimulant effects without action on blood pressure, exhibited a limited potency similar to that of the beta 2-agonist, terbutaline. Zinterol, another beta 2-agonist (30- to 100-fold, beta 2-selective) promoted a clear lipolytic action which was 30–40% that of isoproterenol. Clenbuterol, a drug recently investigated for its action as a "repartitioning" agent influencing muscle accretion and fat deposition in farm animals (26), was weakly active in human fat cells. The potency, defined by K_a values, was in a rank order that was slightly different from that defined for maximal efficiency. The beta 2-agonists clenbuterol and zinterol had K_a values similar to that of isoproterenol, while other beta 2-agonists had lower ones (terbutaline and salbutamol; Table 4, Fig. 5B). Moreover, when associated at submaximal concentrations, dobutamine (10^{-6} M) and zinterol (10^{-6} M) or dobutamine (10^{-6} M) and clenbuterol (10^{-6} M) did not show any additive action; the response initiated by both compounds together was no higher than the response promoted by the most potent agonist alone (dobutamine) (not shown).

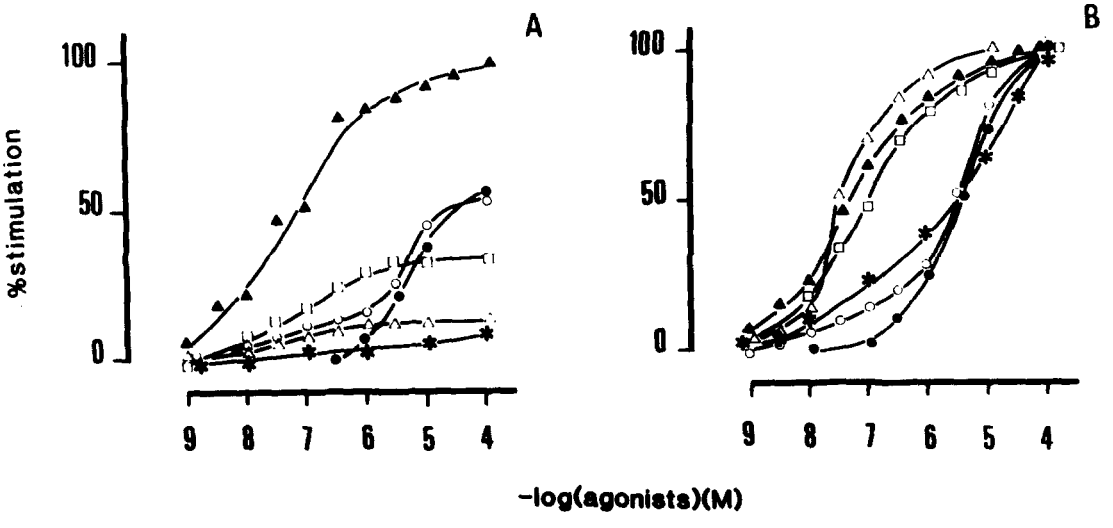


Fig. 5. Effect of beta-agonists on glycerol release by subcutaneous abdominal fat cells: (▲) isoproterenol, (●) dobutamine, (○) terbutaline, (□) zinterol, (△) clenbuterol, and (*) salbutamol. Human fat cells (20–25 mg total lipid) were incubated in 1 ml of KRBA containing 3.5% bovine serum albumin and 6 mM glucose as described in Materials and Methods. Panel A: Lipolysis was expressed as a percentage of the maximum lipolytic response to isoproterenol. Panel B: Lipolysis was expressed as a percentage of the maximum lipolytic response initiated by each agent. The K_a values (agonist concentration required for half-maximal stimulation of lipolysis) given in Table 4 were calculated from dose-response curves as shown in panel B.

TABLE 4. Comparison of activation constants (K_a) and mean intrinsic activities for various beta agonists estimated from in vitro studies on lipolysis in intact abdominal isolated fat cells

Agonists	n	K_a[a]	Intrinsic Activity[b]
		nM	
Isoproterenol	15	60 ± 15	1.00
Dobutamine	8	5920 ± 1200	0.60
Terbutaline	9	5440 ± 2500	0.54
Zinterol	11	100 ± 16	0.34
Clenbuterol	5	40 ± 10	0.15
Salbutamol	4	5200 ± 2000	0.10

Lipolysis studies were performed as described in Materials and Methods. Values are means ± SEM of (n) separate experiments.

[a] K_a, Agonist concentrations required for half-maximal stimulation of lipolysis were calculated from dose-response curves (from 10^{-10} M to 10^{-5} M of various agonists).

[b] Intrinsic activity (mean value of n experiments) was calculated by comparison with the maximal effect initiated by isoproterenol (at 10^{-5} M) which was taken as a reference (1.00). All the compounds tested behaved as partial agonists.

Adenylate cyclase stimulation

The human fat cell adenylate cyclase system has been described as being rather unstable and exhibiting reduced activity in thawed broken cell preparations (27, 28). However, under our working conditions, with inclusion of antiprotease compounds in the buffers used for the preparation of membranes and assays, functionality and sensitivity of the adenylate cyclase system was maintained as assessed by isoproterenol dose-response studies performed on five different fat cell batches (**Fig. 6**). Although there were some noticeable differences in the extent of the maximal response (from 1.2- to 5-fold enhancement of basal activity), the relative order of potency of the agents tested was equivalent to that described in lipolysis studies, i.e, isoproterenol > dobutamine > zinterol > clenbuterol with similar maximal stimulation (Fig. 6). It is noticeable that the relative potency of the agonists was preserved in the adenylate cyclase assays, although the buffer was strikingly different from the KRBA used in lipolysis studies. Thus, the results obtained on adenylate cyclase activation confirm the validity of the investigations based on the lipolysis measurements.

Antagonism of isoproterenol- and zinterol-stimulated lipolysis

In these experiments, several subtype and non-subtype selective compounds were used to inhibit isoproterenol-stimulated lipolysis. The non-selective beta-antagonists were the most powerful agents to suppress isoproterenol-induced lipolysis (**Fig. 7**). Amongst the subtype-selective compounds, and inside the same family, there were noticeable differences in their potencies to inhibit isoproterenol-induced lipolysis. Although all blockers totally inhibited lipolytic activity initiated by 10^{-6} M isoproterenol, the relative order of potency (not all statistically different) of the beta antagonists was: betaxolol > atenolol > LK-204,545 > CGP-20,712A > bisoprolol > IPS-339 > acebutolol > ICI-118,551. In fact, beta 1-antagonists (except acebutolol) were more potent than both the beta 2-antagonists tested. Competition curves were shallow with low Hill coefficients. Thus, to improve the analysis of the curves, as performed for binding assays, computer-assisted non-

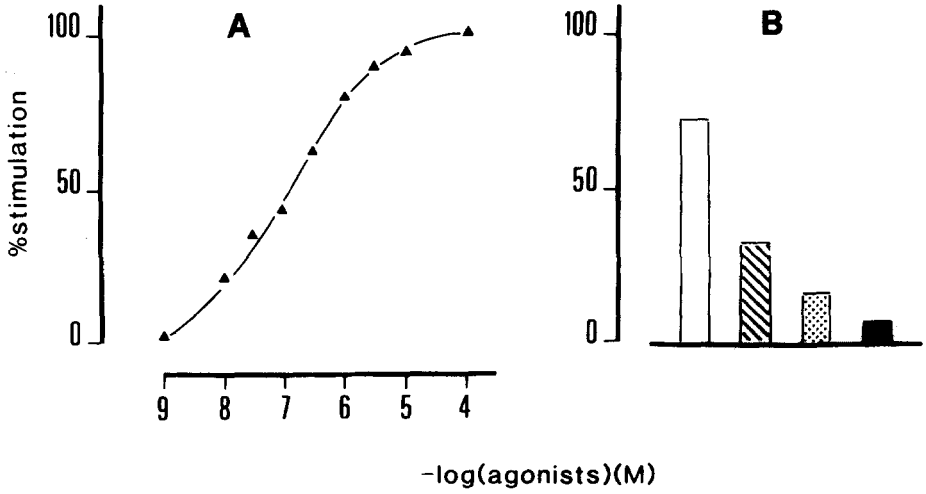


Fig. 6. Typical dose-effect curve of adenylate cyclase activation of human fat cell membranes by isoproterenol under our experimental conditions. Panel A: Human fat cell membranes (20–50 μg) were incubated in the assay buffer for adenylate cyclase as described in Materials and Methods. The results are the means of five experiments (SEM: 10–15%) performed in duplicate. Panel B. Effect of 10^{-6} M of full (isoproterenol □) and partial beta adrenergic agonists (dobutamine ■, zinterol ▨, clenbuterol ⊡). At the concentration used (10^{-6} M) for the assays shown in panel B, the order of potency (zinterol > clenbuterol > dobutamine) corresponds to that described, at this concentration in the experiments on lipolysis (Fig. 5).

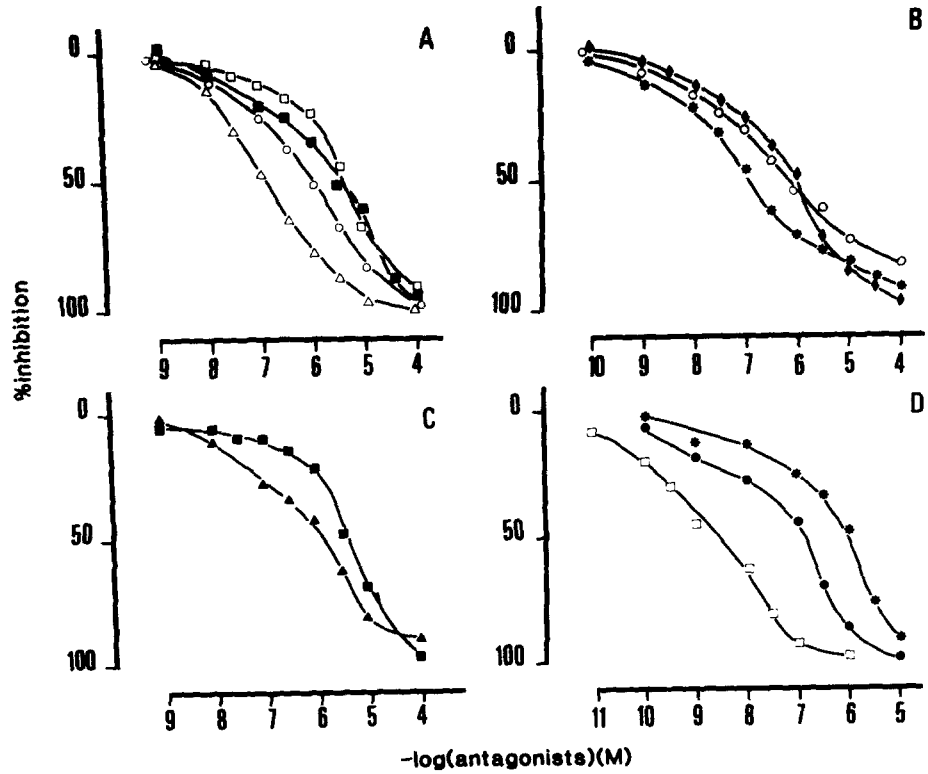


Fig. 7. Dose-effect curves of inhibition of 1 μM isoproterenol-stimulated lipolysis in the presence of beta-1 selective (A, B), beta-2 selective antagonists (C), and nonselective (D); A: (■) acebutolol, (○) atenolol, (△) betaxolol, and (□) metoprolol; B: (♦) bisoprolol, (○) CGP-20,712A, and (*) LK-204,545; C: (■) ICI-118,551, (▲) IPS-339; and D: (□) CGP-12,177, (*) pindolol, and (●) propranolol. Lipolysis studies were performed as described in Fig. 5 and in Materials and Methods. The results expressed as percentage of lipolytic activity observed in the presence of 1 μM isoproterenol are the means of four to eight experiments performed in duplicate.

linear regression analysis was used to analyze the inihibition of isoproterenol-induced lipolysis by selective beta 1-antagonists on five to nine different batches of membranes (**Table 5**). The computer-aided nonlinear regression analysis of the competition curves showed that a two-site model fits the data better than a one-site model ($P < 0.01$), and a mean relative percentage of receptor subtype was defined through this method (around 30% beta 1- versus 70% beta 2-sites with an inherent variability due to differences between patients).

Concerning the zinterol-stimulated lipolysis, the beta 1-antagonists were weakly efficient in the suppression of its lipolytic action while beta 2-antagonists were more potent (not shown). Such a result suggests a specific action of the compound on lipolysis through beta 2-site activation.

Since the incubation buffer used for lipolysis studies contains a mixture of ions and rather large amounts of bovine serum albumin, some differences noticed here could arise from a differential binding of some compounds to albumin (29). Differences in the relative lipid solubility of the agents can also be at the origin of some discrepancies. In fact, the most striking exception among the various drugs was only found with acebutolol, a cardioselective antagonist possessing low lipid solubility. Nevertheless, it is clear that beta 1-antagonists are more efficient than beta 2-antagonists in the suppression of the lipolytic effect of isoproterenol, while the opposite potency was seen with zinterol.

TABLE 5. Inhibition of isoproterenol-stimulated lipolysis by selective beta 1-antagonists: quantitative determination of the percentage of beta 1- and beta 2-adrenergic receptors and of the coupling of each receptor to lipolysis induction

Antagonists	n	K_i Beta 1[a]	K_i Beta 2	% Beta 1[b]
		nM	*nM*	
Betaxolol	8	3.2 ± 2.0	610 ± 410	51 ± 19
Metoprolol	6	2.8 ± 1.6	4200 ± 1200	23 ± 12
CGP-20,712A	9	8.8 ± 4.4	4560 ± 1100	26 ± 7
Bisoprolol	8	0.2 ± 0.1	1020 ± 320	17 ± 4
LK-204,545	5	0.2 ± 0.1	820 ± 270	29 ± 3

Data represent the mean ± SEM of (n) separate experiments.

[a] K_i values for each receptor subtype were calculated according to the method of Cheng and Prusoff as described in Table 3.

[b] The percentages of each receptor subtype and the proportions of the effect of isoproterenol on lipolytic activity attributable to each receptor subtype were determined by computer-aided nonlinear regression analysis as described under Materials and Methods.

DISCUSSION

The present study was undertaken to improve the definition of human fat cell beta-adrenoceptor properties on crude plasma membranes since there is only a small number of pharmacological studies on this family of receptors (see introduction).

In the first set of investigations, the results obtained from binding analyses reveal that (−)^{125}I-labeled CYP, a recently developed radioligand, can be used for the identification of beta-sites on human fat cell membranes. The sites identified with ^{125}I-labeled CYP possess all the properties of beta-adrenoceptors. The number of binding sites determined with this radioligand closely agrees with that determined using two other tritiated radioligands, [^{3}H]DHA and [^{3}H]CGP (Table 1). In the rat, investigators have reported striking differences in the number of sites defined by ^{125}I-labeled CYP or [^{3}H]DHA which have been attributed to the use of high concentrations of lipophilic antagonists for the definition of specific binding (5, 30, 31); this phenomenon was not observed in the present experiments.

Finally, ^{125}I-labeled CYP appears to be a suitable ligand in the identification of human fat cell beta-adrenergic receptors. It represents a useful compound due to its inherently high specific activity and offers an advantage in the quantification of beta-adrenergic receptors on a small amount of membrane proteins, a situation commonly found in clinical investigations when the obtainable amount of fat cells is very limited.

The second set of investigations was devoted to the definition of the beta-adrenoceptor subtypes (beta 1 or beta 2) coexisting in human fat cell membranes. Previous estimates of beta 1- and beta 2-adrenoceptors using graphic analysis of concentration–effect curves generated by beta-adrenoceptor drugs having limited selectivity revealed a higher proportion of beta 1-sites in human fat cells of normal-weight patients (10). It must not be forgotten that the validity of the determination of the number of beta 1- and beta 2-adrenoceptors in a given tissue is based on the specificity of the compounds used for the test. Very recently, several compounds have been identified as highly specific beta 1-adrenoceptor antagonists in various biological systems. We used bisoprolol (18), LK-204,545 (16), CGP-20,712A (17), and ICI-89,406 (14, 15). Bisoprolol and CGP-20,712A have been identified as specific beta 1-adrenoceptor antagonists lacking intrinsic sympathomimetic activity and membrane stabilizing properties; they have a 2,000- (LK-204,545) (16) to 10,000- (CGP-20,712A) (17) times higher selectivity in antagonizing isoproterenol-induced beta 1-adrenoceptor activation than in antagonizing beta 2-adrenoceptor activation.

The estimation of the percentage of putative beta 1- and beta 2-adrenoceptors in human fat cell membranes was conducted using computer-assisted analysis (23). Displacement of ^{125}I-labeled CYP binding by beta 1-antagonists yielded flat displacement curves (Fig. 3) with low Hill coefficient values. The relative proportion of each beta subtype defined in such conditions was practically identical with the most beta 1-selective compounds whatever the radioligand used in the binding assay as shown in Table 2. Unexpectedly, when beta 2-antagonists (ICI-118,551 and IPS-339) were used for subtype delineation, they gave a higher proportion of beta 1-sites; such a discrepancy is not explainable (Table 2).

Finally, the presence of a heterogeneous population of beta-sites is without question in fat cells. On the basis of the present knowledge in the field of beta-adrenoceptors, the existence of the two "classical" subtypes seems to be a reasonable proposition. It is now well accepted that beta 1- and beta 2-adrenergic receptors coupled to adenylate cyclase can coexist on a single cell type (32–34). From the results given by various selective beta 1-antagonists, it seems reasonable to propose that beta 2-sites predominate over beta 1-sites in fat cell membranes of the patients used in the present investigations. The apparently sparse innervation of white fat cells fits well with the predominance of beta 2-adrenoceptors which might be "hormonal" receptors mediating responses to circulating catecholamines (35). It must also be kept in mind that we are working on abdominal fat deposits of slightly overweight women (mean body mass index of 26.3 ± 1.3; n = 30). Since we are working on a specific tissue localization (abdominal subcutaneous fat pad), we cannot exclude the possibility that the relative proportion of each subtype could vary according to the extent of the fat deposits or according to their anatomical location, as previously shown by us in humans for alpha 2-adrenergic receptor sites (4). Moreover, a selective alteration of the binding of one beta receptor subtype over the other during adipose tissue and membrane preparation cannot be completely excluded, beta 1-site binding being lost more rapidly than beta 2-site binding, as previously shown for cardiac beta adrenoceptors (36).

The results of the present binding studies also reveal that the relative affinity of some antagonist compounds for ^{125}I-labeled CYP sites is modified in the presence of Na^+ ions (Fig. 4; Table 3). Reports have mentioned that the selective affinity of agonists at beta 1- and beta 2-sites was markedly influenced by the buffer used in the binding assays (37, 38). Moreover, inclusion of Na^+ in the binding buffer can lower the affinity of beta adrenoceptors for agonists (39–41); there is only one report of changes in the affinity for antagonists (41). Since most of the buffers used for biological assays contain sodium ions to reach isoosmolarity, it must be remembered that absence of Na^+ in binding buffers promotes alteration in antagonist binding as shown in saturation and displacement studies (Fig. 4). This observation seems to be important for receptor definition, the relative order of potency of some com-

pounds is changed in the presence of Na^+ (without changes in the ratio of beta-site subtypes) and can lead to misleading interpretations of receptor properties when the determination of beta-receptor characteristics is based only on binding studies on membranes.

Although binding experiments bring direct evidence for the coexistence of beta 1- and beta 2-adrenoceptor subtypes in human fat cell membranes, they provide no evidence of the functional roles of the individual subtypes. For these different reasons, we felt that the improvement of our analysis on providing some physiological correlation to the binding data was important.

In the metabolic studies described in this report, we mainly focused our attention on the evaluation of the lipolytic responses of isolated fat cells initiated by various synthetic compounds possessing a certain level of subtype selectivity with special attention for some beta 2-agonists. We have previously demonstrated the equipotency of physiological amines (in the presence of alpha 2-antagonists) in the initiation of lipolysis (25). Such a result provided arguments to suggest that the beta-receptor involved in the initiation of lipolysis was of the beta 1- subtype (42).

In the present study, we found that several subtype-selective beta-agonists were able to stimulate adenylate cyclase (Fig. 6) and to promote lipolysis in human fat cells (Fig. 5; Table 4). The response occurred with a potency order that is not characteristic for a typical beta 1- or beta 2-adrenoceptor. Most noticeable is the effect of the beta 2-agonists; although being far less efficient than isoproterenol, they exhibited significant adenylate cyclase-stimulating effect and lipolytic properties. They had a high affinity for fat cell adrenoceptors as assessed by K_a values (Table 4). The disparity of activity of the beta 2-agonists is not readily explained. It cannot be excluded that the beta-receptor of the human fat cell may be unique in its specificity and that, depending on their chemical nature, the beta-agonists interact in an abnormal fashion with the receptor glycoprotein. The receptor can also exhibit some level of microheterogeneity which has become established along the evolutionary processes, the receptor having kept its full recognition ability for the physiological amines alone. It should be pointed out that there is some relationship between the three synthetic compounds exhibiting clear lipolytic potencies on human fat cells: they possess a catechol or resorcinol nucleus, an ethanolamine side chain (β-OH group), and a large substituent on the amine. In summary, the study of the differently active synthetic agonist compounds does not permit the classification of the beta-adrenoceptor of the human fat cell implicated in the induction of lipolysis into a "classical" beta 1- or beta 2-subtype. However the studies reveal some peculiarities of the beta-adrenergic control of lipolysis in human fat cells; both beta 1- and beta 2-subtypes seem to be implicated in the induction of lipolysis, although an atypical nature of the beta receptor cannot be rejected from the examination of the results given by the agonists. The specific behavior of zinterol and clenbuterol, which are able to stimulate lipolysis moderately, focuses attention on such agents having minimal cardiac impact. Such an observation can also explain the lipid-mobilizing properties previously reported for beta 2-agonists under in vivo conditions (43). Beta 2-adrenergic compounds, devoid of (or with a limited) cardioselectivity, can represent putative lipid mobilizing drugs; they require an extended investigation.

Several antagonists were used to counteract the lipolytic effects of isoproterenol and zinterol. All the subtype-selective compounds were able to block isoproterenol-induced lipolysis and the specifically classified beta 1-antagonists were more potent than the beta 2-antagonists, although some exceptions were observed (Fig. 7; Table 5). The heterogeneity of the inhibition curves and their computerized analysis indicate that both receptor subtypes are involved in the induction of the lipolytic effect of isoproterenol. The contribution of each receptor subtype to the activation of lipolysis paralleled the relative proportions of the two receptor subtypes defined in binding assays (Tables 2 and 5).

Thus, the in vitro data support the idea that a mixture of beta 1- and beta 2-adrenoceptors is involved in the lipolytic process. The conclusions raised here are in good agreement with the idea that both beta 1- and beta 2-adrenoceptors could play a role in triglyceride breakdown in vivo (1). Beta 1- as well as beta 2-agonists have been found to promote an increment of plasma free fatty acid levels in humans and it has also been shown that some cardioselective beta blocking agents only exert weak anti-lipolytic effects by comparison with nonselective antagonists (propranolol, pindolol) which were more potent (1, 44–47). Beta 2-antagonists have not been tested under such conditions. Since the in vivo studies on catecholamine-induced lipid mobilization are complicated by various factors (e.g., heterogeneity of the fat deposits, modification of the blood flow dependent on adrenoceptor equipment of the vascular bed, impact on insulin secretion), the studies on intact cells and membranes offer a unique opportunity for the clarification of the pharmacological characterization of human fat cell beta adrenoceptors and for the definition of the role of each receptor subtype.

This study also focuses on the great variability of impact of beta-antagonists on human fat cells. It is to be noted that some cardioselective compounds seem to have a minor impact on adipocytes; this property should be kept in mind when such compounds must be administered chronically, in the treatment of hypertension for example. It is not really understood whether the lipid disturbances promoted by some beta antagonist treatments are connected with their putative impact on fat cells. Nevertheless, it is clearly established that acute administration of

a single dose of beta-antagonist generally lowers the plasma free fatty acid levels (45–47).

In conclusion, the present study was an attempt to delineate and clarify the characterization of human fat cell beta adrenoceptors with the highly selective tools provided recently. The data reported here: *1*) demonstrate that the iodinated (^{125}I-labeled CYP) as well as the tritiated beta antagonists ([^{3}H]DHA and [^{3}H]CGP) represent valuable tools for the quantification of beta adrenoceptors and the delineation of beta receptor subtypes on human fat cell membranes; *2*) reveal that the presence of Na^{+} in binding buffers can cause a modification of the affinity of beta sites for certain beta-antagonists and can provoke some misleading interpretations on beta receptor characterization; *3*) demonstrate that the human fat cell beta-adrenoceptor population defined by nonselective radioligands is composed of two subtypes that can be mainly interpreted in terms of classic beta 1- and beta 2-adrenoceptor subtypes as assessed by the competition studies conducted with highly selective beta 1- antagonists; *4*) support the idea that both beta 1- and beta 2-adrenoceptors are coupled with adenylate cyclase and involved in the induction of lipolysis subsequent to sympathetic activation; *5*) focus on the interest of some beta 2-agonist drugs (zinterol, clenbuterol) in the initiation of lipolysis; this family of compounds can offer some new opportunities as lipid-mobilizing agents.

The essential limits that can be laid down for the present findings concern the clinical status of our patients (slightly overweight women exclusively). It is open to speculation that the relative proportion of beta 1- and beta 2-adrenoceptors can exhibit some variability depending on the sex, the age, the anatomical location and the extent of the fat deposits. Integrated studies on a much wider range of patients and tissues will be necessary before solving these problems. Hence, in our laboratory, work is in progress on human adipose tissue from different anatomical locations in women. Nevertheless, the demonstration of the existence and of the involvement, in human fat cells, of two different types of beta-adrenoceptors mediating the same kind of response opens some perspectives of research towards the understanding of their physiological recruitment, the mechanisms implicated in their physiological or pharmacological regulation, and the definition of new strategies for the synthesis of new lipid-mobilizing drugs having a minimal impact on cardiac function.

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