A reliable assay for beta-adrenoceptors in intact isolated human fat cells with a hydrophilic radioligand, [3H]CGP-12177

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Abstract The beta-adrenergic receptors of isolated human fat cells were identified using a new hydrophilic beta-adrenergic radioligand ($\pm$)[3H]CGP-12177. The results were compared with those from [3H]dihydroalprenolol binding to fat cells and membranes. [3H]CGP-12177 binding to isolated fat cells showed lower nonspecific binding (less than 15% of total binding) than the lipophilic [3H]dihydroalprenolol (40–60%) at 3 times the K_D. At 37°C, [3H]CGP-12177 binding was rapid, reversible, of high affinity (1.2 ± 0.3 nM) and saturable. The total number of binding sites per cell in subcutaneous adipocytes was 25,000 $\pm$ 6,000 and was equivalent to that found using membrane fractions. Displacement of [3H]CGP-12177 bound to adipocytes by propranolol was stereoselective, consistent with competition at a single site, and had the same characteristics as in membranes. The displacement curves of the beta$_1$-selective antagonists (atenolol and betaxolol) were biphasic, the high affinity displacement accounting for 70% of the total binding sites. Beta-adrenergic agonists also competed with [3H]CGP-12177 binding in the order of potency: ($-$) isoproterenol > ($-$) norepinephrine > ($-$) epinephrine, similar to that found in membranes and in in vitro studies on the lipolytic activity of isolated fat cells.▥ This study demonstrates that the sites specifically labeled by [3H]CGP-12177 are the physiological beta-adrenoceptors and also shows that the ligand is better than [3H]dihydroalprenolol for the accurate identification of these receptors in intact human adipocytes. The methodology, which requires biopsies of less than 1 gram of adipose tissue, can be of potential interest for clinical studies investigating the status of fat cell beta-adrenoceptors in various pathophysiological situations. — Lacasa, D., P. Mauriège, M. Lafontan, M. Berlan, and Y. Giudicelli. A reliable assay for beta-adrenoceptors in intact isolated human fat cells with a hydrophilic radioligand, [3H]CGP-12177. *J. Lipid Res.* 1986. **27:** 368–376.

Supplementary key words lipolysis • adipocytes

The main function of adipose tissue is to supply fatty acids to be utilized by different peripheral tissues, and glycerol, which is a gluconeogenic substrate in the liver. In human adipose tissue, the most important, if not the only, physiological lipolytic hormones are the catecholamines (1, 2). These hormones also play a role in diabetes mellitus (3) and hyperthyroidism (4), two common disorders that are associated with adipose tissue dysfunctions (5–8). Knowledge of the basic mechanisms of the lipolytic action of these hormones is, therefore, of considerable interest.

In fat cells, it is now well recognized that catecholamines exert their lipolytic action through their binding to beta-adrenergic receptor sites located on the plasma membrane (9, 10). Since the regulation of lipid mobilization depends both on the activation of the sympathetic nervous system and on the effects of the physiological amines at the receptor level on the adipocyte, knowledge of receptor properties is essential for the understanding of the regulating events controlling human fat cell function.

Changes in adrenergic receptor density on the adipocytes, as well as modifications in their affinity for physiological amines, may have a major impact on the regulation of lipid mobilization in various pathophysiological conditions. The search for reliable methods to characterize fat cell adrenoceptor properties has been within the scope of various laboratories during the last years.

Recent binding studies utilizing the beta-adrenergic receptor radioligand [3H]dihydroalprenolol (3H-DHA) have been successful in characterizing the beta-adrenergic receptors in broken cell membrane preparations from human fat cells (11–13). However, due to possible alterations in the receptors as a result of cell disruption, such receptor studies may not be adequate for valid comparison with the molecular events resulting from the interaction of catecholamines with intact adipocytes (7, 8, 11–14).

Thus, identification of beta-adrenergic receptors on intact fat cells is a useful methodology allowing the investigation of the factors involved in receptor regulation under more suitable physiological conditions. There are few studies devoted to beta-adrenergic receptor identification on intact cells (15–17). In human fat cells, abnormally high values of nonspecific binding have been reported (15). Moreover, in some experiments, the authors could not accurately calculate the affinity of the sites for the radioligand or the maximum binding capacity from the Scatchard curves (16). In rat fat cells, saturable specific binding could not be obtained without drastic treatment of the cells with valinomycin, a K^+-ionophore which depletes intact cells of ATP (17). Several recent studies (18–21) performed on other cell lines with ^{3}H-DHA have demonstrated the inadequacy of this ligand for the quantification of the functional beta-adrenergic receptors in intact cells (i.e., the receptor population located on the cell surface). The most plausible hypothesis explaining abnormal ^{3}H-DHA binding to intact cells is the high lipophilicity of this ligand (20–23) which diffuses easily across the plasma membrane and becomes entrapped within the cell in such a way that the resulting binding partly mimics specific binding. This phenomenon has been described in HeLa adenocarcinoma cells (21).

Recently, with the introduction of the new beta-adrenergic antagonist [^{3}H]CGP-12177 (^{3}H-CGP) different investigators have succeeded in the characterization of the functional beta-adrenergic receptor population in intact cells of different origins (20, 24–27).

In the present studies, an assay of human fat cell beta-adrenoceptors with ^{3}H-CGP was developed and adapted for use after removal of adipose tissue by a simple biopsy procedure. Following this procedure, we show that the binding characteristics of these receptors, although markedly different from those found by Engfeldt et al. (15) studying ^{3}H-DHA binding, are consistent with those of the human adipocyte beta-adrenoceptors defined on crude membranes or in biological assays.

MATERIALS AND METHODS

Materials

[^{3}H]CGP-12177 (40 Ci/mmol), [^{3}H]dihydroalprenolol (80 Ci/mmol), [^{3}H]norepinephrine (50 Ci/mmol), and PCS were purchased from the Radiochemical Centre, Amersham. (–)Epinephrine bitartrate, (–)norepinephrine hydrochloride, (–)isoproterenol hydrochloride, yohimbine hydrochloride, ascorbic acid, catechol, chloroquine, and pargyline were obtained from Sigma Chemical Company. The following compounds were provided as gifts: (–) and

(+) propranolol, ICI-118551, practolol, and atenolol (ICI Ltd., Alderly Park, England); salbutamol from Glaxo Group Research (Ware, England); betaxolol from Synthelabo (Paris, France); and phentolamine methane sulfonate from Ciba Geigy Corporation (Basel, Switzerland). Collagenase came from Worthington, Freehold, NJ. All other chemicals were reagent grade.

Isolation of human fat cells and preparation of fat cell ghosts

Abdominal subcutaneous adipose tissue samples were obtained from unselected patients (33 to 65 years old) undergoing elective abdominal surgery. Subjects with diabetes, obesity, or other endocrinological disorders were excluded from this study. Patients fasted overnight before undergoing surgery and general anesthesia was induced with thiopental. The patients had not received drugs active on the autonomic nervous system.

Fat cells were obtained by collagenase digestion of adipose fragments in Krebs-Ringer bicarbonate buffer (KRB) containing albumin (3.5 g/100 ml) and glucose (0.6 mmol/100 ml) using the method of Rodbell (28). The isolated fat cells were washed three times in KRB buffer and then suspended in the medium used for the binding studies. Cell lysates were prepared by incubating the isolated fat cells in a lysing medium (2.5 mM $MgCl_2$; 1 mM $KHCO_3$; 1 mM ATP; 2.5 mM Tris-HCl, 100 μM PMSF; 3 mM EGTA; pH 7.5). Crude adipocyte ghosts were pelleted by centrifugation (30,000 g, 15 min) at 4°C, washed twice in a buffer (5 mM Tris-HCl, 1 mM EGTA, pH 7.6), resuspended in the buffer used for fat cell membrane binding studies (10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5) at a final concentration of 2.5 mg of protein/ml, immediately frozen, and used within 1 week.

Binding assays with intact adipocytes

Washed fat cells were suspended in Hank's balanced salt solution (HH solution) containing 20 mM HEPES, pH 7.4, and 0.05% bovine serum albumin. In some experiments, this buffer was supplemented with different compounds to inhibit various metabolic events such as the possibility of ligand uptake and internalization (chloroquine, 0.03 mg/ml), the degradation of the amines (ascorbic acid, 0.1 mM; pargyline, 10 μM; catechol, 0.1 mM), and even a putative interaction with alpha$_2$ sites (phentolamine 1 μM). We did not find any noticeable differences in the binding results whichever incubation medium was used; thus we kept the simpler one (HH solution) for the saturation studies. When the fat cells were incubated in the presence of physiological agonists (in competition studies), the complex medium (with the compounds mentioned previously) was used.

^{3}H-CGP binding was assessed by incubating 0.3–0.5 ×

10^6 cells in 450 μl of HH solution for 20 min at 37°C with 50 μl of different concentrations of radioligand (or with radioligand and competing drugs for the competition studies). Incubations were stopped by a 10-fold dilution of the samples with ice-cold filtration buffer (1 mM MgSO$_4$; 20 mM potassium phosphate, pH 7.4) followed by rapid vacuum filtration of the suspension through Whatmann GF/C glass-fiber filters. The filters were washed twice with 5 ml of ice-cold filtration buffer and put wet into 10 ml of PCS scintillation cocktail. After gentle shaking (30 min) the vials were counted at 46% efficiency. Nonspecific binding was defined as the binding observed in the presence of 1 μM (−)propranolol and averaged 10–15% of total binding at 3 nM ^{3}H-CGP. Nonspecific binding increased linearly with ligand concentration (0.2 to 10 nM) and specific binding was linear with the cell number within the range 0.1 to 1 × 10^6 cells. In some experiments, binding of ^{3}H-CGP to adipocytes was measured at low temperature (13°C) following the same procedure as above except that the time of incubation was 180 min. In other experiments, ^{3}H-DHA specific binding to intact adipocytes was studied under the same experimental conditions as above. Nonspecific binding, defined as the binding observed in the presence of 1 μM (−)propranolol averaged 50 to 60% of total binding at 3 nM ^{3}H-DHA.

Catecholamine stability was assessed with [^{3}H]norepinephrine, as previously described (29), in the absence or presence of adipocytes; under these conditions no detectable degradation was found as judged by the radioactivity present in the single peak obtained in the chromatograms.

Binding assays with crude membranes

Thawed crude adipocyte membranes were rehomogenized with four pestle strokes in a Potter apparatus immediately before use and diluted in the incubation buffer commonly used for binding studies (10 mM MgCl$_2$, 50 mM Tris-HCl, pH 7.5). Binding assays were performed in a final volume of 400 μl as previously reported (13) using either ^{3}H-CGP or ^{3}H-DHA and 1 μM (−)propranolol to define the nonspecific binding.

Other determinations

Measurements of lipolysis. Isolated human fat cells were incubated in 500 μl of KRB buffer containing 3% bovine serum albumin, 0.6 mmol/100 ml glucose, 2 μg/ml adenosine deaminase, and the pharmacological agents at suitable dilutions (13). The glycerol released in the incubation medium was determined as previously reported (13).

Protein was determined according to Lowry et al. (30) and cell number was estimated as previously described (6).

Analysis of the competition binding curves was performed according to the one- and two-site models on a Commodore CBM computer, as reported (6, 31, 32).

RESULTS

Kinetic analysis of [^{3}H]CGP-12177 binding to human adipocytes

Binding of ^{3}H-CGP to human adipocytes at 37°C was rapid, with half-maximal specific binding (t$_{1/2}$) of 4 min (**Fig. 1A**), reaching equilibrium within 16–20 min. Binding remained stable for 30 min at 37°C. At 13°C (which was the lowest temperature that could be used without cell coagulation), binding of ^{3}H-CGP was slower, reaching equilibrium within 180 min (data not shown). In the subsequent experiments, incubation times of 20 and 180 min were therefore chosen to represent equilibrium binding at 37 and 13°C, respectively.

At 37°C, the initial rate constant (K$_{ob}$) for the association reaction obtained from the slope (Fig. 1A, inset) was 0.22 min^{-1}. The dissociation of bound ^{3}H-CGP at 37°C was determined by incubating adipocytes to equilibrium and then adding 1 μM (−)propranolol at time zero and measuring residual specific binding at subsequent time intervals (Fig. 1B). Dissociation was rapid and the first-order dissociation rate constant K$_2$ was 0.03 min^{-1}. Calculation of the second-order association rate constant K$_1$ yielded the value of 0.237 × 10^8 × M^{-1} × min^{-1}. The equilibrium dissociation constant K$_D$ determined from the ratio of K$_2$/K$_1$ was 1.27 nM.

Comparison of the equilibrium specific binding of [^{3}H]CGP-12177 and ^{3}H-DHA to human fat cells

The binding characteristics of ^{3}H-CGP to intact human adipocytes at 37°C is described in **Fig. 2**. Specific binding was clearly saturable and of high affinity. Apparent saturation was observed at a ligand concentration of 4–5 nM with half-maximal binding occurring at about 1.5 nM. Scatchard analysis (31) of these data (Fig. 2, inset) yielded a straight line, indicating that the ligand binds to a single class of receptors. The dissociation constant K$_D$ was 1.2 ± 0.3 nM (n = 8) and the total number of binding sites was 40 ± 9 fmol/10^6 cells, which is equivalent to 25,000 ± 6,000 sites per adipocyte. Essentially the same results were found at 13°C (data not shown). In agreement with previously reported data (15, 16), the equilibrium specific binding of ^{3}H-DHA to intact adipocytes was nonsaturable at 37°C and exhibited higher values of nonspecific binding as compared with ^{3}H-CGP. Moreover, neither the affinity of the sites for ^{3}H-DHA nor the maximum binding capacity could be evaluated accurately from the curvilinear Scatchard plots.

Inhibition of [^{3}H]CGP-12177 binding to human adipocytes by competing ligands

Displacement of specific binding by beta-adrenergic antagonists was studied at equilibrium and 37°C. As
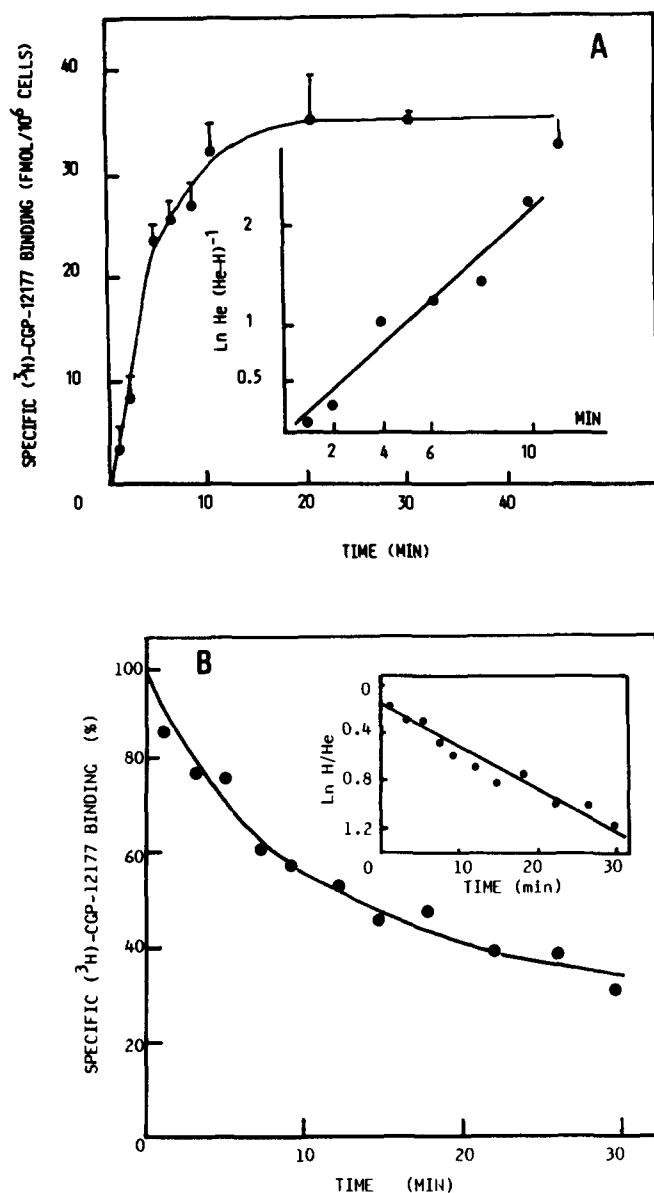
Fig. 1. Kinetics of association (A) and dissociation (B) of [³H]CGP-12177 binding to intact human adipocytes. Association (A): Intact adipocytes (0.5 × 10⁶ cells) were incubated at 37°C for the indicated times with 1 nM [³H]CGP-12177 in the absence or presence of 1 μM (−)propranolol. The inset shows the pseudo-first order rate plot of [³H]CGP-12177 binding of the same data. (He) is the amount of ligand bound at equilibrium and (H) is the amount of ligand bound at each time. The slope of the line (kob) was determined by linear regression analysis (r = 0.97). The data presented at means ± SEM of triplicate determinations from one experiment repeated twice with identical results. Dissociation (B): Cells were incubated with 8 nM [³H]CGP-12177 for 20 min at 37°C. Binding was reversed by adding 1 μM (−)propranolol at time zero and specific binding was determined at the indicated times and expressed as a percentage of maximal binding. The inset shows the first-order rate plot of [³H]CGP-12177 dissociation from the cells. (He) represents the amount of ligand bound at equilibrium before addition of propranolol, and (H) is the amount of ligand bound at the indicated time after dilution. The slope of the line, determined by regression analysis (r = 0.98), represents the dissociation constant K₂. Each point is the mean of triplicate determinations from one experiment repeated twice.

shown in **Fig. 3**, ³H-CGP binding was stereospecifically inhibited by the nonselective beta-antagonist propranolol (the (−) isomer being 30 times more potent than the (+) isomer) and was consistent with competition at a single site. On the other hand, displacement curves of the beta₁-selective antagonists (atenolol and betaxolol) best fitted to two affinity states, the high affinity state accounting for about 70% of the total binding sites. In contrast, displacement curves with the beta₂-selective antagonist ICI-118551 were consistent with competition at a single site. (**Table 1**).

Beta-adrenergic agonists also competed with ³H-CGP binding in the following order of potency: (−) isoproterenol > (−) norepinephrine > (−) epinephrine (**Fig. 4**). Computer analysis of the norepinephrine and epinephrine displacement curves studied at 37°C suggested interaction at one single site with pseudo-Hill coefficients of 0.89 ± 0.09 and 0.93 ± 0.08, respectively. In contrast, the (−) isoproterenol displacement curves at 37°C were shallow and fitted a two-site model better ($P < 0.02$), with the high affinity component representing 30% of the total binding site population. Interestingly, the proportion of these high affinity sites increased twofold (**Table 2**) when the competition experiments were performed at 13°C. At the same temperature, the norepinephrine and epinephrine displacement curves also fitted a two-site model better and computer analysis revealed that 30–35% of the binding sites were displaced with high-affinity (**Table 2**).
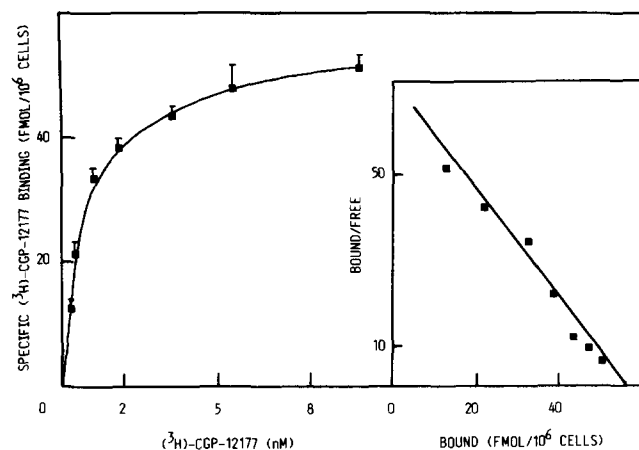

Fig. 2. Concentration-dependence curve of specific binding of [³H]-CGP-12177 to human adipocytes at equilibrium. Intact adipocytes (0.4 × 10⁶ cells) were incubated for 20 min at 37°C with the indicated concentrations of [³H]CGP-12177 as described in Materials and Methods. Nonspecific binding was determined in the presence of 1 μM (−)propranolol. The inset shows the Scatchard plot of the same data. Each point is the mean ± SEM of triplicate determinations from one experiment. The equilibrium dissociation constant, K_D = 0.75 nM, and the maximal number of binding sites, B_{max} = 54 fmol/10⁶ cells, were calculated by regression analysis (r = 0.98) and were consistent in eight separate experiments.
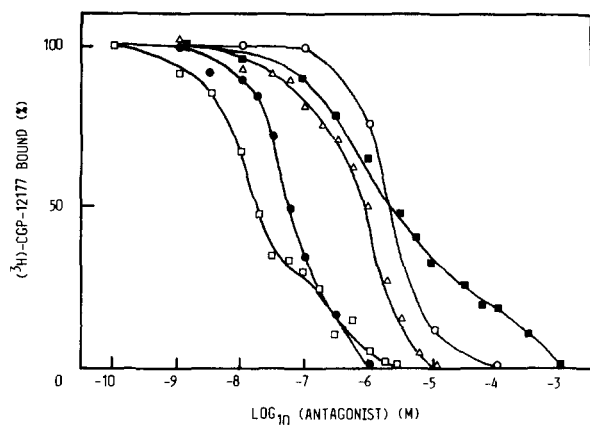
Fig. 3. Displacement curves of specific [³H]CGP-12177 binding to intact human adipocytes by beta-adrenergic antagonists. Human adipocytes (0.5 × 10⁶ cells) were incubated for 20 min at 37°C with [³H]-CGP-12177 (4 nM) and the indicated concentrations of (−)propranolol (●), (+)propranolol (○), (±)atenolol (■), (±)betaxolol (□), and (±)ICI 118551 (△). Binding assays were performed as described in Materials and Methods. Results are expressed as a percentage of radioligand specifically bound in the absence of drugs and are means of three separate experiments performed in triplicate.

[³H]CGP-12177 binding to human adipocyte membranes

In separate experiments, we compared the above binding data with the characteristics of ³H-CGP and ³H-DHA binding to membranes of the same fat cell batch. Saturation binding experiments revealed that there was no noticeable difference in the B_{max} values whatever the ligand or the technique used (26,000 ± 7,000 sites per adipocyte were determined from ³H-CGP binding on membranes and 27,500 ± 7,000 with ³H-DHA; n = 4). From these experiments, the K_D values for ³H-CGP and ³H-DHA were 1.4 ± 0.7 and 2.1 ± 0.9 nM, respectively.

Adrenergic antagonists competed with ³H-CGP binding

sites in the same order of potency as that described in isolated fat cells (betaxolol > ICI-118551 > atenolol >> practolol).

Beta agonists and physiological amines competed with ³H-CGP binding to membranes in the order of potency found in intact cells: (−) isoproterenol > (−) epinephrine and norepinephrine > (+) epinephrine and norepinephrine. Moreover, the competition curves were shallow with slope factors less than 1, suggesting agonist interactions at two affinity sites. When the same experiments were repeated in the presence of the non-hydrolysable guanine nucleotide analog (10⁻⁴ M, GppNHp), the competition curves became steeper and shifted to the right (slope factor = 0.90), data that are consistent with displacement at a single class of binding sites. The epinephrine and norepinephrine K_i values derived from these analyses were similar (5,240 ± 1,300 nM; n = 4) and very close to the values found at 37°C in intact adipocytes and shown in Table 2.

Relationship between [³H]CGP-12177 binding properties and the lipolytic response of intact human adipocytes to catecholamines

In order to compare agonist binding on intact fat cells to cellular beta-adrenergic responses, experiments were conducted to determine the relative potencies of agonists and antagonists in the control of the lipolytic activity of human fat cells. This test is more suitable than adenylate cyclase measurements in fat cell ghosts since smaller amounts of agonists are required (33). We compared the ability of various agonists to stimulate the lipolytic activity of human fat cells incubated in the presence of 2 μg/ml adenosine deaminase. The beta-lipolytic component of physiological amines was tested in the presence of 10⁻⁵ M yohimbine (alpha₂-antagonist) in order to suppress the alpha₂-component of the amines (34, 35). The order of

TABLE 1. Affinities of different beta-adrenergic antagonists for the specific [³H]CGP-12177 binding sites of intact human adipocytes

	K_D (nM)[a]		
Drugs	K_D1	K_D2	R_H[b]
(−) Propranolol	15 ± 6		100
(+) Propranolol	500 ± 40		100
(±) Betaxolol	2.6 ± 0.5	93 ± 8	71 ± 2
(±) Atenolol	250 ± 12	31,500 ± 2,700	67 ± 5
(±) ICI 118551	200 ± 45		100

Each value is the mean ± SEM of the three separate experiments depicted in Fig. 3.

[a] K_D1 and K_D2 values for (±) atenolol and (±) betaxolol were calculated from computer analysis as described in Materials and Methods and represent, respectively, the dissociation constants of the high affinity and low affinity states of the [³H]CGP-12177 binding sites for these drugs. The K_D1 values for (−) and (+) propranolol and for (±) ICI-118551 were calculated from the median effective concentration (EC₅₀) values according to Cheng and Prusoff (32).

[b] R_H values represent the percentage of binding sites having high affinity.
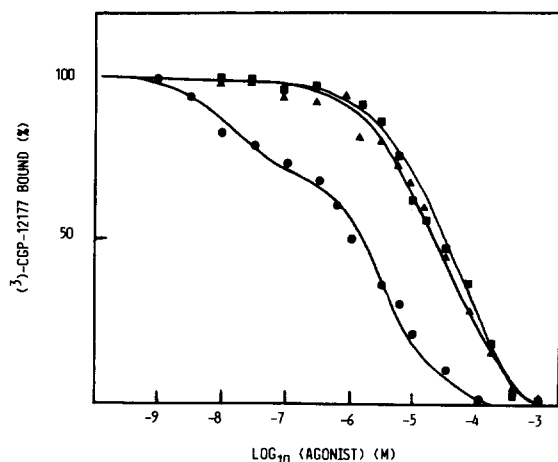
Fig. 4. Displacement curves of specific [³H]CGP-12177 binding to intact human adipocytes by beta-adrenergic agonists. Human adipocytes were incubated at 37°C as in Fig. 3, except that the competitors added were (−)isoproterenol (●), (−)norepinephrine (▲), and (−)epinephrine (■). Results are expressed as a percentage of radioligand specifically bound in the absence of agonists and are means of at least three experiments performed in triplicate.

potency of the drugs in the initiation of the lipolytic response was: isoproterenol > norepinephrine > epinephrine > salbutamol. The reference beta₂-agonist was a poor lipolytic agent while physiological amines, in the presence of an alpha₂-antagonist, had a maximal activity equivalent to that of isoproterenol.

Furthermore, it must be noted that the half-maximal stimulation of lipolysis by isoproterenol, norepinephrine, and epinephrine (**Table 3**) occurred at concentrations similar to the agonists K_{DH} values derived from the competition experiments performed on intact cells at 13°C (Table 2).

DISCUSSION

To our knowledge, the only other study of beta-adrenergic receptors in intact human adipocytes is that reported by Engfeldt et al. (15). In their studies the radioligand used was [³H]dihydroalprenolol, which is a valuable tool for receptor investigations in membrane preparations. However, some of the results obtained in their studies were in direct contrast to the beta-adrenergic receptor characteristics found by others in human adipocyte membranes. For instance, the affinity for beta-agonists was 25 to 50 times less than that reported in membranes (12, 13) and far lower than the sensitivity of the biological response of human adipocytes to catecholamines (adenylate cyclase, cyclic AMP production, lipolysis) (1, 2, 11, 13, 34, 35). Moreover, nonspecific binding was very high and the Scatchard plots were curvilinear, two findings that were confirmed in the present study. Finally, the number of binding sites per cell seemed to be unreasonably high compared to the number reported by others in membranes (11–13).

Recently, different authors (18–21) have postulated that equilibrium binding studies with ³H-DHA may not be appropriate to assess the density and the agonist affinity of the population of the functional beta-receptors in intact cells. In fact, due to its high lipophilicity, this membrane-permeable ligand not only labels the receptors located on the cell surface but also the receptor population that is internalized upon exposure to beta-agonists and which is no longer accessible to hydrophilic catecholamines (20). Therefore, it cannot be excluded that in their equilibrium binding studies Engfeldt et al. (15) might have not only overestimated the number of functional receptors, but also underestimated the agonist binding affinity of these receptors.

Unlike ³H-DHA, a potent new beta-adrenergic antagonist, [³H]CGP-12177 is a highly hydrophilic drug (20). Recent studies performed in different cell lines (20, 24–26) including the rat adipocyte (27), have shown that this ligand is a valuable tool for the characterization of beta-adrenoceptors on intact cells. The present results extend these findings to human adipocytes by showing that the sites specifically labeled by ³H-CGP satisfy all the criteria required for the characterization of functional beta-adrenergic receptors. In fact, nonspecific binding was low;

TABLE 2. Influence of temperature on the characteristics of beta-agonist binding to [³H]CGP-12177 specific binding sites in intact human adipocytes

Beta-agonists	Temperature	K_DH	K_DL	R_H
	°C	nM	nM	%
(−) Isoproterenol	37	6.2 ± 3.8	1,240 ± 60	36 ± 3
	13	14.1 ± 5.7	1,520 ± 160	64 ± 1
(−) Norepinephrine	37		5,030 ± 1,023	0
	13	114 ± 15	9,500 ± 800	34 ± 3
(−) Epinephrine	37		4,200 ± 400	0
	13	450 ± 64	10,500 ± 700	31 ± 2

Intact adipocytes were incubated for 20 min at 37°C or for 180 min at 13°C with [³H]CGP-12177 and various concentrations of beta-agonists. The binding parameters K_DH and K_DL derived from computer analysis of the resulting competition curves represent the affinity of the high and low affinity states of the binding sites for beta-agonists. R_H is the percentage of binding sites in the high affinity state. Each value is the mean ± SEM of three different experiments performed in triplicate.

TABLE 3. Comparison of activation constants for isoproterenol and for the physiological agonists (epinephrine and norepinephrine) estimated from in vitro studies on lipolysis in intact abdominal subcutaneous fat cells

	K_a Values[a]	
	nM	
(−)Isoproterenol	40 ± 15	(4)
(−)Norepinephrine	350 ± 70	(4)
(−)Epinephrine	468 ± 80	(4)

Lipolysis studies were performed as described in Materials and Methods. Isolated fat cells were incubated in Krebs-Ringer bicarbonate buffer containing 2 μg/ml adenosine deaminase. Yohimbine (alpha$_2$-antagonist) was included in the incubation medium at the concentration of 10^{-5} M to suppress the alpha$_2$-component of the physiological amines (epinephrine and norepinephrine). Values are means ± SEM. Numbers in parentheses represent the number of separate experiments performed in duplicate.

[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).

the specific binding was rapid, reversible, stereospecific, and displaceable by the beta-adrenergic agonists and antagonists with potencies consistent with those described in membranes and biological assays. Moreover, the mean density of specific binding sites was around 25,000 per cell, a value which is ten times less than the number previously reported (15). Furthermore, the full beta-agonist, isoproterenol, competed at 37°C with the ^{3}H-CGP binding with high and low affinity, and at lower temperature, the proportion of high affinity binding was increased. This finding is consistent with the accepted concept that exposure of intact cells to catecholamines promotes a rapid and temperature-dependent conformational change in the beta-receptors which involves several steps: first, a decrease in their binding affinity for agonists; second, the internalization of these low affinity receptors (19, 24, 25, 36–39); and third, the desensitization of the system. It must be noted, however, that at 37°C, we were unable to show any high affinity binding sites for norepinephrine or epinephrine; the K_DL values were closer to those found in membranes in the presence of 10^{-4} M GppNHp. Although we have no clear explanation for this, we cannot firmly exclude that such sites exist at 37°C for the following reasons. First, it has been demonstrated that the proportion of receptors having high affinity for agonists is directly dependent on the beta-adrenergic potencies of the agonist (40). With isoproterenol, which is at least 10–20 times more potent than epinephrine and norepinephrine (34, 35), we found only 36% of high affinity sites at 37°C. Therefore, if high affinity sites for epinephrine and norepinephrine exist at 37°C, their proportion would be less than 36% and may be too small (less than 5%) to allow the computer to distinguish between these sites and the low affinity sites. Second, at low temperature, our experiments provided clear evidence for the existence of high affinity norepinephrine and epinephrine binding (Table 2).

The experiments also showed that ^{3}H-CGP binding

was completely displaced by a selective beta$_2$-antagonist at one apparent class of site, whereas 70% of this binding was displaced with high affinity by two selective beta$_1$-antagonists (Table 1). These puzzling observations, though consistent with lipolysis studies (10, 41) may indicate that the beta-receptors of human white adipocytes, rather than being a mixture of beta$_1$- and beta$_2$-subtypes as previously suggested (15, 41), are atypical. It should be noted, however, that this suggestion is based on experiments using pharmacological agents, the preferential beta$_1$- or beta$_2$-selectivity of which has been established on tissues other than adipose tissue. Thus it cannot be excluded that the adrenergic selectivity of these drugs is different in human adipocytes, which is indeed suggested by some of the present results. For instance, salbutamol, a potent beta$_2$-agonist in several tissues, is only a weak lipolytic drug in human adipocytes, whereas ICI-118551, a potent beta$_2$-antagonist in various systems, clearly competes with [^{3}H]CGP-12177 binding and elicits a marked inhibiting effect on catecholamine-stimulated lipolysis (unpublished observation). Therefore, many more experiments with an extended number of selective beta$_1$- and beta$_2$-adrenergic antagonists and agonists remain to be performed before concluding that the beta-adrenergic receptor of human fat cells does not fit the current beta$_1$/beta$_2$ subdivision. However that may be, the present study clearly shows that the sites identified by ^{3}H-CGP are the true beta-adrenoceptors involved in the control of the lipolytic activity of the human fat cell (Table 3).

One major concern of receptor studies in membrane preparations is that cell disruption could induce alterations in receptors and membrane transducing systems. Direct binding studies on intact cells can easily circumvent these problems and offer the possibility of comparing, under the same conditions, the characteristics of agonist binding to the receptor with those of subsequent responses. Furthermore, with this simple and rapid technique, it would be interesting to determine whether the adipocyte beta-adrenergic receptor changes found in animal models under various physiopathological conditions (6, 8, 9, 14, 42–44) are also to be found in the intact human fat cell. In man there are indeed few convincing studies concerning putative variations of these receptors in physiological conditions such a fasting, exercise, sex-related anatomical fat depositions (16, 45–48), and pathological situations such as thyroid dysfunction, diabetes, pheochromocytoma, and obesity which are known to alter the adrenergic control of lipolysis. The emergence of this kind of microassay of beta-adrenoceptors, performed on adipose tissue subcutaneous biopsies of less than 1 gram, should open new fields for such investigations. ▮▮

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